Chapter 2

Literature Review

2.1 Acetone- butanol Fermentation

2.1.1 Microorganism. (3)

Cl.acetobutylicum is straight rod shape of 0.6-0.9 by 2.4-4.7 μm. It is often granulose positive and gram positive. It move with peritrichous flagella. Spores are oval, subterminal; no exosporium and no appendages. Surface colonies are circular, 3-5 mm in diameter, raised, irregular margin, grayish white, translucent, and glossy surface.

Abundant growth in nutrient broth containing fermentable carbohydrate that it produces fermentation products including of acetone, butanol, ethanol, acetic acid and butyric acids. Optimum conditions for growth are 37°C, pH 4.8-5 at atmospheric nitrogen.

2.1.2 Morphology. (4)

In fermentation processes, Cl.acetobutylicum ATCC 824 was different morphology that changes with the ages of bacteria.

Table 2.1 Morphology of Cl.acetobutylicum ATCC 824 during batch fermentation.

Time (hrs.)	Morphology
0-10	vegetative cell is a rod shape.
10-15	Active motility and many of the rod occurred.
15-18	Granulose in the cell or bright phase
18-25	cigar shaped clostridial forms
25-35	endospores
35	spores and cell lysis

The production of acetone and butanol by anaerobic fermentation has traditionally been carried out in a batch system, the whole process normally lasts between 30-48 hours.

During the batch fermentation, the first phase is characterized by exponential growth of the cell population. Active growth is accompanied by the fermentation of substrate to produce acetic acid and butyric acid which cause a drop in the pH of fermentation broth.

About 15 hours after inoculation, exponential cell growth and motility began to decrease. About 2 hours before the break in the pH occurred, the pH of the fermentation broth reached its lowest point and then began to rise again, granulose was first detected in the cell.

After 18 hours, solvent production firstly occurred only at the stage when the culture entered the stationary phase. The solvent formation has been found to be linked to the conversion of vegetative rods into the clostridial stage. In the industrial fermentation processes, high solvent yields are associated with a high proportion of clostridial forms in the culture.

2.1.3 Fermentation processes. (5)

In a industrial fermentation of acetone-butanol (Figure 2.1), butanol, acetone and ethanol are produced by the selective bacterial fermentation (Table 2.2) of carbohydrate-containing material (Table 2.3). It is necessary to feed the raw material which is passed through some kinds of preliminary treatment. When corn is used as the source of carbohydrate, the germ (24% oil) is removed and sold for coproduct value, and karnels are ground to a coase meal. The ground meal is diluted with water to a sugar concentration of 6 to 8 percent approximately, is sterilized, is cooled to 30°C, and is pumped to a fermentor where it is inoculated with a culture of bacteria (Clostridium acetobutylicum) and allowed to ferment for 48 to 72 hours at 37°C (Table 2.4). Protein nutrients and an alkali buffer that is

used to control the pH are also added to improve yields. It is essential to sterilize all the fermentors, pipes, pipe connections and equipments that grain or butanol organisms may come into contact, since contamination in this fermentation is usually a very serious matter that may involve considerable losses.

After fermentation is completed, the beer (fermentation mixture) containing 1.5 to 2.5% mixed solvent is pumped to a column. When a 50% solvent mixture is stripped overhead, distiller slop is removed as bottoms waste. This slop is dried and sold as a high protein meal coproduct. It is a source of riboflavin and other components of the vitamin B complex to be used as animal feed. Another by-product is a mixture of carbon dioxide and hydrogen evolved during the fermentation.

The mixed-solvent vapor from the beer column are led to a batch fractionating column from which three fractions (acetone, ethanol and butanol) are removed as top product, leaving the water as bottom product. The acetone and ethanol fraction, containing about 15% water, is led to a column from which vapors containing 70% butanol and 30% water are removed as top product. On condensation, two layers are formed. The top layer (80% butanol and 20% water) is recycled to the butanol column, and the bottom layer (4% butanol and 90% water) is recycled to the beer column.

The yield of mixed solvents is approximately 30% by weight based on the sugar content. The ratio of solvent yield are 60-65% butanol, 30-35% acetone and 5-10% ethanol by weight (Table 2.4, 2.5). The biochemical pathway scheme for the butylalcohol fermentation and glucose balance of acetone-butanol fermentation are shown in Figure 2.2, 2.3 and 2.4, respectively.

Table 2.2 Microorganisms which can product butanol by fermentation. (6)

U.S.patent no.	Name of bacteria	Substrate	Solvent ratio (%)			Isopropanol
			Butanol	Ethanol	Acetone	
1,725,083	Bacillus saccharobutylicum-beta	Inverted molasses and $CaCO_3$	75	-	3	35
1,908361	CL. saccharobutylicum-gamma	Blackstrap molasses and $CaCO_3$	65-80	-	18-34	1-2
1,922,921	CL. saccharobutyl-acetonicum	Blackstrap molasses, corn gluten and $(NH_4)2\ SO_4$	64	-	36	-
2,017,572	CL. viscifaciens	Inverted molasses and $CaCO_3$	66	-	3	31
2,050,219	CL. saccharoacetobutylicum-beta and gamma	Cone molasses and degradedz protein such as ammonia, steep water or distillery slop	68-73	1-3	26-32	-
2,063,448	CL. propyl butylicum	Inverted molasses, NH_3 and $CaCO_3$	69-70	-	4-17	14-28 (mixture isopropyl and ethyl)
2,073,125	CL. invertoacetobutylicum	Louisianna molasses (inverted) and ammonium salt or	66-70	2-3	27-31	-

		alkalies				
2,089,522	CL. saccharoacetobutylicum	Louisianna molasses $(NH_4)2SO_4$ and $CaCO_3$	68-73	1-3	26-32	-
2,132,039	CL. propyl butylicum-alpha	Inverted molasses,$(NH_4)2SO_4$ and $CaCO_3$, K_2HPO_4 and $MgSO_4$	65-70	-	5-10	16-26
2,139,108	CL. saccharoacetobutylicum-liquefaciens-gamma and delta	Blackstrap molasses $(NH_4)2SO_4$, $CaCO_3$ and P_2O_5	58-74	2-6	24-36	-
2,139,111	CL. saccharoacetobutylicum-liquefaciens-gamma and delta	Cuban molasses ,$(NH_4)2SO_4$, $CaCO_3$ and P_2O_5	60-69	3-4.5	26-35	-
2,147,487	B.butacone	Blackstrap molasses and animal and vegetable protein	65	-	28	-
2,169,246	CL. celerifactor	Inverted molasses, N_{H3} and $CaCO_3$	60	2	38	-
2,195,629	CL. granulobacter acetobutyricum	Molasses, corn gluten ,ammonium salt and $CaCO_3$	60-75	1-10	25-30	-
2,219,426	CL. saccharobuyl-isopropyl-acetonicum-beta	Cane and beet molasses ,$(NH_4)2SO_4$ and $CaCO_3$	60-85	-	15-40	0.1-4
2,308,837	CL. esdisonii	Cuban blackstrap, $(NH_4)2SO_4$,$CaCO_3$ and NH_4OH	75-76	4-6	17-20	-
2,420,998	CL. amylosaccharobutyl-propylicum	Inverted molasses,$(NH_4)2SO_4$ and $CaCO_3$ and P_2O_5 or NH_4OH and P_2O_5	65-72	Trace	2-4	26-32
2,430,791	CL. saccharoacetoperbutylicum	Inverted molasses,$(NH_4)2SO_4$ and $CaCO_3$ and P_2O_5 or NH_4OH and P_2O_5	60-76	2-7	18-25	-

Table 2.3 Raw material which can be used as carbon source in acetone-butanol fermentation. (7)

Raw material	Microorganism	Fermentation Time (hr)	% Production yield	Total Solvent (g/l)	Solvent (g/l)		
					Butanol	Acetone	Ethanol
Whey	CL. acetobutylicum P 262	39	42	9.5	7	2.5	-
Corn starch	CL. acetobutylicum No.105	72	26.7	13.3	7.2	4.3	1.8
Molass	CL. acetobutylicum P 262	30-36	31-32	16.18	N*	N*	N*
Tapioca Starch	Clostridium No. 8P-2	25-30	29.22	14.03	9.82	3.95	0.25
Tapioca Starch	CL. butylicum	68.5	27.83	14.63	9.51	4.86	0.26
Glucose	CL. acetobutylicum ATCC 824	96	32	20.8	15	4.5	1.3
Glucose	CL. butylicum NRRL B 592	110	28.2	14.1	N*	N*	N*
Glucose	CL. acetobutylicum P 262	58	32	12.7	9	3.4	0.3
Xylose	CL. acetobutylicum ATCC 824	144	28	14.1	8.9	3.9	1.3
Arabinose	Cl.acetobutylicum ATCC 824	99	29	16.5	10.5	4.5	1.5
Lactose	Cl.acetobutylicum P 262	96	39	9.5	6.7	2.6	0.2
Galactose	CL. acetobutylicum P 262	42	31	10	7.1	2.7	0.2

N* = no reported data

Table 2.4 Mass balance of fermentation

Components	% of Fermented Sugar
Carbon dioxide	57
Hydrogen	2
ABE	32
Biomass	6
Acetic acid and Butyric acid	2
Other metabolism	1

Table 2.5 Solvent ratio

% n-Butanol	% Acetone	% Ethanol
60-65	30-35	5-10

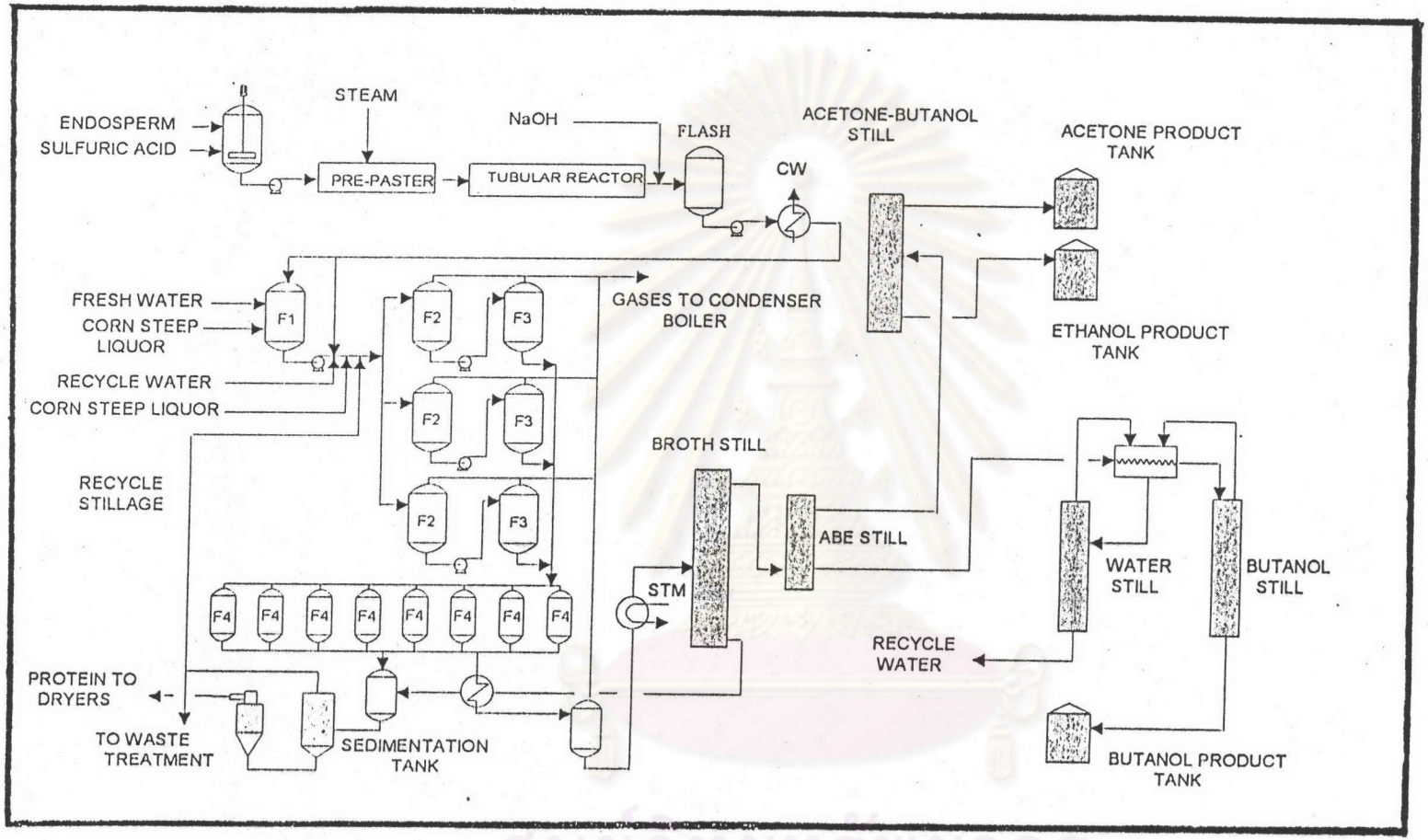

Figure 2.1 Process for n-butanol production fermentation (8)

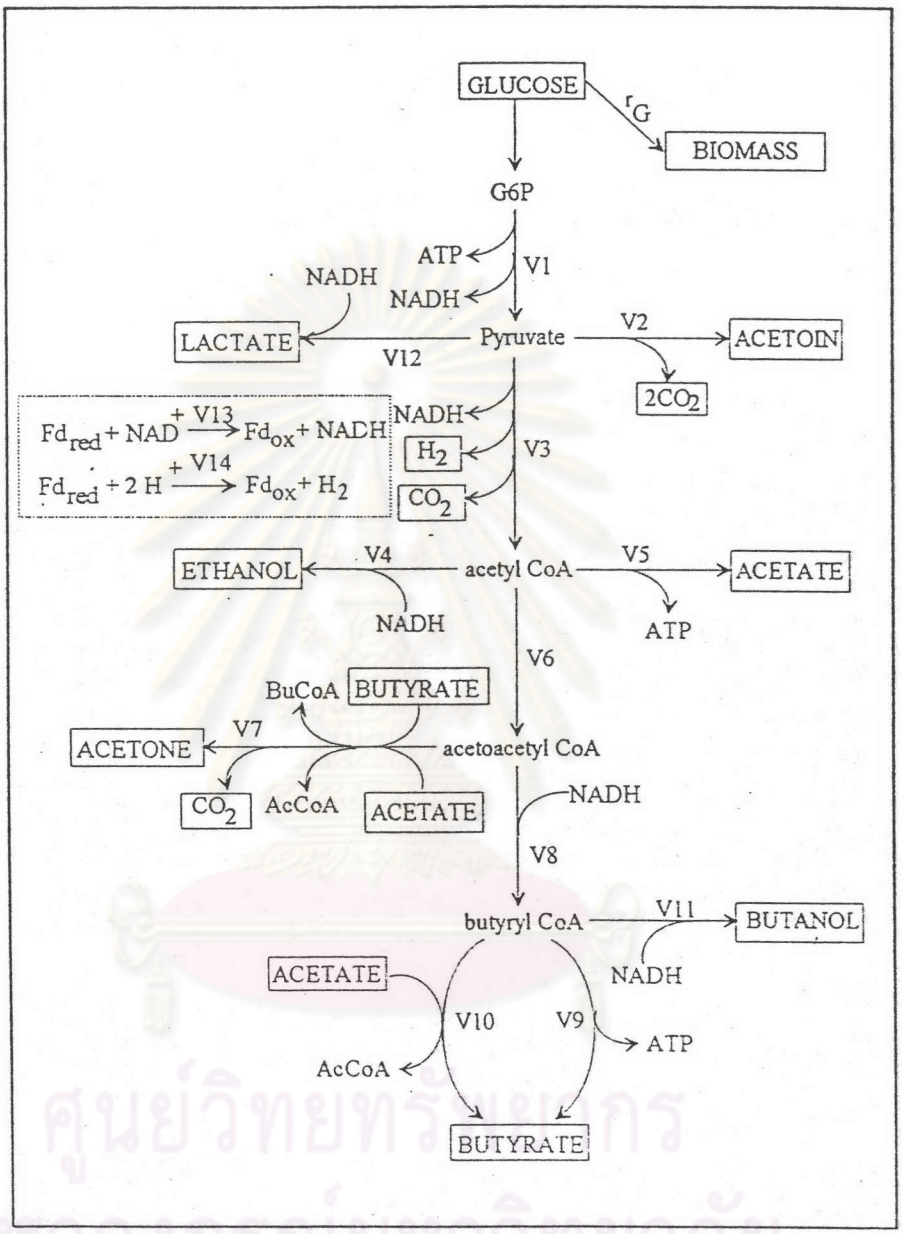

Figure 2.2 Biochemical pathway for conversion of sugar into organic solvents by Cl.acetobutylicum (9)

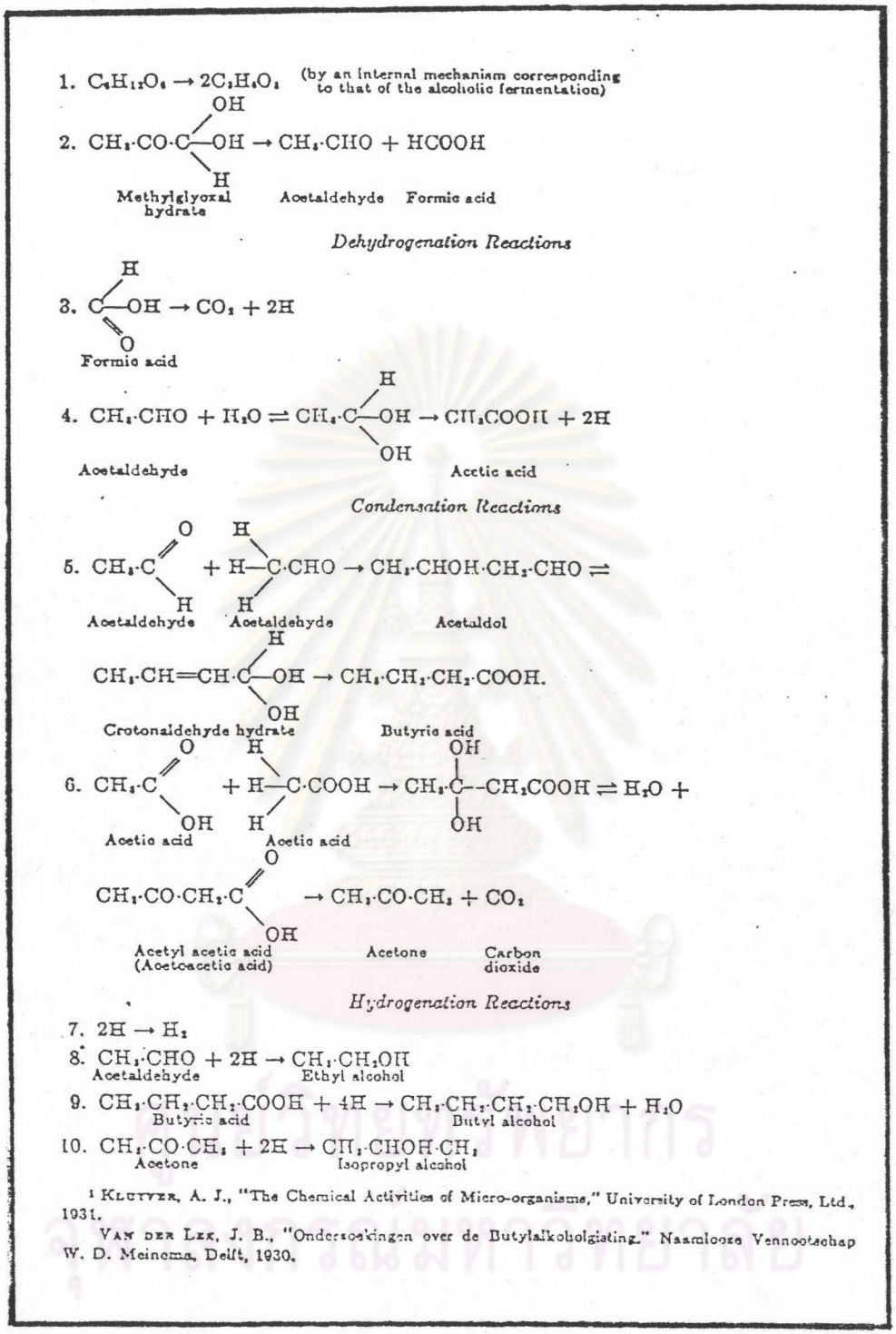

Figure 2.3 The scheme for the butanol fermentation (10)

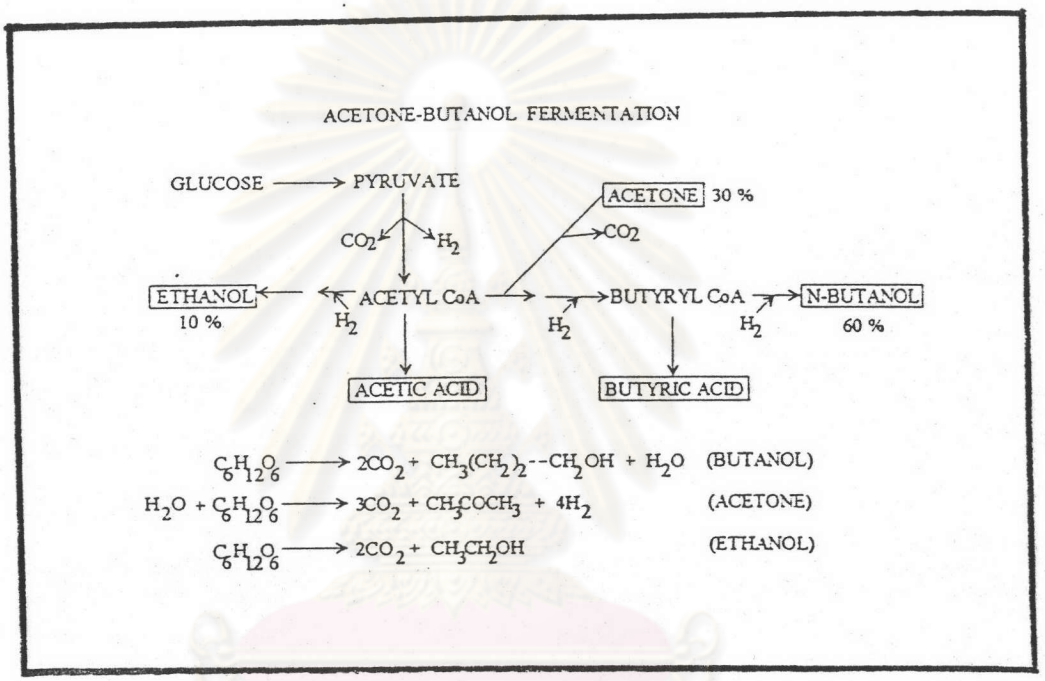

Figure 2.4 Glucose balance of acetone-butanol fermentation. (10)

2.1.4 Culture Methods

The continued transfer of a seed culture in the usual media results in the culture becoming sluggish, with corresponding decreased yield of solvents. It has been shown that the most prodigious producers of solvents are in general the most heat resistance. " Heat Shocking " is a method that the vegetative cells and the weaker spores of a culture are destroyed. By subjecting a culture of the organism, the development of spore has been favored at temperature of 100°C for 1 to 2 minutes, heat shocking is effected.

The treatment bears a direct relation to the temperature employed, the size and nature of the tube containing the seed culture and the characteristics of medium. If one is to use a thin-walled wafer tubing and a very small amount of the culture, it is obvious that neither the time of shocking nor the temperature should be excessive, since even the resistant spores may be destroyed.

Alternate heat shocking and successive subculturing are commonly used to activate a culture. The medium containing the seed culture is allowed to stand at room temperature for a few days to encourage sporulation. New medium is inoculated from the spore-containing culture and heat shocked. The surviving spores are permitted to germinate under favorable conditions and subcultures are made successively at daily intervals for 4 to 7 days. At the end of this, the culture is again permitted to stand at

room temperature to encourage spore formation. The cycle is then repeated heat-treatment, repeated subculturing, rest for sporulation, heat shocking, and so on.

2.1.5 Optimum Conditions for Fermentation (5).

Temperature - The most favorable temperature range is 37°C to 42°C. Since the neutral solvents, especially acetone, are volatile at these temperatures it is necessary to take precautions to avoid losses during the fermentation processes.

Oxygen Relationship - Since the organism best suited to the production of acetone and butanol are anaerobic in nature, the highest yields will be obtained when anarobiosis is maintained.

pH - Growth may be obtained in corn mashes between a pH of 4.7 and 8 by the butanol organisms, but there is a low production of solvent at both of the extremes. The pH range of 5 to 7 is satisfactory in most cases.

2.1.6 Uses for Products and By-Products of Acetone-butanol Fermentation

Butanol - Butanol is used primary in the manufacturing of lacquers which are utilized on automobiles, rayon, detergent, brake fluids, amine for gasoline additive. It is also used as a solvent for fats, waxes, resins, rubber, plastics, shellac and varnish. Large quantities of butanol and its derivatives are used in other industrial processes.

Acetone - Acetone is used mostly as a solvent for fats, oils, waxes, resins, rubber plastics, lacquers, varnishes. Moreover, it is used in the manufacturing of artificial silk and leather, photographic film, airplane dopes, rubber cement and other products.

Ethanol - Ethanol is used in pharmaceutical industrials and is also used for chemical material synthesis such as ether or chloroform.

Gases - The gases , consisting of 60% CO_2 and 40% H_2 , can be used to produce methanol or ammonia. When the two gases are separated, the hydrogen can be used in chemical synthesis as burned for energy and the carbon dioxide solidified into dry ice.

Solid Residues - The acetone-butanol fermentation produces a beer which contain riboflavin, complex vitamins B, protein and certain unknown growth factors in relatively large concentrations. After drying, the solid residues was used as a vitamin supplement for animal feeds.

2.1.7 The Petrochemical Processes

In the United States, 70% of butanol is made by the Oxoprocess. The oxo or hydroformylation process has been developed into a 3 MMt/y industry. Most of the aldehydes produced are either reduced to alcohols directly or subjected to alcohol condensation prior to hydrogenation. A limited amount of aldehyde is oxidized to the corresponding acid. Propylene is the highest volume feedstock for Oxo plant.

The overall reaction is

$$2CH_3CH{=}CH_2 + 2CO + 4H_2 \longrightarrow CH_3\text{-}CH_2\text{-}CH_2OH + CH_3\text{-}\underset{\displaystyle CH_3}{\underset{|}{C}}HCH_2OH$$

Besides normal and isobutylaldehydes, varying amounts of normal and isobutyl alcohols, propane and heavy ends are produced in two major reaction steps. In the first step, the olefin are reacted with hydrogen and carbon dioxide using cobalt carbonyls as catalyst. In the second step, the oxo-products, largely aldehydes are hydrogenated using supported metallic cobalt as catalyst. The hydrogenated product is caustic mashed to remove organic acid and formats. The crude alcohols are purified in two tower fractionation system. The process is shown in Figure 2.5

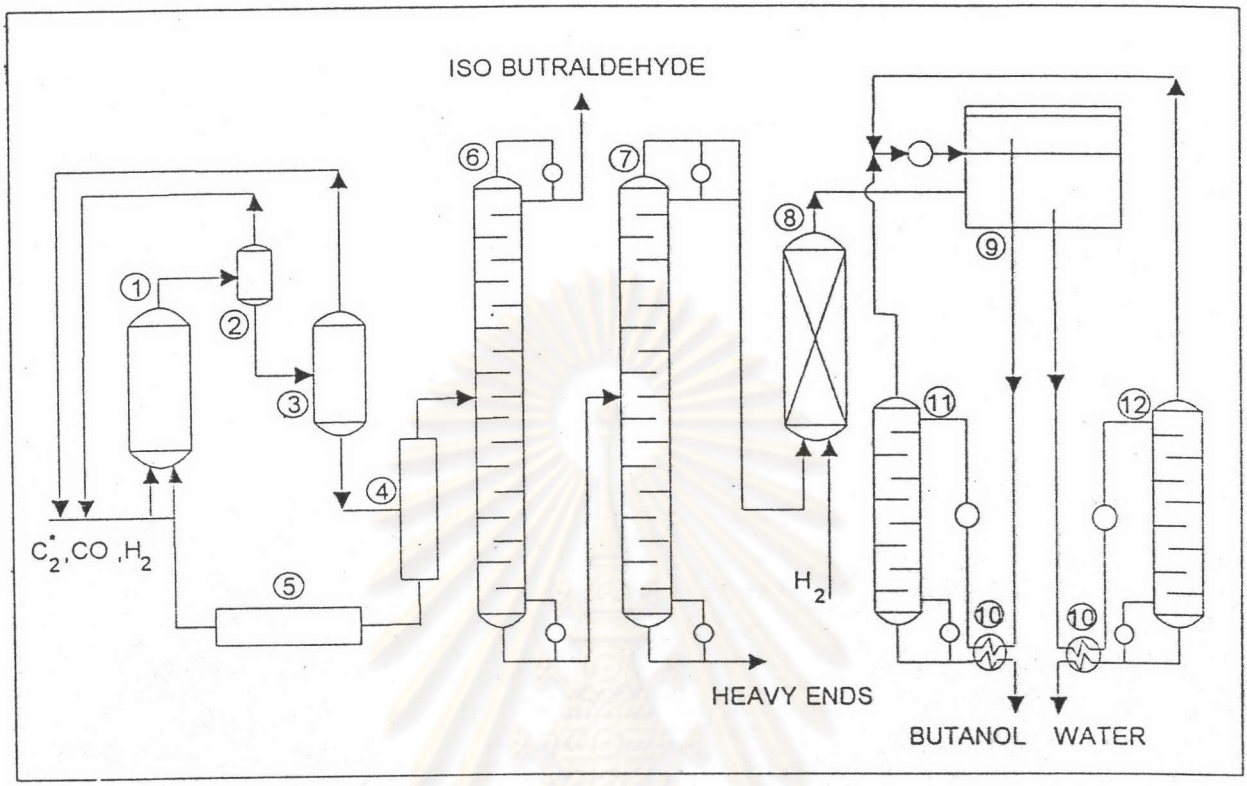

Figure 2.5 Process of n-butanol production by hydroformulation of propylene. (11)

1. oxo reactor
2. low pressure separator
3. high pressure separator
4. catalyst removal
5. catalyst storage
6. iso-butylaldehyde column
7. normal-butylaldehyde column
8. hydrogenation reactor.
9. decanter.
10. condenser
11. butanol column.
12. aqueous column.

2.2 Development of Acetone-butanol Fermentation Processes

2.2.1 Single-stage Continuous Fermentation

Leung and Wang (12) attained a solvent productivity of 2.5 gl^{-1} h^{-1} at solvent concentration of 12 gl^{-1} in a continuous culture of Clostridium acetobutylicum ATCC 824 which is growthing in a complex medium with 15 gl^{-1} yeast extract.

M.Fick, P.Pierrot and J.M.Engasser (13) obtained a solvent productivity of 0.75 gl^{-1} hr^{-1} with an end concentration 13 gl^{-1} solvent from a continuous culture of Clostridium acetobutylicum ATCC 824 on a complex medium containing 40 gl^{-1} glucose. This process can be maintained for 2 months, at an optimal dilution rate of 0.06 hr^{-1}. For batch culture, butanol toxicity limited high product concentration and volumetric productivities. However, the continuous culture was usually limited by high concentration of butanol which had a strong toxic effect and inhibits cell growth.

2.2.2 Immobilization

C.Muangnapoh and G.Goma (14) reported a solvent productivity of 0.75 gl^{-1} hr^{-1} in a two-stage continuous process when an immobilization technique was used in

the second stage fermentor. Spore and vegetative cells of Cl. acetobutylicum were immobilized in pored-volcanic rock.

Ch.Frick and K.Schugerl (15) studied a two-stage continuous fermentation with and without immobilized ATCC 824 in calcium alginate gel of the second fermentor for comparison between the two systems. The systems without immobilization obtained a solvent productivity of 1.93 gl^{-1} hr^{-1} with an end concentration 15.4 gl^{-1} solvent while the systems with immobilization obtained a solvent productivity of 4.02 gl^{-1} hr^{-1}

The single-stage continuous process of butanol using immobilized Clostridium acetobutylicum (L.Haggtrom and N.Molin, (16)) in calcium alginate gel was reported that a solvent productivity was 0.48 - 0.64 gl^{-1} hr^{-1}. However, since butanol toxicity rapidly reduced cell activity within the immobilization matrix, extensive investigation will be required to establish economical working condition for such a process.

2.2.3 Microfiltration

The application of microfiltration (MF) to fermentation processes has been studied to improve productivity in acetone-butanol fermentation. Continuous ethanol fermentation, using crossflow microfiltration (CFM) to recycle cell back to the fermentor, has been reported to increase the biomass per unit volume, facilitate an

increase in productivity. In MF and CFM, fermentation broth flows tangentially across the membrane surface with cell-free liquid permeating through the membrane. Accumulated cells are swept away from the membrane surface using a high recirculation flow rate. Concentrated cells and a portion of the cell-free liquid stream are returned to the fermentor.

M.Minier, E.Feras, G.Goma and P.Soucallce (17) improved productivity in acetone-butanol fermentation by coupling continuous fermentation with microfiltration. The membrane was a carbon tube with a microfiltering ceramic coat inside. With total recycle of biomass, a dry weight concentration of 125 gl^{-1} was obtained, which enhanced a solvent productivity of 4.5 gl^{-1} hr^{-1} at dilution rate 0.33 hr^{-1}.

D.Schlote and G.Gottschalk (18) improved a productivity of acetone-butanol fermentation by using cellulosetriacetate membrane to separate and recycle cells in a continuous fermentation of Clostridium acetobutylicum ATCC 824 under phosphate limitation ($0.74x10^{-3}$ molar) at dilution rate 0.40 hr^{-1}. A solvent productivity of 4.1 gl^{-1} hr^{-1} was maintained for three months.

M.Pisalaphoge (19) studied application of microfiltration for improved productivity in continuous acetone-butanol fermentation. A multitubular ceramic microfilter was used to separate and recycle cells of Cl.acetrobutylicum ATCC 824.

With total recycle of biomass, a dry weight concentration of 80 gl^{-1} and a solvent productivity of 6.06 gl^{-1} hr^{-1} were obtained at dilution rate 0.55 hr^{-1} on a complex medium containing 42.4 gl^{-1} glucose.

2.2.4 Control of Biomass Concentration in Continuous Fermentation.

Afschar et.al (20) obtained significant high productivity by using CFM (hollow fiber configuration) in a continuous fermentation and by Clostridium acetobutylicum ATCC 824 fermenting a complex glucose medium. At biomass concentration of 8 gl^{-1} was obtained in a single-stage cell recycle fermentor using turbidostatic cell concentration control and a dilution rate of 0.64 h^{-1}, a solvent productivity of 5.4 gl^{-1} h^{-1} was attained.

M.Fick, P.Pierrot and J.M.Engasser (21) did the experiments at dilution rate of 0.30 hr^{-1} by using a microfiltration which was coupled with continuous fermentor at dilution rate 0.30 hr^{-1}, a biomass concentration of 20 gl^{-1}, a solvent productivity of 4.5 gl^{-1} hr^{-1} and an end concentration 16 gl^{-1} solvent were obtained from this process.