CHAPTER II

LITERATURE REVIEW

2.1 Acetone-Butanol Production

2.1.1 The fermentation process (3)

Butanol, acetone and ethanol are produced by the selective bacterial fermentation (see table 2.1) of carbohydrate-containing materials (see table 2.2, 2.3) such as molasses and grain. Molasses is dituted with water to a concentration of approximately 5% sugar, sterilized, cooled to 30 °C, and pumped to a fermenter. A culture of bacteria (Clostridium gaccharobutyl acetonicum liquefaclens, grown in sterile molasses is added to start the fermentation. Protein nutients and an alkaline buffer to control the pH are also added to improve yields.

After fermentation has proceeded for 36-48 hours, the beer (fermentation mixture) containing 1.5 to 2.5% mixed solvents is pumped to a column. When a 50% solvent mixture is taken off overhead, distillers slop is removed as bottoms. This slop may be dried and sold as animal feed for utilization as a source of riboflavin and other components of the vitamin B complex, since it has been discovered that the bacteria used in the fermentation synthesizes vitamins. Another by-product is a mixture of carbon dioxide and hydrogen evolved during the fermentation.

The mixed-solvent vapors from the beer column are led to a batch fractionating column from which three fractions (acetone, ethanol and butanol) are removed overhead, leaving water as bottoms. The acetone and ethanol fraction, containing about 15% water, is led to a column from which vapors containing 70% butanol and 30% water

U.S. patent no.	Names of bacteria	Substrate	Solvent ratiosx			
			Butyl alcohol	Ethyl alcohol	Acetone	Isopropyl alcohol
1,725,083	Bacillus saccharobutylicum-beta	Inverted molasses and $CaCO_3$	75	-	3	35
1,908,361	Clostridium 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	64	-	36	
2,017,572	Cl. viscifaciens	Inverted molasses and $CaCo_3$	66	-	3	31
2,050,219	Cl. saccharoacetobutylicum-beta and gamma	Cane molasses and degraded protein, such as ammonia, steep 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 salts or alkalies	66-70	2-3	27-31	
2,089,522	Cl. saccharoacetobutylicum	Louisiana molasses, $(NH_4)_2$ SO_4 and $CaCO_3$	68-73	1-3	26-32	
2,132,039	Cl. propyl butylicum-alpha	Inverted molasses, $(NH_4)_2$ SO_4 $CaCO_3$, K_2HPO_4 and $MgSO_4$	65-70		5-10	16-26
2,139,108	Cl. saccharobutyl-acetonicum-liquefaciens -gamma and delta	Blacktrap molasses, $(NH_4)_2$ SO_4 $CaCO_3$ and P_2O_5	58-74	2-6	24-36	
2,139,111	Cl. saccharobutyl-acetonicum-liquefaciens -gamma and delta	Cuban molasses, $(NH_4)_2$ SO_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, ammonia and $CaCO_3$	60	2	38	
2,195,629	Cl. granulobacter acetobutylicum	Molasses, Corn gluten, ammonium salts, and $CaCO_3$	60-75	1-10	25-30	
2,219,426	Cl. saccharobuly-isopropyl-acetonicum -beta	Cane and beet molasses, $(NH_4)_2$ SO_4 and $CaCO_3$	60-85	-	15-40	0.1-4.0
2,398,837	Cl. madisonii	Cuban blackstrap, NH_4OH $(NH_4)_2$ SO_4 and $CaCO_3$	75-76	4-6	17-20	
2,420,998	Cl. amylosaccharobutyl-propylicum	Invert molasses, $(NH_4)_2$ SO_4 $CaCO_3$, and P_2O_5, or NH_4OH and P_2O_5	65-72	Trace	2-4	26-32
2,439,791	Cl. saccharoacetoperbutylicum	Invert molasses, $(NH_4)_2$ SO_4 $CaCO_3$ and P_2O_5 or NH OH and P_2O_5	69-76	2-7	18-25	

Table 2.1 Microorganisms which can produce butanol by fermentation (4)

Table 2.2 Raw material which can be used as carbon source in acetone-butanol fermentatoion (5)

Raw material	Microorganism	Fermentation time (hr)	% Production yield	Total solvent (g/lit)	Solvent (g/lit)		
					Butanol	Acetone	Ethanol
Whey	Cl. acetobutylicum P 262	39	42	9.5	7.0	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	A*	A*	A*
Tapioca Starch	Clostridium No. 8P-2	25-30	29.22	14.03	9.82	3.95	0.25
Tapioca Starch	Cl. butylicum NRRL B592	68.5	27.83	14.63	9.51	4.86	0.26

A* = No reported data

TABLE 2.3 Sugars which can be used as carbon source in acetane-butanol fermentation (5)

Substrate	Microorganism	Fermentation time (hr)	% Production yield	Total solvent concentration (g/l)	Solvent concentration (g/l)		
					Butanol	Acetone	Ethanol
Glucose	Cl. acetobutylicum ATCC 824	96	32	20.8	15.0	4.5	1.3
Glucose	Cl. butylicum NRRL B592	110	28.2	14.1	A*	A*	A*
Glucose	Cl. acetobutylicum P 262	58	32	12.7	9.0	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	38	9.5	6.7	2.6	0.2
Galactose	Cl. acetobutylicum P 262	42	31	10.0	7.1	2.7	0.2

A* = No reported data

are removed overhead. On condensation, two layers are formed. The top layer (80% butanol and 20% water) is returned to the butanol column, and the bottom layer (4% butanol and 90% water) is returned to the beer column.

The yield of mixed solvents is approximately 30% by weight based on the sugar charged. Solvent yield ratios are 60-65% butanol, 30-35% acetone, and 5-10% ethanol by weight.

Sterilized degermed cornstarch mash may be substituted for the molasses, in which case the fermentation is carried out at 37 °C using bacteria of the Clostridium genus such as Clostridium acetobutylicum. Figure 2.1 is the flow diagram of Acetone-butanol fermentation. The biochemical pathway and scheme for the butyl alcohol fermentation are shown in Figure 2.2, 2.3 respectively.

2.1.2 Products and By Products of Acetone-Butanol Fermentation and their Uses.

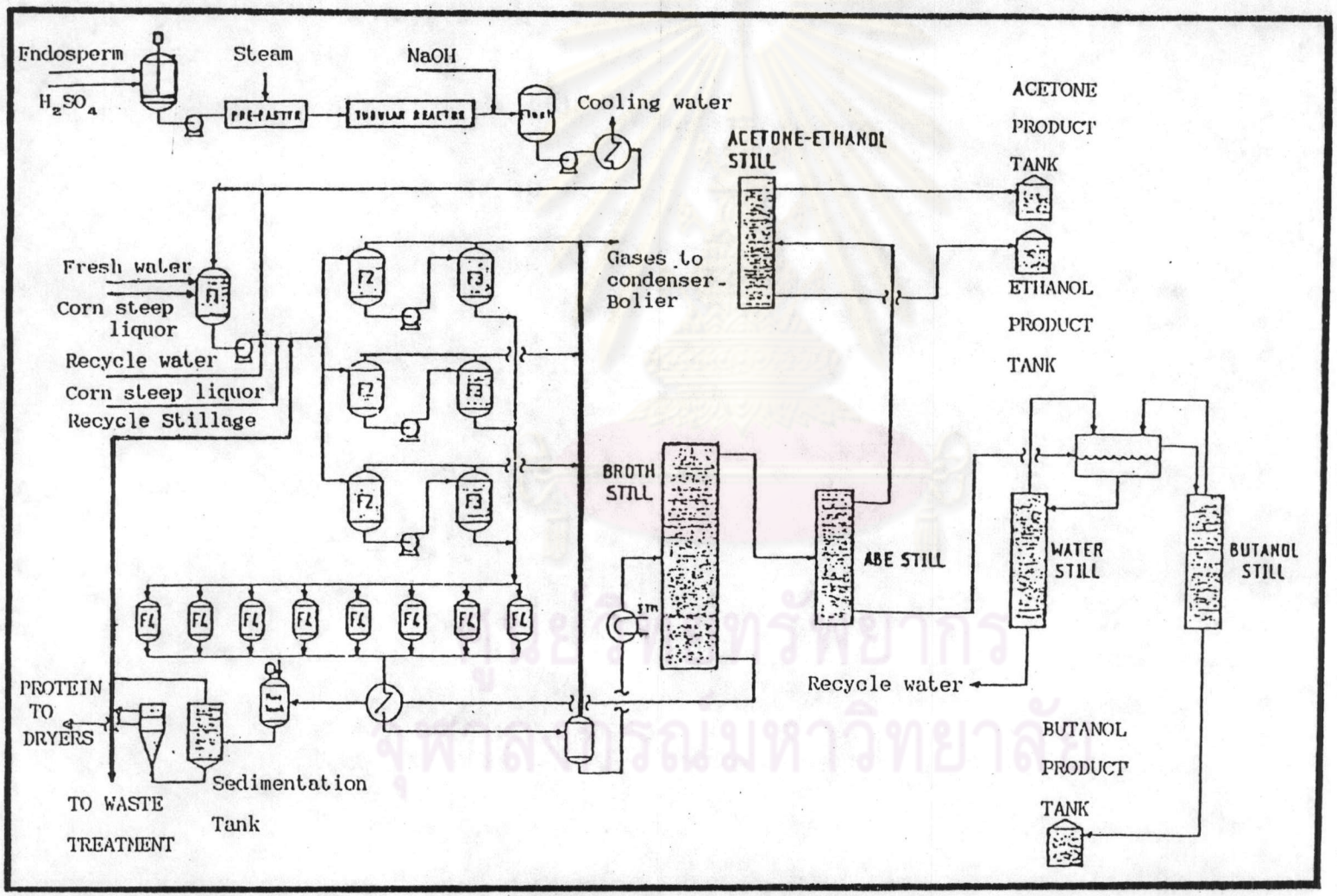
Butanol : Butanol is used primarily in the manufacture of lacquers, rayon, detergents brake fluids, amines for gasoline additives and it's also used as a solvent for fats, waxes, resins, shellac and varnish.

Acetone : Acetone is used mostly as a solvents for fats, oils, waxes, resins, rubber plastics, lacquers, varnishes and rubber cements.

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

Gas : The gas produced by this fermentation consists of 60% CO_2 and 40% H_2. About 60-70 g. of gas is produced from 100 g. of glucose. The gases 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 CO_2 solidified into dry ice.

Figure 2.1 Process for n-butanol production fermentation (3)



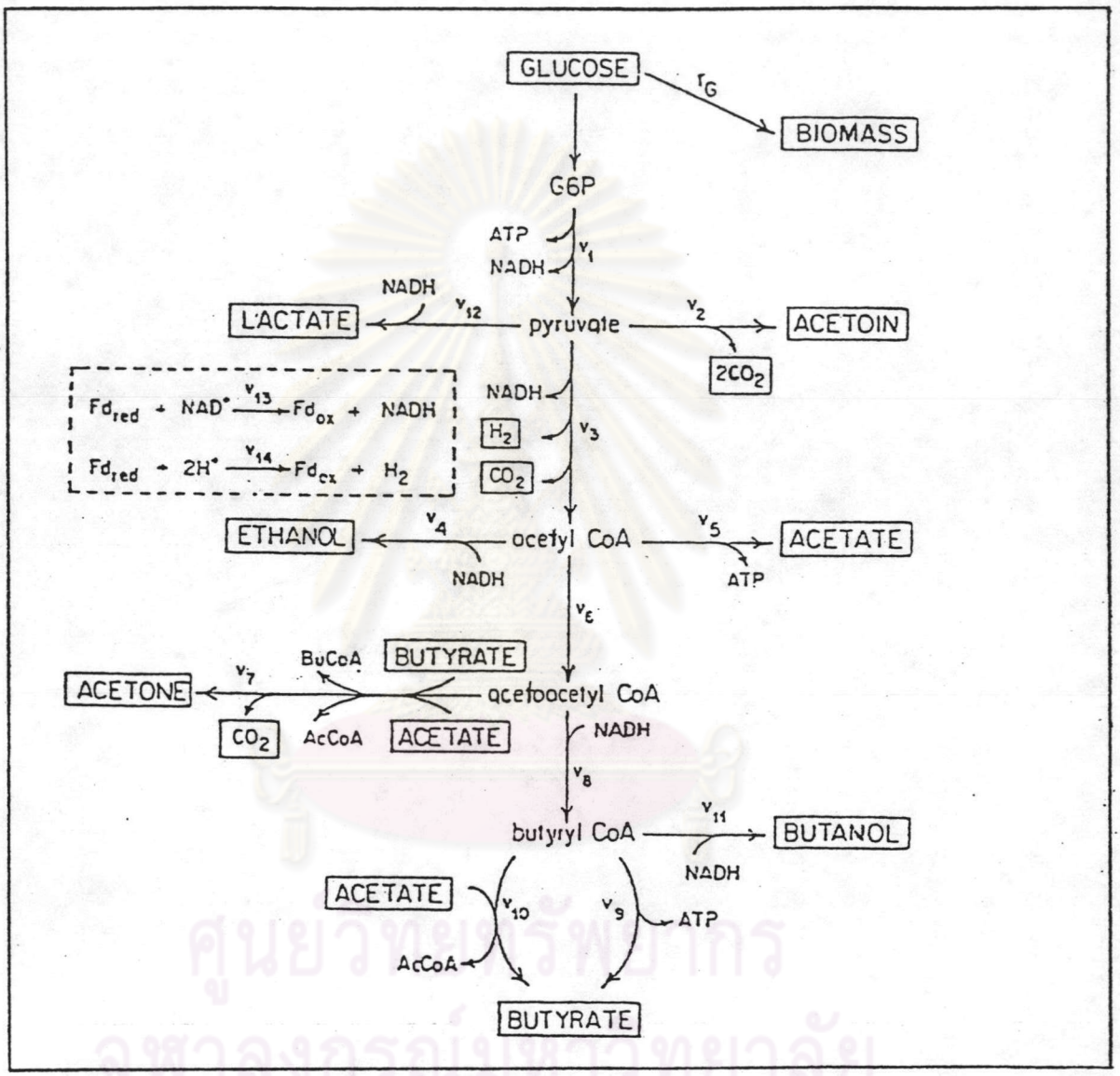
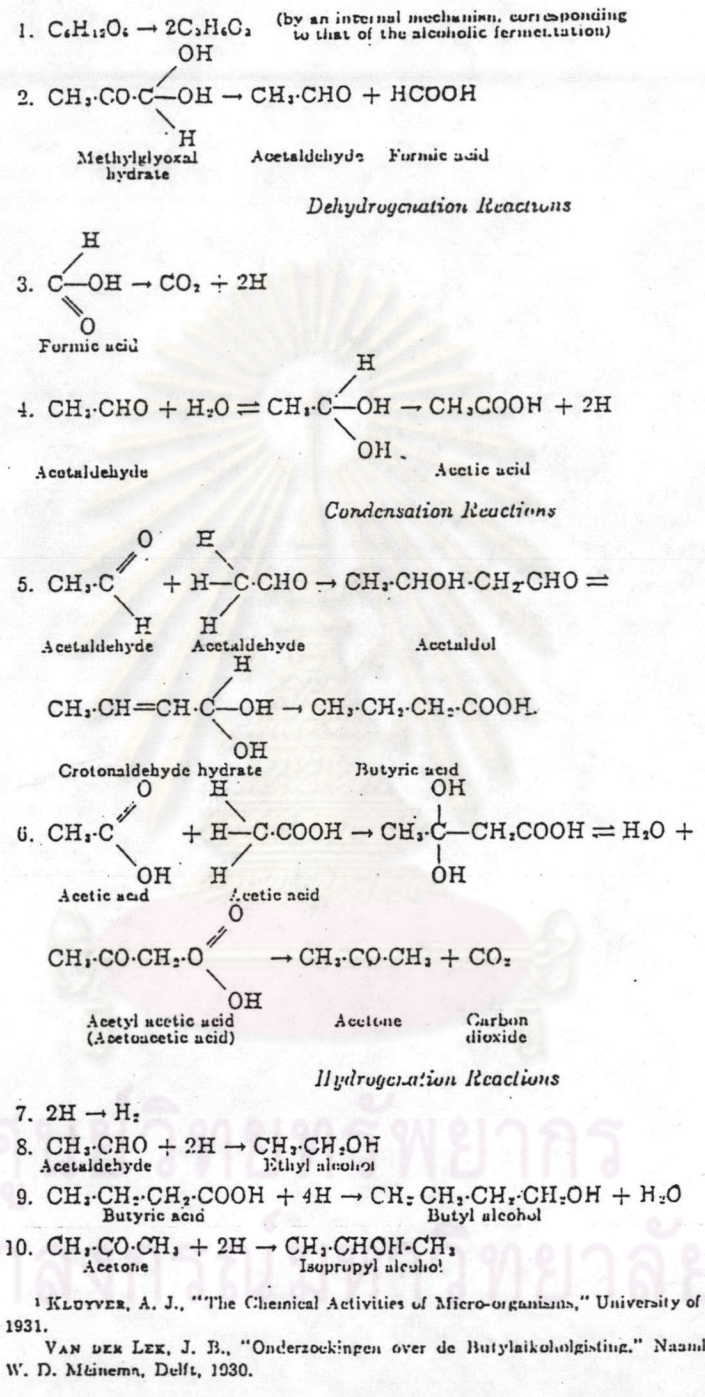


Figure 2.2 Biochemical pathway for conversion of sugar into organic solvents by Cl. acetobutylicum (6)



[1] KLUYVER, A. J., "The Chemical Activities of Micro-organisms," University of London Press, Ltd., 1931.

VAN DER LEK, J. B., "Onderzoekingen over de Butylalkoholgisting," Naamlooze Vennootschap W. D. Meinema, Delft, 1930.

Figure 2.3 The scheme for the butyl alcohol fermentation (6)

Solid Residues : The acetone-butanol fermentation produces a beer which contains riboflavin (60-100 g. per g. dry wt.), B complex vitamins, protein (20-30% dry wt.) and certain unknown growth factors in relatively large concentrations. The solid residues after drying by spray or drum drying was used as a vitamin supplement for animal feeds.

The mass balance of fermentation material is in table 2.4, 2.5., and the figure 2.4 (9).

Table 2.4 : Mass balance of fermentation

Component	% of Fermented Sugar
CO_2	57
H_2	2
A B E	32
Biomass	6
Acetic and butyic acids	2
other metabolism	1

Table 2.5 : Solvent ratio

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

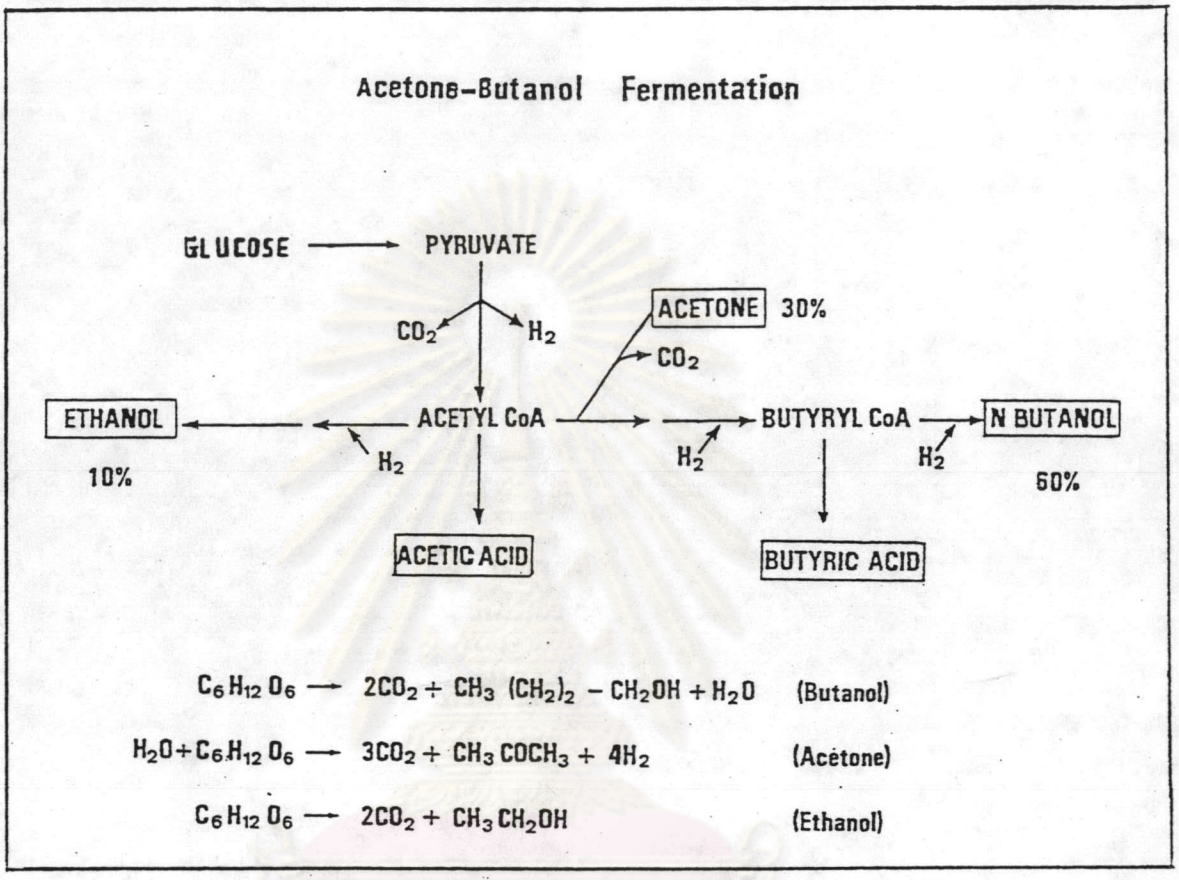


Figure 2.4 Glucose balance of acetone-butanol fermentation

2.1.2 The Petrochemical Process

In the United States 70% of butanol is being made via the oxo process. The oxo or hydroformylation process has been developed into a 3 MM t/y industry. Most of the aldehydes produced are either reduced to alcohols directly or subjected to aldol condensation prior to hydrogenation. A limited amount of aldehyde is oxidized to the corresponding acid. Propylene is the highest volume feedstock for oxo plants.

The overall reaction is

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

Besides normal and isobutyraldehydes, varying amounts of normal and isobutyl alchols, propane, and heavy ends are produced. In the case of Shell's phosphine-modified cobalt catalyzed oxo process (vide infra), alcohols are made in a separate hydrogenation step. A number of transition metal carbonyls, e.g., those of Co, Fe, Ni, Rh, Ir, and cobalt carbonyls can catalyze olefin hydroformylation. The process is showed in Figure 2.5.

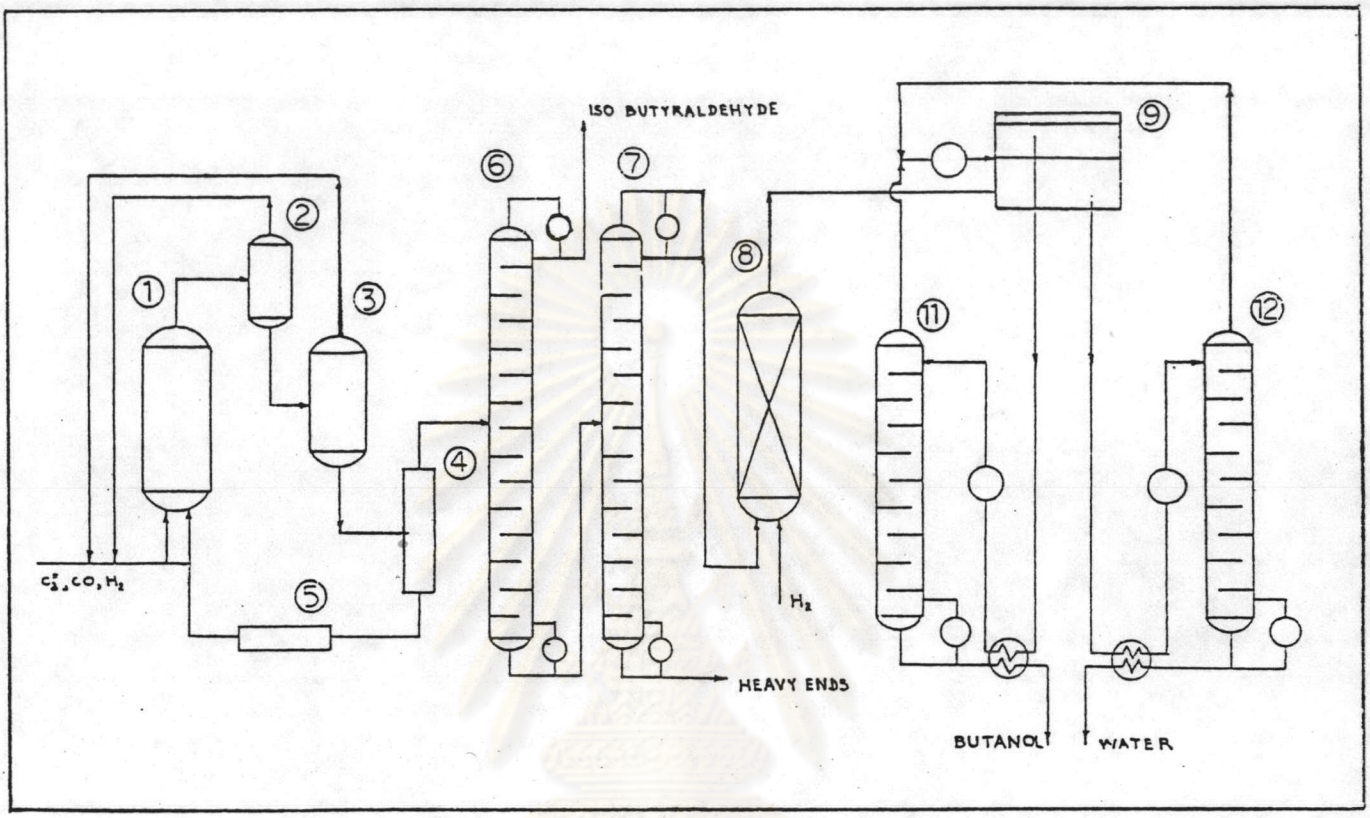


Figure 2.5 Process of n-butanol production by hydroformylation of propylene (11).

1. OXO REACTOR
2. LOW PRESSURE SEPARATOR
3. HIGH PRESSURE SEPARATOR
4. CATALYST REMOVAL
5. CATALYST STORAGE
6. ISO BUTYRALDEHYDE COLUMN
7. NORMAL BUTYRALDEHYDE COLUMN
8. HYDROGENATION REACTOR
9. DECANTER
10. CONDENSOR
11. BUTANOL COLUMN
12. AQUEOUS COLUMN

2.2 Development of Acetone-Butanol Fermentation Processes

2.2.1 Two-Phase Fermentation

The use of aqueous two-phase fermentation systems for in situ extraction of butanol has been demonstrated. The phase system consisted of a complex glucose medium supplied with 6% (w/w). Dextran T-40 (Pharmacia) and 25% (w/w) carbopeg 8000 (Union Carbide) resulting in a top to bottom phase ratio of 6:1. Cl. acetobutylicum was completely partioned in the bottom phase and solvents partitioned in the top and bottom phases. Solvents were recovered by distillation of the top phase, and replaced to subsequent restart the batch fermentation. Similar yield results were obtained with a controlled batch fermentation. The mean reactor productivity was 0.24 kg $m^{-3}hr^{-1}$ which compares favourably with the ordinary batch process productivity of 0.26 kg $m^{-3}hr^{-1}$. Long incubation times resulted in a decreased butanol concentration in the phases system, attributed to a reduction in water activity and hence alteration of cell metabolism.

2.2.2 Continuous Fermentation

Fermentation operated continuously or semi-continuously can improve reactor productivity. Leung and Wang (1981) reported that volumetric butanol productivity could be increased three-fold in continuous culture over that obtained in batch culture (2.5 $kgm^{-3}hr^{-1}$ versus 0.8 $kgm^{-3}hr^{-1}$). It was also shown that specific productivities of acetone and butanol increased with dilution rate to maximum values of 0.3 and 0.2 g g^{-1} cell hr^{-1}, respectively, at the dilution rate of 0.22 hr^{-1}; above that dilution rate, the fermentation favored butyric acid production. Fick, Pierrot and Engasser (1985), obtained a

productivity of 0.75 $kgm^{-3}hr^{-1}$ solvent from a stable continuous culture of ATCC 824 on a complex medium containing 40 g/l glucose. This process can be maintained for two months, at an optimal dilution rate 0.06 hr^{-1}, the solvent concentration was 13 g/l. For continuous culture, near theoretical conversion of glucose into solvents has been obtained, but as in batch culture, butanol toxicity limits high product concentrations and volumetric productivities.

2.2.3 Immobilization

The continuous production of butanol has been reported from immobilized Cl. acetobutylicum (Haeggstroem and Molin, 1979) and Cl. butylicum (Krouwel et al., 1980). Spores and vegetative cells were immobilized in calcium alginate gel and studied under what were termed as non-growth conditions. The productivity of these cells was found to be reasonable (1.0 g. butanol $l^{-1}hr^{-1}$) but because butanol toxicity rapidly reduced cell activity within the immobilization matrix, extensive investigation will be required to establish economical working conditions for such a process.

2.2.4 Ultrafiltration

The application of ultrafiltration (UF) and crossflow microfiltration (CFM) to fermentation processes has been studied to improve productivity in acetone-butanol fermentation. Continuous ethanol fermentation, using CFM to recycle cells back to the fermentor has been reported to increase the biomass per unit volume, facilitate an increase in productivity. In UF 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. Afschar et al (1985) obtained significant productivity increases using CFM (hollow fibre configuration) in a continuous fermentation using Cl. acetobutylicum ATCC 824 fermenting a complex glucose medium. A biomass concentration of 8 kg m^{-3} was obtained in a single-stage cell recycle fermentor using turbidostal cell concentration control at a dilution rate of 0.64 hr^{-1}.

Ferras, Minier and Goma (1986), improve productivity in acetone-butanol fermentation by coupling continuous fermentation and ultrafiltration. The membrane was a carbon tube with an ultrafiltering ceramic coat inside. With total recycle of biomass, a dry weight concentration of 125 g/l was obtained, which enhanced the volumetric solvent productivity in averaging 4.5 $gl^{-1}hr^{-1}$ at dilution rate 0.33 hr^{-1} for significant periods of time (>70 hr).

Schlote and Gottschalk (1986), improved the productivity of the acetone-butanol fermentation, a cellulosetriacetate ultrafiltration membrane was used to separate and recycle cells in a continuous fermentation of Clostridium acetobutylicum ATCC 824 under phosphate limitation (0.74 mM), at a dilution rate of 0.40 hr^{-1}, a solvent productivity of 4.1 g $l^{-1}hr^{-1}$ was maintained over three months.