CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Theory

2.1.1 Lactic acid [10]

Lactic acid is a three carbon organic acid: one terminal carbon atom is part of an acid or carboxyl group, the other terminal carbon atom is part of a methyl or hydrocarbon group, and a central carbon atom having an alcohol carbon group. Lactic acid exists in two optically active isomeric forms (Figure 2-1).

L (+) Lactic acid D (-) Lactic acid

Figure 2-1 Optical isomeric forms of lactic acid [10]

Lactic acid is soluble in water and water miscible organic solvents but insoluble in other organic solvents. It exhibits low volatility. Other properties of lactic acid are summarized in Table 2-1.

Table 2-1 Physical properties of lactic acid [10]

Molecular weight	90.08
Melting point	16.8°C
Boiling point	82°C at 0.5 mm Hg 122°C at 14 mm Hg
Dissociation constant, Ka at 25°C	1.37×10^{-4}
Heat of combustion, ΔHc	1361 KJ/mole
Specific Heat, Cp at 20°C	190 J/mole/°C

2.1.2 Lactic acid fermentation

Lactic acid fermentation is the type of fermentation in which lactic acid is the end product. It is carried out by some bacteria (e.g. lactic acid bacteria), and also by animals.

1. Lactic acid bacteria

The lactic acid bacteria are a group of Gram positive bacteria, non-respiring, non-spore forming, cocci or rods, which produce lactic acid as the major end product of the fermentation of carbohydrates [11]. All lactic acid producers are non-motile gram positive rods that need complex carbohydrate substrates as a source of energy. The two main groups of lactic acid bacteria are

The homofermenter : only produce lactic acid

The heterofermenter : produce lactic acid plus other volatile compounds and small amounts of alcohol

The pathways of lactic acid production differ for the two. Homofermenters produce mainly lactic acid, via the glycolytic (Embden – Meyerhof pathway) [Figure 2-2]. Heterofermenters produce lactic acid plus appreciable amounts of ethanol, acetate and carbon dioxide, via the 6-phosphoglucanate/phosphoketolase pathway.

Homolactic fermentation:

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

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

Glucose Lactic acid

Heterolactic fermentation:

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

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

Glucose lactic acid ethanol carbon dioxide

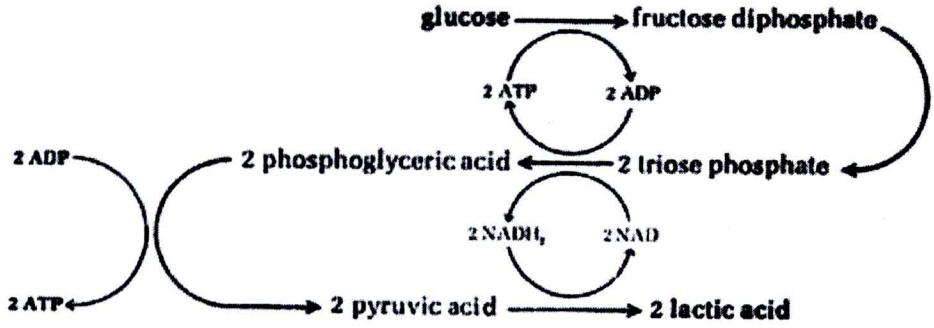

Figure 2-2 The pathway of lactic acid production

Table 2-2 Major lactic acid bacteria in fermented plant products. [9]

Homofermenter	Facultative homofermenter	Obligate heterofermenter
Enterococcus faecium	*Lactobacillus bavaricus*	*Lactobacillus brevis*
Enterococcus faecalis	*Lactobacillus casei*	*Lactobacillus buchneri*
Lactobacillus acidophilus	*Lactobacillus coryniformis*	*Lactobacillus cellobiosus*
Lactobacillus lactis	*Lactobacillus curvatus*	*Lactobacillus confusus*
Lactobacillus delbrueckii	*Lactobacillus plantarum*	*Lactobacillus coprophilus*
Lactobacillus leichmannii	*Lactobacillus sake*	*Lactobacillus fermentatum*
Lactobacillus salivarius		*Lactobacillus sanfrancisco*
Streptococcus bovis		*Leuconostoc dextranicum*
Streptococcus thermophilus		*Leuconostoc mesenteroides*
Pediococcus acidilactici		*Leuconostoc paramesenteroides*
Pedicoccus damnosus		
Pediococcus pentocacus		

2. Conditions Required for Lactic acid Fermentations [11]

1.) Temperature

Different bacteria can tolerate different temperatures, which provide enormous scope for a range of fermentations. While most bacteria have a temperature optimum of between 20 to 30°C, there are some (the thermophiles) which prefer higher temperatures (50 to 55°C) and those with colder temperature optima (15 to 20°C). Most lactic acid bacteria work best at temperatures of 18 to 22°C. Temperatures above 22°C, favour the lactobacillus species, such as *L. salivarius subsp. salivarius* that exhibited the highest yields and productivities at 45 °C [7].

2.) Hydrogen ion Concentration (pH)

The pH is one of the main factors influencing lactic acid production by fermentation process because the catalytic activity of the enzymes and the metabolic activity of the microorganisms depend on the extra cellular pH [12]. The fermentation pH

is both set at the beginning and then left to decrease due to acid production, or it is controlled by base titration, or by extraction, adsorption, or electrodialysis of LA. In all cases, titration to a constant pH resulted in higher or equal LA concentration, yield and productivity in comparison with no pH control [13]. The optimal pH for LA production varies between 5.0 and 7.0. A pH below 5.7 was only optimal for Lb. strains, which are known to tolerate lower pH than *Lactococci* [13].

3.) Oxygen Availability

Some of the fermentative bacteria are anaerobes, while others require oxygen for their metabolic activities. *L.salivarius subsp. salivarius* is the one of anaerobe fermentative, which is the amounts of oxygen isn't the limiting factor for fermentation [7].

4.) Nutrients

All bacteria require a source of nutrients for metabolism. The lactic acid bacteria require carbohydrates – either simple sugars such as glucose and fructose or complex carbohydrates such as starch or cellulose [11]. The addition of nutrients (such as brewer's yeast autolysate, yeast extract, corn steep liquor and peptone) and higher nutrient concentrations generally had a positive effect on the lactic acid production [13]

2.1.3 Fermentation process and mathematical analysis

Fermentation is a bioprocess involving microbial cells. Raw materials, usually biomass, are treated and mixed with other ingredients that are required for cells to grow well. The liquid mixture, the medium, is sterilized to eliminate all other living microorganisms and introduced to a large cylindrical, bioreactor or fermentor, typically equipped with agitators, baffles, air spargers, and various sensing devices for the control of fermentation conditions. A pure strain of microorganisms is introduced into the vessel. The number of cells will start to multiply exponentially after a certain period of lag time and reach a maximum cell concentration as a medium is depleted. The fermentation will be stopped and the contents will be pumped out for the product recovery and purification. This process can be operated either by batch or continuously [14].

1. Basic mathematical equation for fermentation process

The general material balance equations for growth, substrate utilization and product formation are defined as the following equations:

Cell (X) growth rate:

cell mass accumulation = cell in - cell out + cell growth - cell death

$$dX/dt = FX_0/V - FX/V + \mu X - \gamma X \quad (3\text{-}1)$$

Substrate (S) utilization rate:

Substrate mass accumulation = substrate in - substrate out - substrate for growth - substrate for M - substrate for P

Where

M = Maintenance

P = Product formation

$$dS/dt = FS_0/V - FS/V - \mu X/Y_{X/S} - mX - q_pX/Y_{P/S} \quad (3\text{-}2)$$

Product (P) formation rate:

Product mass Accumulation = product in - product out + product synthesis - product destruction

$$dP/dt = FP_0/V - FP/V + q_pX - KP \quad (3\text{-}3)$$

Where

X = Cell concentration (g-cell/l)

F = Volumetric flow rate (l/h)

V = Working volume (l)

K = Product destruction rate (h^{-1})

S = Substrate concentration (g-substrate/l)

μ = Specific growth rate (h^{-1})

γ = Specific death rate (h^{-1})

q_p = Specific product rate (g-product/g-cell-h)

m = Maintenance factor (h^{-1})

$Y_{X/S}$ = cell yield (g-cell/g-substrate) = $\Delta X/\Delta S$

$Y_{P/S}$ = Product yield (g-product/g-substrate) = $\Delta P/\Delta S$

Subscript (0) stands for initial condition

2. Mathematical equation for batch fermentation

For simplicity, some assumptions are made as the following items.

1. at the exponential growth phase:

$$\mu >> \gamma \quad (3\text{-}4)$$

2. at the product formation phase:

$$q_p >> K \quad (3\text{-}5)$$

3. at the substrate consumption phase:

$$\mu X/Y_{X/S} >> mX \qquad (3\text{-}6)$$

There are no inlet and outlet feed rate in batch operation hence F is zero. And together with equation (3-4), the equation (3-1) can be simplify as follow

$$\frac{1}{X}\frac{dX}{dt} = \mu \qquad (3\text{-}7)$$

Integration of equation (3-7) yields;

$$\ln\frac{X}{X_0} = \mu t \qquad (3\text{-}8)$$

This equation can be applied to obtain the doubling time (td) for the binary fission of microorganisms in log phase as follows

$$td = \frac{0.693}{\mu} \qquad (3\text{-}9)$$

With the same fashion, the equation (3-2) and (3-3) can be simplify as follows

$$q_p = \frac{1}{X}\frac{dP}{dt} \qquad (3\text{-}10)$$

$$qs = \frac{1}{X}\frac{dS}{dt} \qquad (3\text{-}11)$$

$$Y_{X/S} = \frac{\Delta X}{\Delta S} \qquad (3\text{-}12)$$

$$Y_{P/S} = \frac{\Delta P}{\Delta S} \qquad (3\text{-}13)$$

where

q_s = Specific comsumption rate (g-substrate/g-cell-h)

3. Phase of batch growth-cycle [15]

When microbial cells are inoculated into a batch fermentor containing fresh culture medium and their increase in concentration is monitored, several distinct phases of growth can be observed (Figure 2-2).

1. Lag phase

After inoculation, cells use new nutrients to regenerate pools of essential nutrients before growth can resume.

2. Log phase

Cells have adapted and are dividing at a constant rate (i.e. the maximum for the species under the given conditions of temperature, pH, nutrients, oxygen, etc.)

3. Declining growth phase

The rate of cell growth is decrease (short phase).

4. Stationary phase

Cell growth is finished as nutrients are exhausted and/or products build up in the media.

5. Death phase

The number of viable (living cells) in the stationary phase culture is decrease (usually due to toxicity of products).

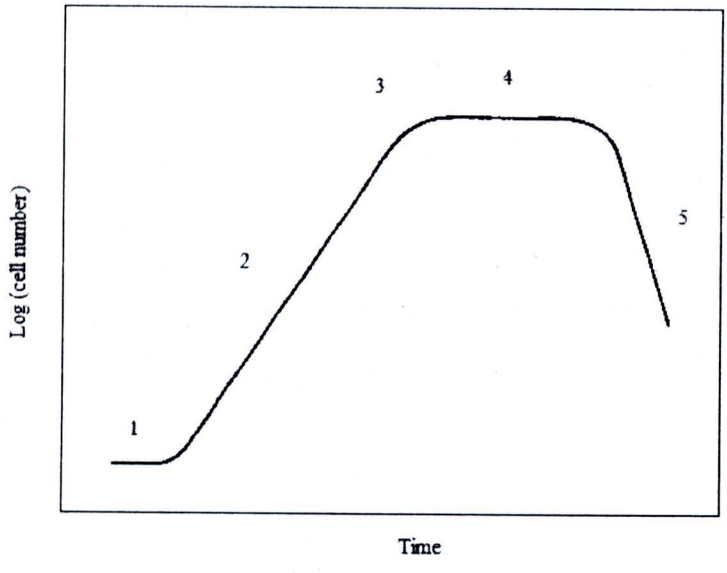

Figure 2-3 Phases of microbial growth in batch culture

4. Product Formation Kinetics

Product formation kinetic is classified into the following three types:

1. Growth Associated Product Formation

Product is formed simultaneously with growth of cells. That is product concentration increases with cell concentration.

$$q_p = \frac{1}{X}\frac{dP}{dt} = Y_{p/x}\mu$$

2. Mixed Mode Product Formation

Product is start forming at log or exponential phase and the product formation is a combination of growth rate and cell concentration.

$$q_p = \alpha\mu + \beta$$

if $\alpha = 0$, the product is completely non-growth associated; if $\beta = 0$, the product is completely growth-associated.

3. Non-Growth Associated Product Formation

Product is start forming at stationary phase and the product formation is unrelated to growth rate, but is a function of cell concentration.

$$q_p = \beta = \text{constant}$$

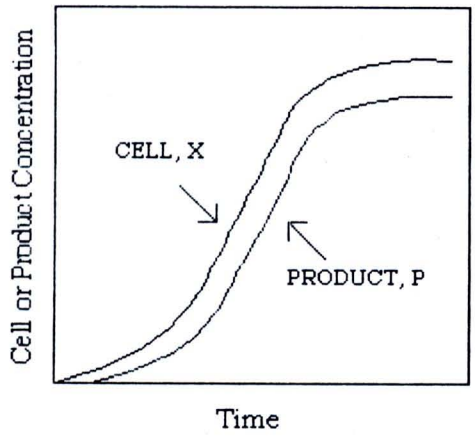

Figure 2-3 Growth Associated Product Formation

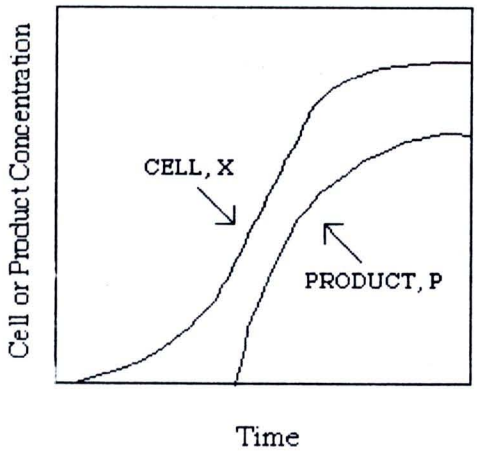

Figure 2-4 Mixed Mode Product Formation

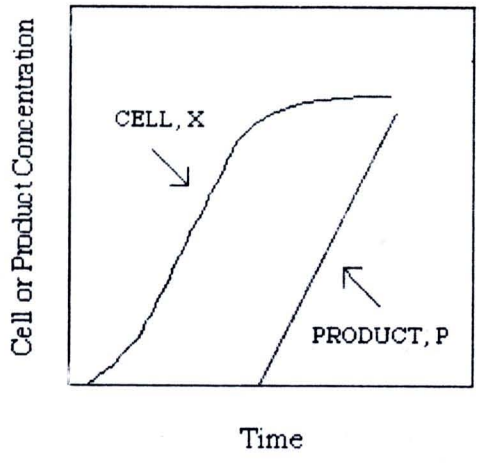

Figure 2-5 Non-Growth Associated Product Formation

2.1.4 Spent brewer's yeast [16]

Spent brewer's yeast (*Saccharomyces uvarum*) is the by-product of beer manufacturing. It is a cream or slurry containing about 15-20% total solids of which approximately two-thirds are yeast solids and one-third are beer solids. Spent excess brewer's yeast is recovered in breweries by sedimentation or centrifugation. The slurry must be kept refrigerated before use because even at 4 °C respiration reduces the carbohydrate fraction [17]. The best condition for storage are 4 °C in the absence of oxygen with intermittent agitation (two hour per day) to achieve homogeneity without seriously affecting yeast quality [18].

The composition of brewer's died yeast is given in table 2-3. This shows that brewer's dried yeast is an excellent source of protein [19]. Yeast are known as excellent sources of the water soluble B-complex vitamins including B1, B2 and nicotinic acid, as shown in the comparison (Table 2-4). Moreover, the protein in brewer's yeast contains relatively high levels of all the essential amino acids, with the exception of methionine (Table 2-5).

Table 2-3 Average composition of dried brewer's yeast [20]

	Chemical composition (% as dry matter)
Protein	48
Carbohydrate	36
Ash	8
Water	7
fat	1

Table 2-4 Typical B vitamin contents of dried brewer's yeast [21]

Vitamin	Range of content (μg/g)
B1 Thiamine	50-360
B2 Riboflavine	36-42
B6 Pyridoxine	9-102
Niacine	310-1000
Pantotenic A	100
Inositol	270-5000

Table 2-5 Approximate an amino acid content in the protein fraction of brewer's dried yeast [20]

Amino acid	%
Alanine	6.0
Arginine [a]	4.3
Aspartic acid	8.4
Cysteine	0.2
Glutamic acid	11.3
Glysine	4.1
Histidine [a]	2.2
Isoleucine [a]	3.9
Leusine [a]	6.3
Lysine [a]	6.5
Methionine [a]	1.2
Phenylalanine [a]	3.9
Proline	3.9
Serine	4.3
Threonine [a]	4.1
Tryptophan [a]	1.4
Tyrosine	2.4
Valine [a]	5.1

[a] Essential amino acid

2.1.5 Brewer's yeast autolysate and bitterness [16]

An autolysate is the total content after autolysis, or self-degradation, of the yeast. It is composed up of the particulate matter which is mainly the cell wall and debris suspended in the soluble fraction of the yeast.

Autolysis is a process by which the cell components are solubilized by activation of the degradative enzymes inherently present within the cell. It is achieved by the application of carefully controlled conditions such as temperature, pH, time and addition of certain enchancing agents under conditions which well kill the yeast population without inactivation the degrading enzymes that remain compartmentalized in live cells [17, 22, 23]. As the cells die under these conditions, disorderliness occurs within the cells, allowing the free degradative enzymes to indiscriminately attack their specific substrates.

This causes the breakdown of the corresponding macromolecules like proteins and nucleic acids to their basic units. Disorderliness within the cell results in leaky cell wall and a loss in the integrity of the semi-permeable membranes.

The bitterness of the spent brewer's yeast is iso-alpha acid, which is isomerized from the alpha acid that contained in hop. The most important class of hop compounds are the hop acids, which are distinguished as alpha-acids or humulones (1) and beta-acids or lupulones (2) (Figure 2-6). The two series comprise, in fact, three constituents differing in the nature of the side chain, which is derived from the hydrophobic amino acids, leucine, valine and isoleucine, for humulone (1a)/lupulone (2a), cohumulone (1b)/colupulone (2b) and adhumulone (1c)/adlupulone (2c), respectively [20].

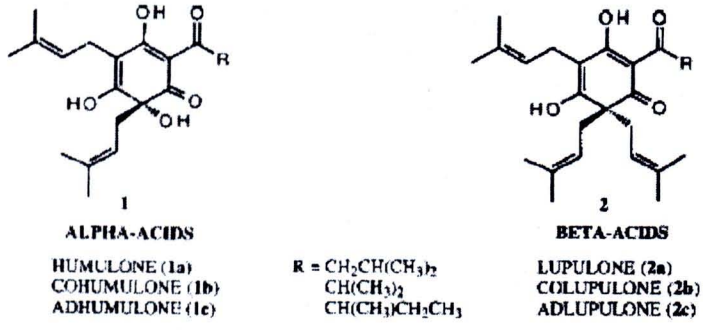

Figure 2-6 Structures of humulones and lupulones [24].

2.1.6 Cassava starch hydrolysate

Cassava is classified as a crop for developing countries and for consumption only by rural people, whereas the large crop of cassava grown annually in the tropics is actually consumed in all its forms at nearly all income levels. Originally the cassava tuber was a main food crop only in South America. Nowadays, however, it is grown as a substitute for rice or alternately with rice on extensive acreages in regions where, for centuries, rice has been the sole food crop.

In many tropical countries cassava as the principal source of carbohydrates occupies much the same position in the diet as potatoes in parts of the temperate zones. The cassava tuber is not a balanced food, consisting as it does largely of starch (Table 2-6); nonetheless, it is the most remunerative of crop plants in the hot climates, yielding perhaps more starch per hectare than any other cultivated crop with a minimum of labour.

Table 2-6 Nutrients in cassava roots [25]

	Percent
Protein	0.8-1.0
Fat	0.2-0.5
Carbohydrate	32
Ash	0.3-0.5
Moisture	65
Fibre	0.8
Calories per 100 g	127

Cassava starch hydrolysates are obtained by controlled acid or enzymatic hydrolysis of starch, which results in the production of D-glucose, maltose and a series of oligosaccharide and polysaccharides [26]. The enzymatic depolymerisation of starch is performed in two steps.

1. The liquefaction process:

For converts the insoluble starch granules into soluble polymer fragments (maltodextrins), the thermostable bacterial alpha-amylase is used in this process.

2. The saccharification process:

For break a polymer fragments into glucose, the glucoamylase is used. The various process alternatives for lactic acid production from starch are shown in Figure 2-7.

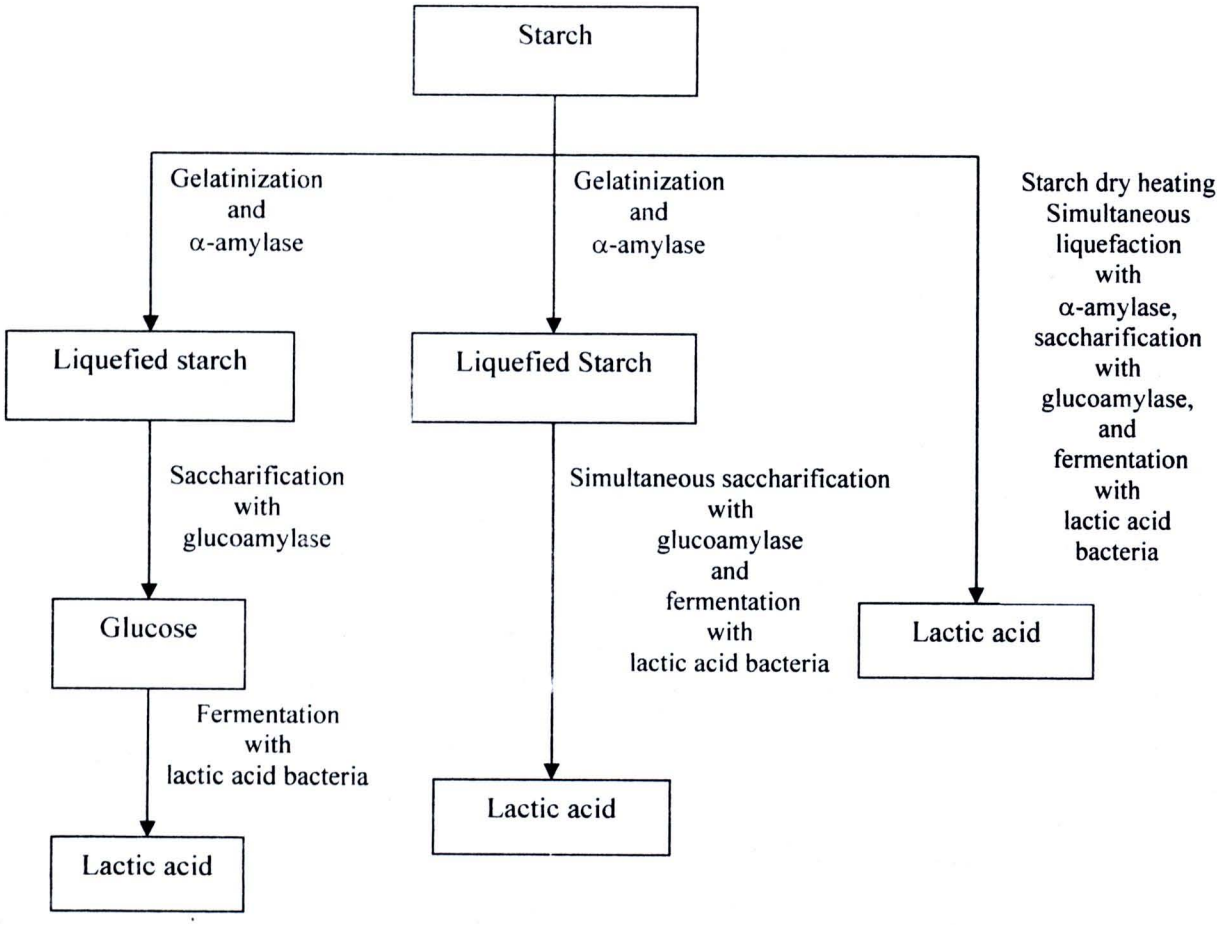

Figure 2-7 Various process alternatives for lactic acid production from starch [27]

2.2 Literature review

2.2.1 Production of lactic acid

Lactic acid is the most widely occurring caboxylic acid in nature, discovered by Swedish chemistry C.W. Scheele in 1780 [10]. It can be manufactured by chemical synthesis or microbial fermentation.

1. Chemical synthesis

M. Bicker et al. [28] studied the catalytical conversion of carbohydrates in subcritical water, which is a new chemical process for lactic acid production. In pure sub- and supercritical water (SCW) only small amouts of lactic acid are produced. But by adding small quantities of metal ions such as Co(II), Ni(II), Cu(II) and Zn(II) to that reaction media, the lactic acid yield is increased up to 42% (g g^{-1}) starting from sucrose, and 86% (g g^{-1}) starting from dihydroxyacetone at 300 °C and 25 MPa. From the result obtained, Zn(II) gave the best results with regard to the lactic acid yield.

2. Microbial fermentation

M. Altaf et al. [29] reported the screening of inexpensive nitrogen sources for production of L(+) lactic acid from starch by *Lactobacillus amylophilus* GV6 in single step fermentation. Seven type of inexpensive organic nitrogen supplement (flour of pigeon pea, red lentil gram, black gram, Bengal gram, green gram, soya bean and baker's yeast) were evaluated for their potential to replace more expensive commercial nitrogen sources of peptone and yeast extract for lactic acid production. L(+) lactic acid yield was about 92% g (lactic acid) / g (starch) utilized in this study.

M.Y. Ha et al. [1] reported the optimal condition of yeast extract, corn steep liquor and glucose concentration for the growth and lactic acid production of *Lactobacillus casei* KH-1 in pH-controlled batch cultures. The effect of yeast extract, corn steep liquor and glucose were significant for the maximum specific growth rate, μ_{max}, and the maximum biomass concentration, x_{max}. The results were shown that the growth and lactic acid production of *L.casei* KH-1 were strongly affected by glucose and the importance of the media composition was demonstrated. The optimal conditions of the growth and lactic acid production of *L.casei* KH-1 were 1.276% and 0.697%(w/v) for yeast extract, 3.505% and 1.708%(w/v) for corn steep liquor, and 2.309% and 2.215%(w/v) for glucose.

A.G. Vara Y.R. et al. [30] reported the production of L(+) and D(-) lactic acid isomers by *Lactobacillus casei subsp. casei* DSM 20011 and *Lactobacillus coryniformis subsp. Torquens* DSM 20004 in a conventional chemostat mode using various dilution rates. The results were shown that the specificity of L(+) lactic acid production *by L.casei* and the specificity of D(-) lactic acid production by *L.coryniformis* were not affected by dilution rate. The mean L(+) and D(-) fraction were 97% and 98% respectively.

S. Mirdamadi et al. [5] reported the comparison of lactic acid isomers produced by fungal and bacterial strains. The optical isomers of lactic acid were examined by L(+) and D(-) lactate dehydrogenase kit. All the *Rhizopus oryzae* strains test produced both optical isomers of lactic acid. The highest fungal and bacterial producer strains were *R.oryzae* PTCC 5263, *Lactobacillus plantarum* PTCC 1058, *L.bulgaricus* PTCC 1332 and *L.delbrueckii subsp. delbrueckii* PTCC 1333. From these lactobacillus strains, *L.casei subsp. casei* produced the low amount of D(-) lactic acid (2%). The optimum concentration of glucose for lactic acid production by R.oryzae and Lactobacillus strains were 180 g/l and 80-120 g/l, respectively.

M. siebold et al. [7] compared the production of lactic acid by three different Lactobacilli on MRS medium and glucose substrate, and its recovery by extraction and

electrodialysis. *L. salivarius ssp. salivarius* ATCC 11742 was used for producing L(+) lactic acid exhibited the highest specific growth rate (1.41 h^{-1}) and the highest specific productivity (5.45 h^{-1}). The products was extracted by four different amine carriers (Amberlite LA2, Hoe F2565, Hostarex A327 and Alamine 366) in the presence of modifiers (long-chain alcohols, alkylphosphates and acidic organic compounds) in kerosene and butylacetate, and a phosphinoxide (Cyanex 923). When using Hostarex A327 in oleylic alcohol or Cyanex 923 (phosphinoxide carrier) in kerosene or in oleylic alcohol, no modifier was necessary. The re-extraction of the free acid was not very successful. A maximum lactic acid yield of 72% was obtained. Except for kerosene and oleyl alcohol, the biocompatibility of the other chemicals was unsatisfactory. Recovery of the free lactic acid by electrodialysis and bipolar membrane is very promising.

I.K. Yoo et al. [3] studied and compared the various nitrogen sources with yeast extract (YE) for lactic acid production by *Lactobacillus casei subsp. rhamnosus*. On the basic of the amount of B vitamins in YE, various amounts of five essential B vitamins known to support the growth of *L.casei* were supplemented together with the nitrogen sourced. The results were shown that half of the YE in a medium supplemented with 10 g/l YE could be replaced by B vitamins at less than 25 mg/l and 3.3 g/l N-Z-soy peptone without a significant decrease in the rate of lactic acid production.

V. Arasaratnam et al. [31] studied the supplementation of whey with glucose and different nitrogen sources for lactic acid production by *Lactobacillus delbrueckii*. When glucose (20 g/l) was added to whey, 20 g/l yeast extract supplementation was found to be most suitable. Among the different nitrogen sources supplemented to whey (yeast extract, peptone, soya flour, and $(NH_4)_2SO_4$), yeast extract was the best. As yeast extract supplementation was not economically attractive, the mixture of $(NH_4)_2SO_4$ and yeast in ratio 3:1 which were the same efficiency of lactic acid production were replaced. From these results, the supplementation of whey with different nitrogen sources and vitamin B has no significant improvement in lactic acid production.

W. Fu and A.P. Mathews [32] studied the kinetic model effects of pH, substrate, and oxygen on the lactic acid production from lactose by *lactobacillus plantarum*. Lactic acid fermentation with this bacterium is found to be homolactic and primarily growth associated. Optimum pH for cell growth and acid production was found to be between 5 and 6. Anaerobic fermentation gave a higher lactic acid yield of about 2.3 times that for aerobic fermentation. The high lactic acid yield (0.95~1.03 w/w), the complete conversion rate is 1.05 (w/w), tolerance of low pH conditions and the high cell growth

L.delbrueckii ssp. bulgaricus was much lower. The productivities and yields increased with the addition of yeast extract for all strains. All four organisms produced mainly L-lactate. The Lactococci produced 100% L-lactate in the presence of yeast extract and *Lactococcus lactis ssp. lactis* ATCC 19435 produced exclusively L-lactate also in the absence of yeast extract.

R.P. John et al. [2] studied the solid-state fermentation (SSF) of lactic acid production from agro wastes (cassava bagasse and sugarcane bagasse) using *Lactobacillus delbrueckii*. Gelatinished cassava bagasse was enzymatically hydrolysed and starch hydrolysate was used to moisten the inert sugarcane bagasse, which was used as the solid support for SSF. This substrate was supplemented with 0.5 g/ 5 g support NH_4Cl and yeast extract. SSF was carried out in 250 ml Erlenmeyer flasks at 37°C using L. delbrueckii as inoculum. A maximum of 2.49 g/l lactic acid was obtained after 5 days of fermentation under the optimized conditions.

T.R. Shamala and K.R. Sreekantiah [36] studied the degration of starchy substrates by a crude enzyme preparation and utilization of the hydrolysates for lactic fermentation. An enzyme preparation obtained from *Aspergillus ustus*, possessing cellulose, α-amylase, amyloglucosidase, proteinase and D-xylanase activities, was used along with commercial bacterial α–amylase and amyloglucosidase for the degradation of ragi (Eleusine coracana) flour and wheat (Triticum vulgare) bran. In the present study it was found that the hydrolysed wheat bran can be used alone or with other hydrolysed starch substrates or glucose without fortification of other nutrients to yield high amounts of lactic acid (97-99%), and the use of A. ustus enzyme preparation in addition to α–amylase and amyloglucosidase for the hydrolysis of the substrates indicated that the hydrolysate could be used at a lower level to obtain higher yields.

B.J. Naveena et al. [37] studied the production of L(+) lactic acid by *Lactobacillus amylophilus* GV6 in semi-solid state fermentation using wheat bran. To maximize the production of lactic acid, process variables like the volume of inoculum and incubation period were optimized keeping mass /volume ratio of wheat bran and $CaCO_3$ at 9 % and 0.375%, respectively, and constant pH 5.5. Maximum starch conversion to lactic acid was observed at process conditions of wheat bran mass /volume ratio 9% at 37 °C, pH 6.5, inoculum volume 3.5 ml and incubation period of 130 h.

Table 2-7 The lactic acid production

organism	FM	Substrate, S	nutrient	operating condition	X (g/l)	S (g/l)	LA (g/l)	μ_{max} (h^{-1})	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	Ref.
L.casei KH-1	batch	glucose	YE 0.697% + CSL 1.708%	37 °C, pH 5.7, 24 h	5.38	22.15	-	0.49	0.31	0.81	[1]
L.casei ssp. casei DSM 20011	cont	glucose	YE	36°C, pH 6.4, 16 h	-	40.00	-	0.47	0.11	0.96	[30]
L.bulgaricus PTCC 1332	batch	glucose	YE	37°C, pH 5.5, 48 h	9.00	6.00	24.00	-	0.15	0.40	[5]
L.plantarum PTCC 1058	batch	glucose	YE	37°C, pH 5.5, 48 h	9.12	6.00	29.00	-	0.15	0.83	[5]
L.delbruekii ssp. delbruekii PTCC 1333	batch	glucose	YE	37°C, pH 5.5, 48 h	8.76	6.00	15.00	-	0.14	0.25	[5]
L.lichmannii PTCC 1057	batch	glucose	YE	37°C, pH 5.5, 48 h	7.86	6.00	1.68	-	0.13	0.02	[5]
L.casei PTCC 1055	batch	glucose	YE	37°C, pH 5.5, 48 h	8.22	6.00	6.18	-	0.13	0.10	[5]
L.casei ssp. casei PTCC 1608	batch	glucose	YE	37°C, pH 5.5, 48 h	8.40	6.00	19.00	-	0.14	0.31	[5]

Table 2-7 The lactic acid production (Cont.)

organism	FM	Substrate, S	nutrient	operating condition	X (g/l)	S (g/l)	LA (g/l)	μ_{max} (h^{-1})	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	Ref.
L.lactis ssp. lactis PTCC 1403	batch	glucose	YE	37°C, pH 5.5, 48 h	7.80	6.00	2.91	-	0.13	0.04	[5]
L.amylophilus GV6	batch	Corn starch	Red lentil 2% + Baker's yeast 1%	37 °C, pH 6.5	-	-	12.20	-	-	0.92	[29]
L.delbrueckii ssp. delbrueckii ATCC9649	batch	Wheat flour hydr	YE	37 °C, pH 6.0, 168 h	-	119.00	109.00	-	-	0.91	[35]
L.delbrueckii ssp. bulgaricus DSM 20081	batch	Wheat flour hydr	YE	45 °C, pH 6.0, 168 h	-	144.00	26.00	-	-	0.18	[35]
L.casei ssp. rhamnosus	batch	glucose	YE	45 °C, pH 6.0	3.17	30.00	27.80	0.68	-	0.90	[7]
L.salivarius ssp. salivarius	batch	glucose	YE	45 °C, pH 6.0	3.05	30.00	27.50	1.41	-	0.99	[7]
L.helveticus	cont, recirc	Whey perm powder	YE	42 °C, pH 5.5, 138 h	-	-	28.29	-	-	0.88	[34]
L.casei NRRL B441	batch	Barley starch	YE	37 °C, 48 h	-	130.00	-	-	-	0.98	[27]
L.casei LA 041	batch	CSL + glucose	YE	42°C, pH 6.25, 84 h	2.55	-	112.5	-	-	0.87	[38]

Table 2-7 The lactic acid production (Cont.)

organism	FM	Substrate, S	nutrient	operating condition	X (g/l)	S (g/l)	LA (g/l)	μ_{max} (h^{-1})	$Y_{X/S}$ (g/g)	$Y_{P/S}$ (g/g)	Ref.
L.plantarum ATCC 21028	batch	lactose	YE	37°C, pH 6.0	-	-	-	0.36	0.39	1.02	[32]
L.rhamnosus NBRC3863	batch	glucose	Spent cells + YE	42 °C, pH 6.0	-	-	-	-	-	0.99	[39]
L.casei ATCC 10863	batch	RHH + glucose	YE	38 °C, pH 6.0, 18 h	6.70	21.00	-	-	-	0.44	[4]
L.delbrueckii NCIM 2365	batch	Cane sugar	YE	42 °C, pH 6.0, 24 h	3.90	80.00	66.00	-	-	0.97	[6]
L.delbrueckii NRRL B445	batch	Whey + glucose	YE	pH 6.5, 48 h	-	-	24.50	-	-	0.82	[31]
L.delbrueckii NRRL B445	batch	Whey + glucose	Soya flour	pH 6.5, 48 h	-	-	23.00	-	-	0.77	[31]
L.delbrueckii NRRL B445	batch	Whey + glucose	$(NH_4)_2SO_4$	pH 6.5, 60 h	-	-	23.50	-	-	0.79	[31]

Abbreviations: FM = fermentation mode; LA = lactic acid; Yp/s = yield of g LA per g substrate provides; Ref = reference; YE = yeast extract; CSL = corn steep liquor; hydr = hydrolysate; perm = permeate; RHH = ram horn hydrolysate; cont = continuous culture; recirc = recirculation of cells

2.2.2 Brewer's yeast autolysate and bitterness

An autolyasate is the total content after autolysis, or self-degradation, of the yeast. It is composed up of the particulate matter which is mainly the cell wall and debris suspended in the soluble fraction of the yeast. Unlike extracts, autolysates do not give a clear solution due to the presence of the insoluble cell material. Thus they can be used in formulations where clarity is of less importance [19].

Bitterness of spent brewer's yeast is attributed to hop constituents adsorbed on the yeast cell wall. During fermentation iso-alpha acids associate with the surface of the yeast cells present rendering brewer's yeast intensely bitter [40]. The acids are thought to be bound to the yeast by adsorptive forces, but the compounds can be removed by dilute sodium carbonate solution, or by urea, thiourea and potassium thiocyanate solution, reagents usually employed to eliminate hydrogen bonds and other weak forces [41].

All beers have in common the property that the flavor is mainly derived from hops. The essential components are the so-called hop alpha-acids or humulones [42]. These alpha-acid are tasteless, but upon prolonged boiling they are isomerized to the very bitter-tasting iso-alpha-acids or iso-humulone. When hops or hop products are boiled in wort, the most important reaction of hop components is the isomerization of alpha-acids to iso-alpha-acids [40].

P. Kittianong. [43] studied the production of yeast extract from spent brewer's yeast using rotary microfiltration as a means to combine debittering and cell debris separation into a single step, without using a toxic alkali wash. The pH of yeast homogenate was found to affect protein yield and bitterness of the product. Rotary filtration of yeast homogenate at various pHs resulted in different percent protein transmissions. These were found to be 5.05%, 9.83%, and 30.83% for pH 5, 6, and 7.5, respectively. The bitterness concentration in the permeate was also found to be higher at higher pHs. Autolysis of the cell homogenate prior to filtration increased protein yield and decreased bitterness considerably. At pH 5.5, the protein transmission was increased to 60% and debittering efficiency was increased from 59% to 86.

P. Boonyeun et al. [44] studied the separation of cell debris from intracellular product by rotating microfiltration. The preparation of feed to microfiltration process was performed by using 13 hour autolysate from yeast cream 22 % dry weight (spent brewer's yeast) and then this autolysate was diluted to 11.25 % dry weight. At 0.28

bar, mass flux of protein and amino acid was maximum and equal to 800 and 1400 g/ 2 h, respectively. The production rate of protein and amino acid from yeast autolysate filtration was 9.71 and 16.01 g/h, respectively. The content of protein and amino acid obtained from the first filtration was 0.339 and 0.574 g/g dry weight, respectively, and the content from the first leaching process was 0.346 and 0.602 g/g dry weight, respectively. The more number of leaching, the more bitterness of product was obtained.

A. Niemthanorm. [16] studied the production of yeast extract from spent brewer's yeast by comparison yeast extract without debittering and yeast extract with debittering by solvent extraction. It was found that the solid yield, the protein recovery, and the level of the bitterness of yeast extract with debittering by solvent extraction (using sodium hydroxide, and sodium carbonate) had less than the yeast extract without debittering. It was also found that using hexane (1:5 v/v) for debittering the yeast extract could had more solid yield and protein recovery comparison with using sodium hydroxide and sodium carbonate.

M. Rakin et al. [8] reported the influence of brewer's yeast autolysate and lactic acid bacteria on the production of a functional food additive based on Beetroot juice fermentation. Comparative investigation of three bacteria cultures (*Lactobacillus plantarum* A112, *L.acidophilus* BGSJ 15-3 and *L.acidophilus* NCDO 1748) during fermentation in two media, beetroot juice and a mixture of beetroot juice with an autolysate of brewer's yeast have been performing. The poorest fermentative activity and growth was observed using *L.acidophilus* NCDO 1748 culture. The acidifying activity (production of lactic acid and decrease in pH) of the *L.acidophilus* BGSJ 15-3 was better than the *L.plantarum* A112. Whereas, the *L.plantarum* A112 shown better growth than *L.acidophilus* BGSJ 15-3. From this result obtained, it has been conclude that the *L.plantarum* A112 and *L.acidophilus* BGSJ 15-3 can successfully used for fermentation of the mixture of beetroot juice and brewer's yeast autolysate in order to obtain a functional food additive.

M. Rakin et al. [45] studied the conditions for lactic acid fermentation based on a mixture of beetroot juice, carrot juice and different content of brewer's yeast autolysate with *Lactobacillus plamtarum* A112 and with *Lactobacillus acidophilus* NCDO1748. The production of lactic acid has been stimulated using a high content of brewer's yeast autolysate. From this result obtained, *L.plantarum* A112 showed better growth and lactic acid production than *L.acidophilus* NCDO 1748 in these mixtures,

and it can be seen that the mixture of beetroot, carrot juice and brewer's yeast autolysate is richer in minerals (Ca, P, Fe) and B-carotene than fermented beetroot juice with the same content of brewer's yeast autolysate.