CHAPTER II

THEORETICAL AND LITERATURE REVIEW

Recent progressive increases in the cost of crude oil have resulted in considerable attention being focused on fermentation technology as a mean to replace many organic chemicals currently derived from petroleum product. The major organic chemicals that are produced from carbohydrate containing raw materials are methane, ethanol, n-butanol, acetic acid, lactic acid and propionic acid. The main carbohydrate sources for fermentation are starch from grains such as corn, wheat and barley, sucrose from beet, sugar cane and sorghum, by-product materials from processing such as fruit and vegetable wastes, dairy whey and molasses, and also waste organic material such as sewage sludge, municipal solid waste, and agricultural plant and animal wastes. The bioconversion of these carbohydrate sources to fermentation chemicals depends upon the basic structural composition and integrity of lignocelluloses, which is the major component of residue biomass.

1. Molasses

Molasses is a by-product of the manufacture or refining sugar either from cane beet or other starch containing pulp. It is a sticky, high sugar but contains low nitrogen. There are three grades of cane molasses; first molasses, second molasses and blackstrap molasses. First molasses is the result of first boiling and extracting process. It has the highest sugar content because comparative little sugar has been extracted from the juice. Second molasses is created from a second boiling and sugar extraction, so there is less sugar. It has a dark color and slightly bitter taste. Blackstrap molasses is the brownish-black liquid separated from the final boiling [11]. It is the heavy, viscous liquid from which no further sugar can be crystallized. It is generally described as inedible because it is not used for human consumption.

Blackstrap molasses can be valuable for numerous uses such as animal feed, food milling ingredient, fermentation, soil amendment and other miscellaneous industrial processes, for example, as a fermentation feedstock in the production of yeast, lysine and ethanol. Blackstrap molasses contains high concentration of C_6 sugar and other fermentable carbohydrates as well as signification concentrations of vitamins. The components of blackstrap molasses are show in table 2.

Molasses can also be used as an environmentally biodegradable agent for dust and wind erosion. Molasses can serve as a relatively low-ash binder in the production of carbon black pellet and charcoal briquettes. As a soil amendment, molasses contains high carbon and nutrient content, which nurtures and composting microbes [10]. There are 46 sugar refinery plants in Thailand, which produce more than $2x10^6$ ton of molasses as a by-product from $47x10^6$ ton of cane press quantity of the refining sugar in Thailand [19]. The components of black strap molasses are shown in Table 1 [17].

Table 1 The components of blackstrap molasses

Component	Quantity (% w/v)
Dry matter	78.0 %
Protein	3.0 %
Carbohydrates	54.0 %
Fat	0.4 %
Ash	9.0 %
Mineral components	
Calcium	0.74 %
Magnesium	0.35 %
Phosphorus	0.08 %
Potassium	3.67 %
Vitamins	
Choline	660 mg/kg
Niacin	46.86 mg/kg
Pantothenic	42.9 mg/kg
Pyridoxine	44.0 mg/kg
Riboflavin	4.4 mg/kg
Thiamine	0.88 mg/kg
Component of amino nitrogen	
Glycine	0.1 %
Leucine	0.01 %
Lysine	0.01 %
Threonine	0.06 %
Valine	0.02 %

2. Uses of Acetic acid

Acetic acid is also known as ethanoic acid, ethylic acid, vinegar and methane carboxylic acid. Acetic acid is a short chain carboxylic acid with the formula of CH_3COOH. Acetic acid is by far the most important of all carboxylic acids because acetic acid is used for several chemical productions. In 1989, the worldwide production of acetic acid by the petrochemical route was approximately 4 million tones [2].

Prices of acetic acid are up 35% on average worldwide this year, due to improving demand, and higher natural gas and methanol prices [18]. Average prices of acetic acid are shown in table 2.

Table 2 Prices of Acetic acid in U.S., Europe and Asia

Region covered	Price (U.S.$/metric ton)	Price (Baht/kg)
U.S.	470 – 480	18.8-19.2
Europe	420 – 450	16.8-18
Asia/Pacific	540 – 550	21.6-22

The major use of acetic acid is for vinyl acetate, which is used for vinyl plastics, adhesives, textiles furnish and latex paints. This market has grown rapidly during the past few years due to the demand for synthetic fiber. In 1978, calcium magnesium acetate (CMA) was identified as a non-corrosive environmental biodegradable alternative to chloride salts for deicing roads. Liquid potassium acetate is being used as a partial replacement for ethylene glycol. In addition, there are reports that CMA or calcium magnesium acetate can also be used as an additive to coal fired combustion unit, for example, boilers used by electrical utilities [12]. Calcium magnesium acetate as a grabber for sulfur in the coal reduces sulfur dioxide emission and partially relieves the problem of acid rain pollution. If these environments related substitution take place, the demand for acetic acid would increase tremendously.

Other commercial uses include the manufacture of vitamins, antibiotics, hormones, organic chemical and food industries. Acetic acid is in the 33rd highest volume chemical produced in the United States [7].

Acetic acid is also used in food industries where it is known as vinegar. The product obtained by acetic acid fermentation of alcohol containing solution. In the food industry, vinegar is used for the production of pickles, other vegetables, fish, mustard, mayonnaise and salad dressings [13]. The industrial importance of acetic acid can be understood from figure 1.

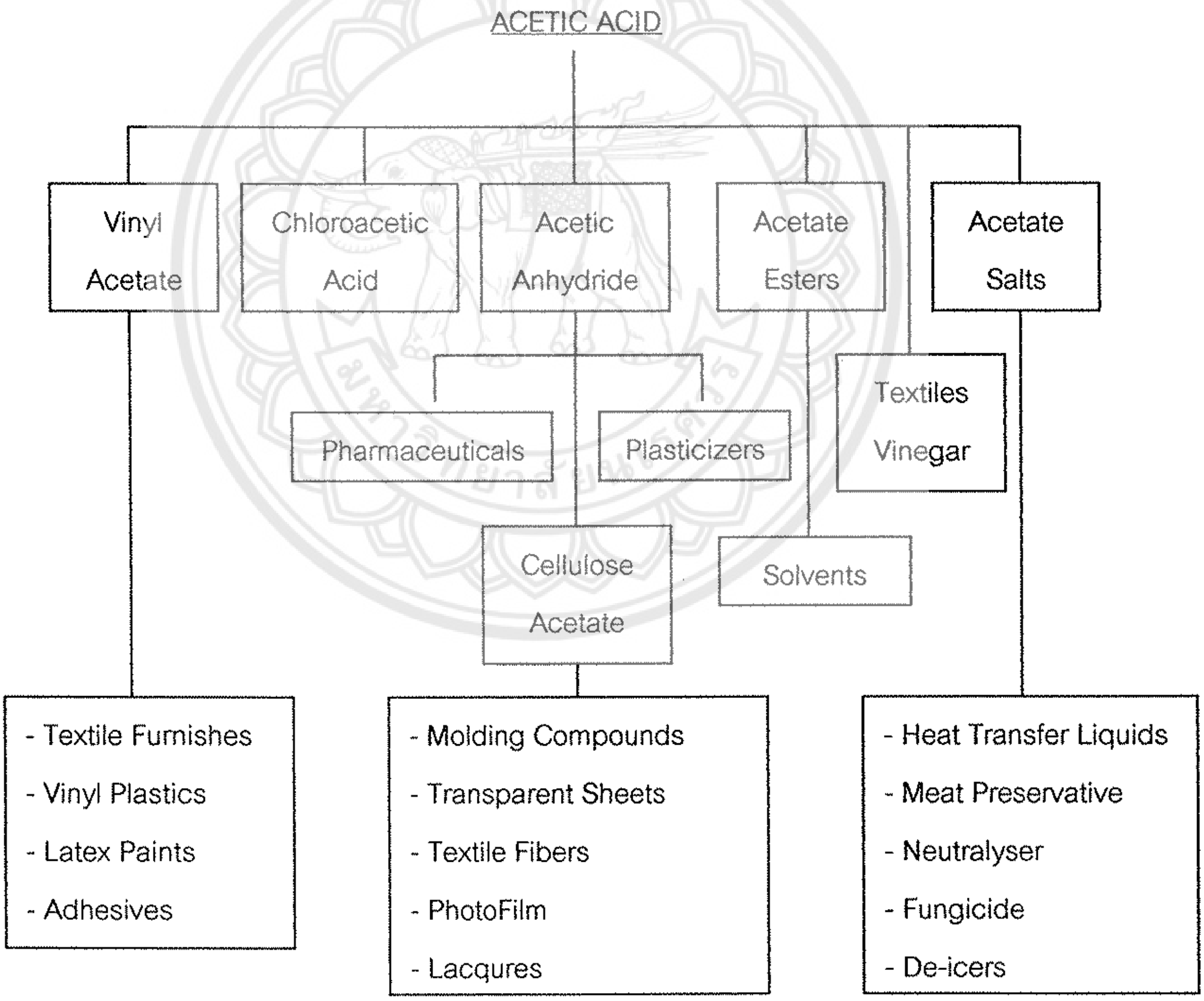


Figure 1 The uses of acetic acid as feedstock for chemical production

3. Production of acetic acid

Acetic acid as an industrial chemical is presently produced from petroleum and chemical. There are three processes: acetaldehyde oxidation, Butane oxidation and methanal carbonylation. However, acetic acid can be produced via microbial fermentation of carbohydrates resources which is considered as petroleum substitutes.

3.1. Petroleum process

Acetic acid is synthesized via three chemical processes, which are from acetaldehyde, butane and methanol and carbon monoxide [4].

3.1.1. Conversion of Acetaldehyde to acetic acid

In this process acetic acid is manufactured by the oxidation of acetaldehyde. The reaction is conducted in a stainless steel reaction kettle. A highly concentrated acetaldehyde solution (99 to 99.8%) is introduced in to the reactor along with 0.1 to 0.5 % manganous acetic acid. The latter is used to decompose the explosive intermediate-per acetic acid. Air at a pressure of 70 to 75 psi is bubbled through the liquid which is kept at a temperature of 50 to 65° C. The oxidation will be completed within 12 hrs. The gases leaving the reactor are scrubbed with water to recover the acetaldehyde entrained in it, releasing nitrogen to atmosphere (figure 2). The dilute acetaldehyde solution is sent to a column for recovery. The crude acetic acid from the reactor is rectified to yield 99 % glacial acetic acid. In modern plants, the oxidation is conducted continuously, in which case, cobalt acetate dissolved in acetic acid is used as the catalyst. The reactor is operated at 15 psi and 70 to 80° C. Approximately 4 moles of air enter in the converter per mole of acetaldehyde. In an alternative process, vapour phase catalytic oxidation of a mixture of alcohol and acetaldehyde is utilized. This process yields both acetaldehyde and acetic acid as products. Acetaldehyde is reused when alcohol is added and acetic acid is obtained.

Process is conducted also in liquid phase, in which the reaction is carried out in an acetic acid solution using a mixture of cobalt and chromium acetates as catalyst. The reactor contains 11 moles of ethyl alcohol to 89 moles of acetaldehyde and yields 95% of acetic acid. The reaction is shown as follow:

$$CH_3CHO + \frac{1}{2}O_2 \xrightarrow[88-89\%\ \text{yield}]{} CH_3COOH \qquad \text{.......... (a)}$$

The process requires 1.1 tones acetaldehyde, 3.3 kilograms Manganous acetate and 8040 cu.ft. air to produce 1 ton of acetic acid.

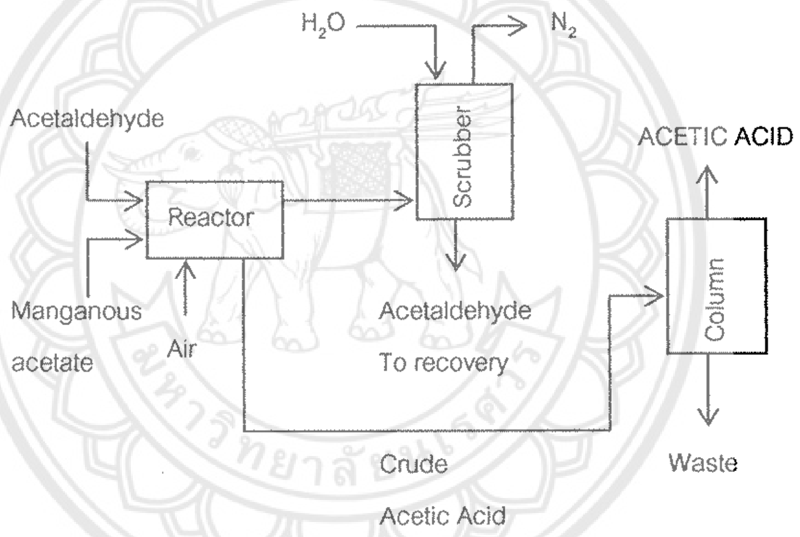


Figure 2 Conversion of Acetaldehyde to acetic acid

3.1.2. Conversion of Butane to acetic acid

Acetic acid is produced as the predominant product by the liquid phase catalytic oxidation of butane. Commercial butane (95% n-butane, 2.5% iso-butane and 2.5% pentane) is charged to a reactor containing cobalt, manganese or chromium acetates. Air is bubbled through the solution at 300 to 475° F (5:1 of air:butane) Vaporous leaving the reactor are condensed and then separated to individual chemicals. Unreacted nitrogen and hydrocarbon are ventilated (Figure 3). Under proper reaction conditions, the principal reaction product obtained is acetic acid. The reaction is shown as follow:

$$2C_4H_{10} + 5O_2 \rightarrow 4CH_3COOH + 2H_2O \qquad \text{.......... (b)}$$

The process requires 965 kgs Butane and 120,000 cu.ft. air to produce 1 Tone of acetic acid (Plus varying amounts of acids, alcohol and ketones).

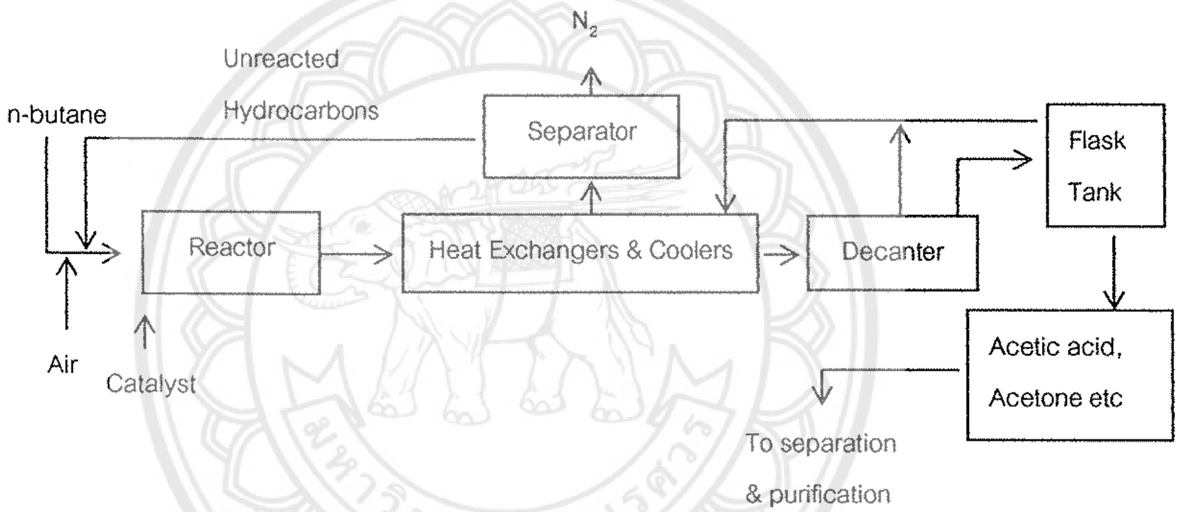


Figure 3 Conversion of butane to acetic acid

3.1.3. Conversion of methanol and carbon monoxide to acetic acid

This process is the major commercial production method for acetic acid. Methanol, which can be generated from synthesis gas (reaction c)("syn gas", a CO/H_2 mixture), is reacted with carbon monoxide in the presence of a catalyst to afford acetic acid. In essence, the reaction can be thought of as the insertion of carbon monoxide into the C-O bond of methanol (reaction d).

$$CO / H_2 \xrightarrow{\text{CU / ZnO}} CH_3OH \qquad \text{.......... (c)}$$

$$CH_3OH + CO \xrightarrow[180^0\text{C},30-40\text{ atm}]{\text{Rh, I}^-} CH_3COOH \qquad \text{.......... (d)}$$

There are two major concerns of using Rhodium and I_2. Rhodium is expensive material (1 mole of $RhCl_3 \cdot 3H_2O$ cost around US$ 30,000) and I_2 is extremely corrosive. Other Halogens do not work nearly as well as I_2 [9]. The catalytic cycle is shown in Figure 4.

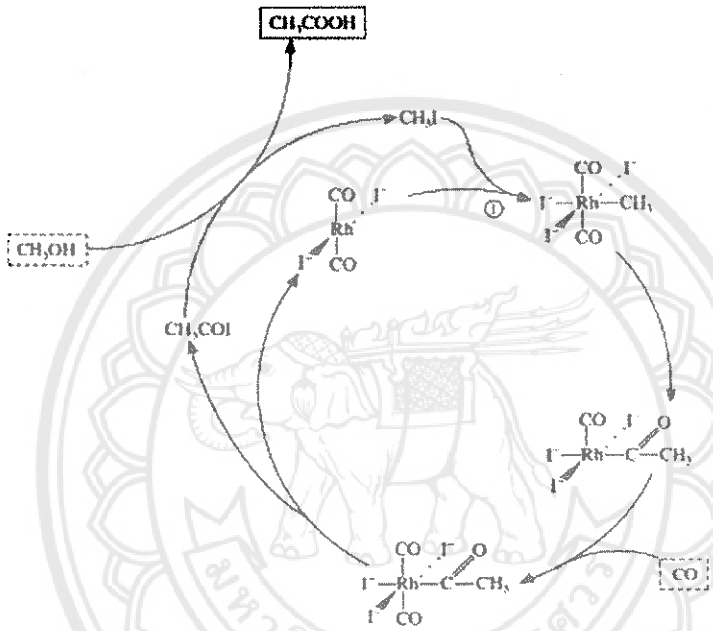


Figure 4 Acetic acid synthesis from carbonylation of methanol with Rh and I^- as catalyst

3.2. Microbial fermentation from carbohydrates

Acetic acid can be produced via microbial fermentation. The substrates used are normally sugar or polysaccharide that is hydrolysed prior to fermentation. There are two fermentation routes for acetic acid production from carbohydrates [13].

3.2.1. Vinegar production

This process is a commercially available technology for acetic acid production which requires two steps. The first step is the conversion of glucose to ethanol by yeast, *Saccharomycetes cerevisiae,* and the second is the conversion of ethanol to acetic acid by a bacterium, *Acetobacter acetic*:

$$C_6H_{12}O_6 \xrightarrow{S.cerevisiae} 2C_2H_5OH + 2CO_2 \qquad \text{.......... (e)}$$

$$2C_2H_5OH + 2O_2 \xrightarrow{A.aceti} 2CH_3COOH + 2H_2O \qquad \text{.......... (f)}$$

The theoretical maximum yield of this conventional route is two moles of acetic acid from one mole of glucose or 0.67 g acetic acid/g glucose. In commercial practice, actual acetic acid yield is 0.50-0.55 g/g glucose or roughly 75-80% of theoretical yield.

3.2.2. Homoacetogenic anaerobic bacteria

Fermentation with *Clostridium thermoaceticum* offers a significant advantage in terms of acetic acid yield compared to the conventional vinegar fermentation *Clostridium thermoaceticum* theoretically can produce 3 moles of acetic acid from 1 mole of glucose. In practice, 85% of sugar is converted to acetic acid. *Clostridium thermoaceticum* also can ferment fructose and xylose which can not be utilized by the yeast currently used in vinegar fermentation:

$$C_6H_{12}O_6 \xrightarrow{\text{C. thermoaceticum}} 3CH_3COOH \qquad \text{.......... (g)}$$

$$2C_5H_{10}O_5 \xrightarrow{\text{C. thermoaceticum}} 5CH_3COOH \qquad \text{.......... (h)}$$

4. Characteristics of *Clostridium thermoaceticum*

Clostridium thermoaceticum is a spore forming, gram positive, obligate anaerobic bacterium. The vegetative cells in a 72-hour culture have an average size of 2.8 X 0.4 μm, and the spores are terminal and very nearly round. Circular, Agar colonies are smooth and opaque. Motility is not observed in liquid culture but flagella stains by Gray's method show perritrichous flagella. The bacterium is an obligate thermophile with an optimum growth temperature between 55 and 60° C and an optimum pH between 6 and 8.5. *Clostridium thermoaceticum* is able to accomplish a unique type of fermentation, which it can homoferment 1 mol of glucose to 3 mol of acetic acid. *Clostridium thermoaceticum* does not ferment any of the disaccharides, trisaccharides, or polysaccharides. There is a lesser fermentation of galactose, mannose, D-arabinose, D-lactic and gluconic acid. Since the bacterium is an anaerobic, spore forming, acid-forming thermophile, it belongs in the genus Clostridium. The species name thermoaceticum was proposed because of its thermophilic nature and because the principle product from its fermentation of carbohydrates is acetic acid [13].

5. Fermentation modes

Microbial fermentations in liquid media can be carried out under different operation conditions. There are three Industrial fermentations, which operated as Batch culture, Fed-batch culture and Continuous cultures [16].

5.1. Batch fermentation

Batch processes, which are closed systems where there are no additions following inoculation, apart from acid or alkali for pH control and input of air for aerobic fermentation (figure 5a). During batch fermentations the population of microorganisms goes through several distinct growth phases: lag, acceleration, exponential growth, deceleration, stationary and death (figure 5b)

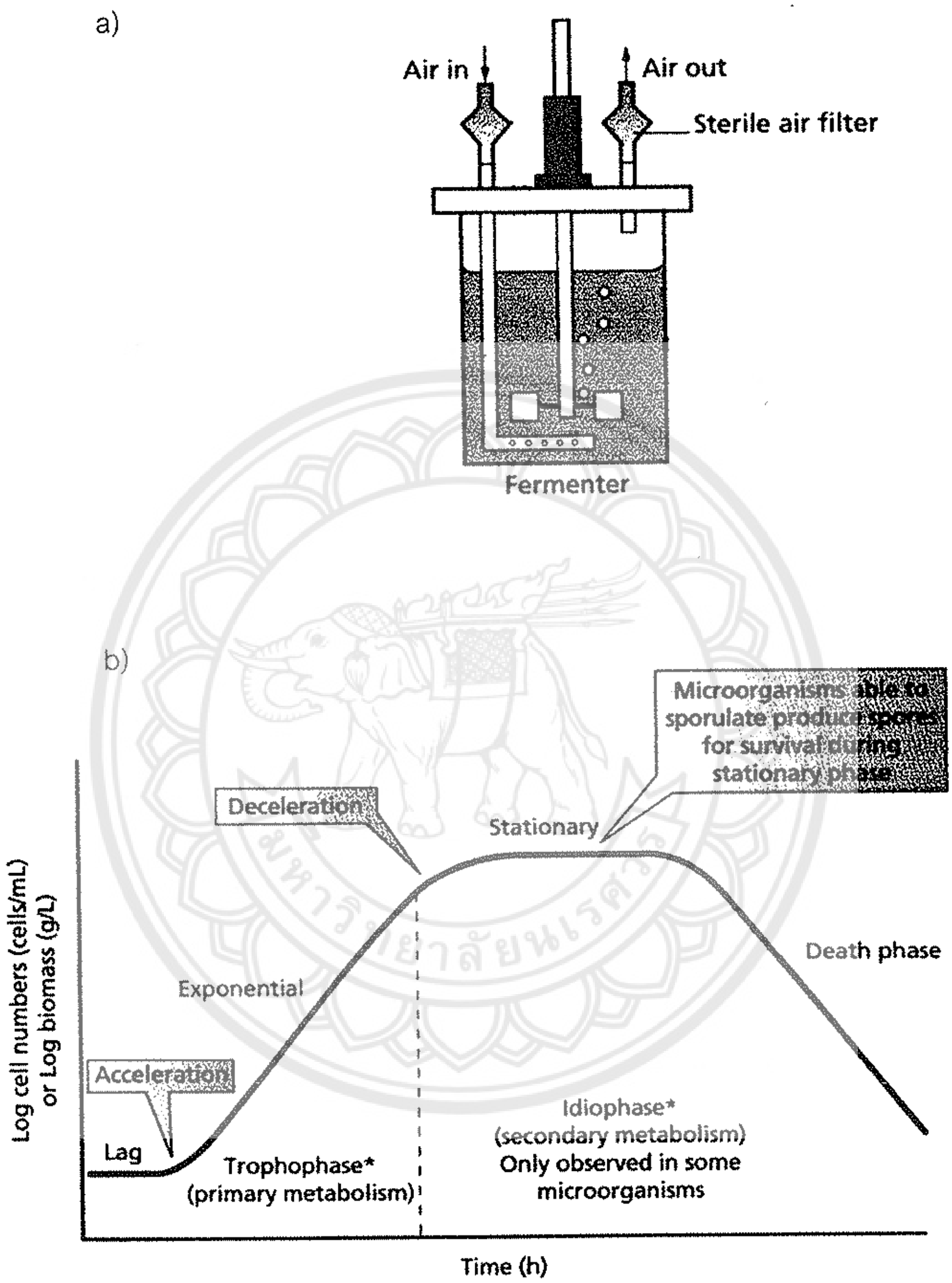


Figure 5 Batch culture a) Batch culture apparatus b) Growth of a microorganism in a batch culture

In the lag phase, virtually no growth occurs and the microbial population remains relatively constant. Nevertheless, it is a period of intense metabolic activity as the microbial inoculum adapts to the new environment. In batch fermentations there is a definite and end to the process. Fermentation is loaded, sterilized and inoculated, and the organism is grown through a typical batch profile. The product is then harvested and the fermenter must be cleaned before starting the cycle. This non-productive phase is referred to as 'down-time'. Examples of this mode of operation include the production of alcoholic beverages, most amino acid, enzymes, organic acid, etc.

Variations include fed-batch systems that have been successfully used for producing baker's yeast and penicillin. For this mode of operation extra nutrients are added as the fermentation progress, which increases the fermentation volume. Additions may be made continuously, intermittently or as a single supplementation, often when a batch culture approaches the end of the rapid growth phase. Fed-batch operation can extend the product formation phase and may overcome problems associated with the use of repressive, rapidly metabolized, substrates. This method is also useful where a substrate causes viscosity problems or is toxic at high concentrations. Fed-batch with recycle of cells (biomass) can also be used for specific purposes, e.g. some ethanol fermentation and waste-water treatment processes.

The advantages of batch systems are that initial capital expenditure is lower and, if contamination occurs, it is relatively simple to terminate and restart a new fermentation cycle. As mentioned above, batch systems are successfully used in the production of many traditional fermentation products; and for producing secondary metabolites, such as antibiotics, where the cells are first grown beyond the rapid growth phase prior to the accumulation of these metabolites. However, batch fermentations are theoretically less effective for the production of biomass and primary metabolic products. Only a small fraction of each batch fermentation cycle is productive, as there may be a considerable lag period and it is only in the later stages of the exponential phase that large quantities of the product are generated. Other disadvantages of batch systems are the batch-to-batch variability of the product; plus increased non-productive down-time, involving, cleaning, sterilizing, refilling and poststerilization cooling. The increased frequency of sterilization may also cause greater stress on instruments and probes. In addition, the running costs are greater for preparing and maintaining stock cultures, and generally more personnel are required for operating batch processes.

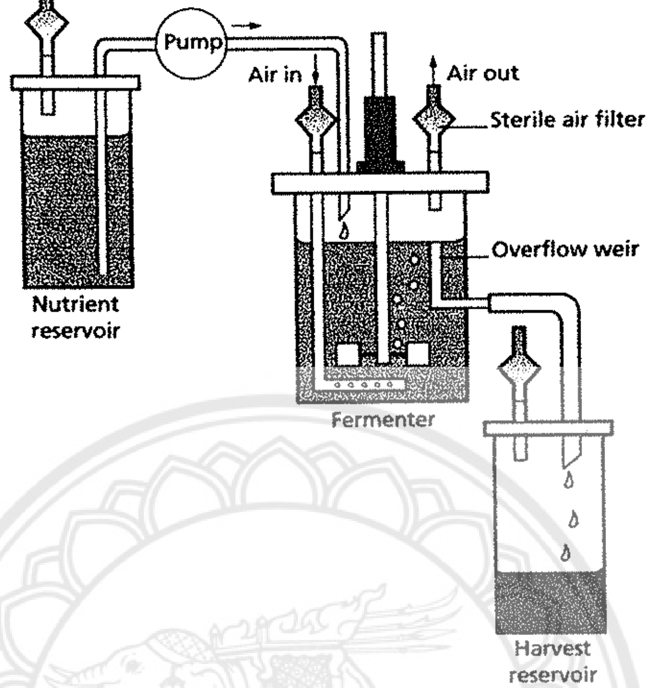
5.2. Continuous culture

Continuous culture is an open system where fresh medium is continuously added and culture is simultaneously removed at the same rate, resulting in a constant working volume (figure 6a). In continuous systems, calls grow exponentially for extended periods at a specified predetermined growth rate. Furthermore, the system has the property of reaching a steady state in which the concentration of limiting nutrient and the cell number does not vary with time (figure 6b)

Consequently, in theory, such systems are more productive than batch systems. Continuous fermentations are particularly well suited for the production of biomass and growth-associated primary metabolites. Their reduced down-time and lower operating costs are also desirable attributes. However, they require higher initial capital expenditure and, to date, relatively few large-scale industrial examples have become established, other than for biomass, fuel industrial ethanol and effluent treatment.

Problems associated with continuous, culture processes, other than waste-water treatment; include the fact that throughout their 20-50 days or longer operation, sterility must be maintained and a continuous supply of media of constant composition provided. However, these difficulties can be overcome by GMP and good microbiological practices. Nevertheless, the operation conditions place strong selection pressure on the organism. Any genetic instability may lead to the generation of low-yielding mutants that may outgrow the original high-yielding strain.

a)



b)

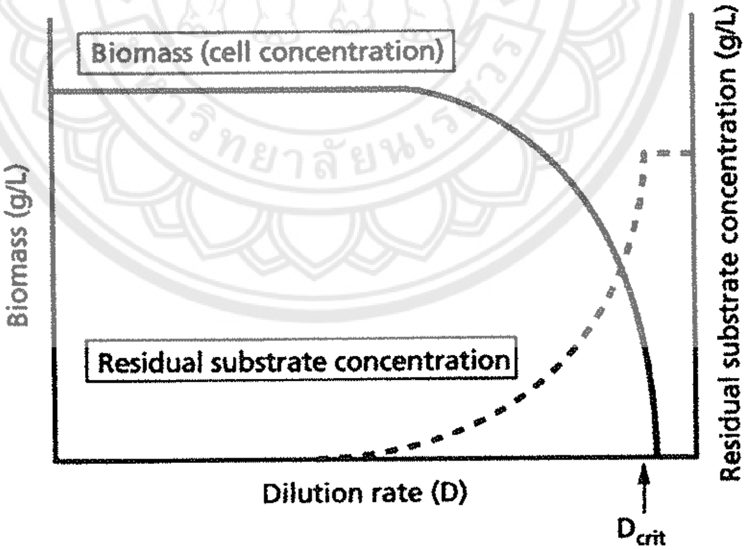


Figure 6 Continuous culture a) Continuous culture apparatus b) Growth of a microorganism in continuous culture