

# Part 5. Biochemical conversion of biomass

## 5.1 Biomethanation

### 5.1.1 What is biomethanation?

“Methane fermentation” or “anaerobic digestion” is usually used to indicate “biomethanation”. Biomethanation is a complex microbial process in which organic compounds are degraded into methane and carbon dioxide by variety of anaerobes. This biogas has a low heating value of 20-25 MJ/m<sup>3</sup>-N (5,000~6,000 kcal/m<sup>3</sup>-N) and can be used for fuel after desulfurization of hydrogen sulfide. Biomethanation is used as a technique of biofuel recovery from biomass and treatment of waste biomass. Fermented residue can be used for liquid fertilizer and raw material of compost.

### 5.1.2 Feature of biomethanation

First of all, organic compounds are decomposed to organic acid or hydrogen by variety of anaerobes. At the final stage, acetate or hydrogen and carbon dioxide are converted to methane. Biomethanation takes place under anaerobic conditions, especially, methanogens require absolute anaerobic conditions for methane production. Biomethanation is a microbiological process; therefore, this process proceeds under normal temperature and pressure. Biomethanation can be applied for variety of biomass compared with ethanol fermentation due to activities of complex microflora.

### 5.1.3 Mechanism of biomethanation

When organic compounds are maintained at 5-70°C and neutral pH under anaerobic conditions, spontaneously biomethanation will happen. Biogas is often produced from underground of landfill. Kitchen garbage and sewage sludge have been used as substrates of biomethanation. Organic wastewater from food factory containing sugars and starch has been also used as substrate for biomethanation.

Biomethanation is composed of hydrolysis, acetogenesis and methanogenesis. Fig. 5.1.1

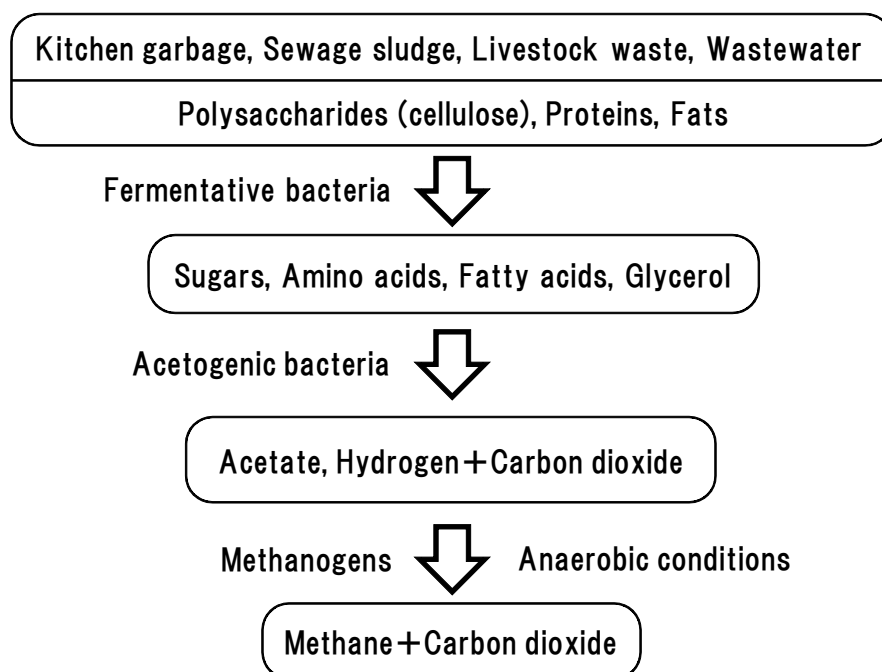
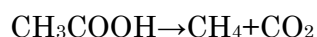
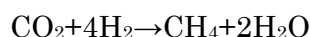


Fig. 5.1.1 Schematic diagram of biomethanation

indicates an outline of biomethanation. Polysaccharides are decomposed to monosugars, proteins are to amino acids, and fats are to fatty acids and glycerol. Fermentative bacteria are for example *Bacteroides* spp. and *Clostridium* spp. Sugars and amino acids are decomposed to acetate and propionate by acidogens. Finally, methanogens convert acetate or hydrogen and carbon dioxide to methane. Acidogenesis is a complex process in which microflora of anaerobes collaboratively decomposes organic compounds to low molecular organic acids. Acetate, lactate, succinate, ethanol, butanol, acetone and etc. can be produced from glucose by acidogens. In wastewater treatment, 70% of methane is produced from acetate, and 30% is produced from hydrogen and carbon dioxide. Formula of acetoclastic reaction is as follows;



Formula of hydrogenotrophic reaction is as follows;



Methanogens are anaerobes which can grow using acetate or hydrogen and can produce methane. Representative methanogens are *Methanobacter* spp. and *Methanosaeta* spp.

Methanogens are killed by exposure to oxygen; therefore, methane formation requires obligate anaerobic conditions. Phylogenetic analyses indicate that methanogens are placed in Archaea group, distinguished from eukaryote and prokaryote. Methanogens can only use hydrogen, formate, acetate, 2-propanol, 2-buthanol, methylamine, methanol, methylmercaptan to produce methane.

#### 5.1.4 Current status

Biomethanation is commercialized for food waste, cattle waste, sewage sludge, and wastewater. European countries have developed biomethanation technology. Biogas plants have been gradually increasing in Japan. The reaction takes place at high, moderate, or low fermentation temperature, and organic content classifies biomethanation into wet and dry fermentation. High temperature systems reveal high gasification performance compared with other temperatures. Disadvantageous points of biomethanation are low digestion ratio, low removal ratios of ammonium and phosphate, long treatment time, and necessity of heat. Fermented effluent and residue should be recycled for agriculture as an organic fertilizer since treatment cost is high. Technological developments is being conducted to overcome these issues.

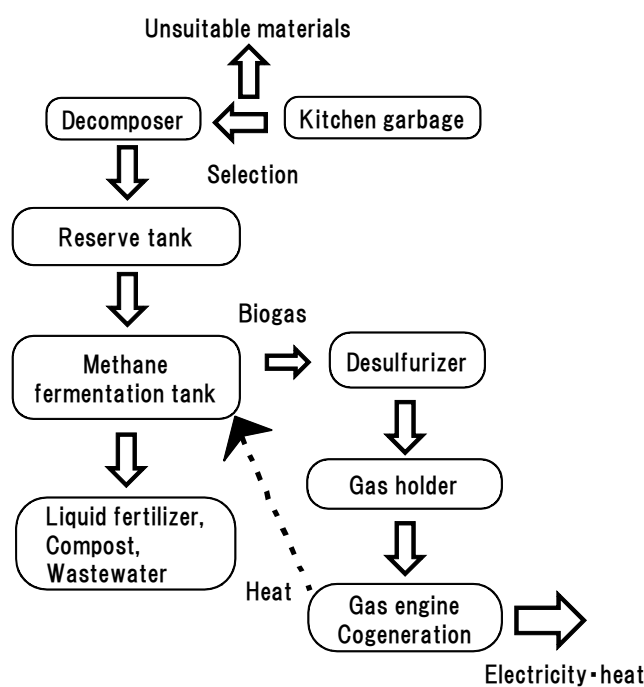


Fig. 5.1.2. Biomethanation flow of kitchen garbage.

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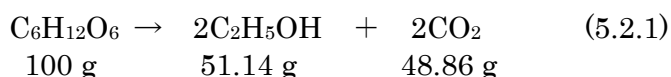
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## 5.2 Ethanol Fermentation

### 5.2.1 General scope

In ethanol fermentation saccharine materials like glucose, fructose, and sucrose are metabolized by yeast strains through glycolysis pathway (Embden-Meyerhof Pathway) to produce ethanol and carbon dioxide in anaerobic condition (Eq. (5.2.1)). In this reaction two molecules of ATP are generated from one molecule of glucose and are used as energy for growth of yeast cells. Mankind has long known and utilized ethanol fermentation for brewery of alcohol drinks, manufacture of fermented food, bakery and so on for several thousands years. In the medieval period, people came to know how to make distilled liquor. Ethanol became available in the field of various chemical industries, beverage and food industry, medical use, as well as fuel, since great progress was achieved with technologies of fermentation and distillation in 19-20th century. Large amount of fuel ethanol has been produced from corn in the USA and from sugar cane in Brazil for the purpose to alternate fossil fuel and to prevent global warming, particularly after two times of oil crisis in 1970's. Extensive research and development are undergoing in the world on technologies for ethanol production from various cellulosic materials which are available in large amount and do not compete with food utilization.



Ethanol fermentation is a biological reaction at room temperatures and under atmospheric pressure. *Saccharomyces cerevisiae* is the yeast which is widely used for industrial and fuel ethanol production, and has excellent ethanol fermenting ability and ethanol tolerance. Yeast strains produce 51.14 g of ethanol from 100 g of glucose according to Eq. (5.2.1). In this reaction nearly 50% weight of glucose is lost as carbon dioxide, but about 91% of energy

contained in glucose (2.872 MJ/mol) is retained in ethanol. Therefore, ethanol fermentation is an excellent biological process to convert biomass to liquid fuel ethanol. Yeast cells were first isolated from beer as pure cultures in 1883 in Denmark and a great deal of work was carried out on their metabolic pathway of ethanol fermentation. *S. cerevisiae* can ferment many sugars including glucose, fructose, galactose, mannose, sucrose, maltose, except pentoses like xylose and arabinose. *Pichia stipitis* and *Pachysolen tannophilus* are known as yeasts capable of fermenting pentoses, but they are not so tolerant to ethanol as *S. cerevisiae* is. Strain improvement research to construct strains of *S. cerevisiae* having pentose fermentability is undergoing in many laboratories.

Next to *S. cerevisiae*, *Zymomonas mobilis* is an excellent bacterium to ferment a limited range of sugars of glucose, fructose and sucrose to ethanol. Fermentation yield and fermentation rate of *Z. mobilis* are supposed to be better than those of yeast *S. cerevisiae*, but *Z. mobilis* is not so tolerant to ethanol as *S. cerevisiae* is. *Zymobacter palmae*, isolated in 1980's in Japan, has ethanol fermenting ability similar to that of *Z. mobilis* and its genome base sequence has been determined recently. Strain improvement of *Z. mobilis* and *Z. palmae* regarding pentose and mannose fermentation has been successfully made in Japan. Pentoses are contained at relatively high concentration in hard woods and herbaceous plants, and mannose is a characteristic component of soft woods.

DNA recombinant strains of *Escherichia coli* and *Corynebacterium glutamicum* having ethanol fermenting ability have been constructed through biotechnology. Other ethanol fermenting bacteria like hetero-lactic acid bacteria (*Lactobacillus*), cellulose degrading *Clostridium* bacteria, and anaerobic thermophilic bacteria *Thermoanaerobacter* have been known so far, but they can produce ethanol at relatively low concentration and with byproducts like organic acids. Therefore, these bacteria are considered difficult for the industrial use at least for the present.

### 5.2.2 Ethanol fermentation of saccharine materials

Saccharine materials used for ethanol production at a large scale are juice and molasses of sugar cane and sugar beet. Molasses is a byproduct which is concentrated mother liquid after sugar crystallization. Sugar concentration of molasses is around 50% and contains glucose, fructose and sucrose as main sugar components. These saccharine materials are good substrate for ethanol fermentation by yeast and *Zymomonas*. A lot of cane juice is used for ethanol production in Brazil and India.

Popular ethanol fermentation process in Brazil is continuous or semi-continuous fermentation process called Melle-Boinot process in which yeast cells are recovered from beer through centrifuge and recycled to fermentation tank after sterilization of contaminated bacteria with dilute sulfuric acid at pH 3. Ethanol fermentation at high concentration of yeast cells can make beer containing 6 to 8 % of ethanol from cane juice (11-17% sugar concentration) in about 15 h of fermentation period. Molasses is used for fermentation after two-times dilution or mixing with cane juice or beet juice.

When fermentation yield is 82% (based on total sugar), and sugar concentration of molasses is 55%, the amount of molasses required to produce 1 m<sup>3</sup> (kL) of 95% ethanol is 3.3 t-wet.

Unique saccharine materials are milk whey and citrus molasses. In dairy industry of New Zealand, for example, a large amount of milk whey containing about 4% lactose is discharged. They use the waste whey for ethanol fermentation to recover value-added byproduct and to reduce BOD.

A large amount of citrus peel is discharged in citrus juice manufacture. Secondary juice from citrus peel containing about 8% sugar and bitter components is concentrated to citrus molasses of sugar concentration of over 40% for ethanol production.

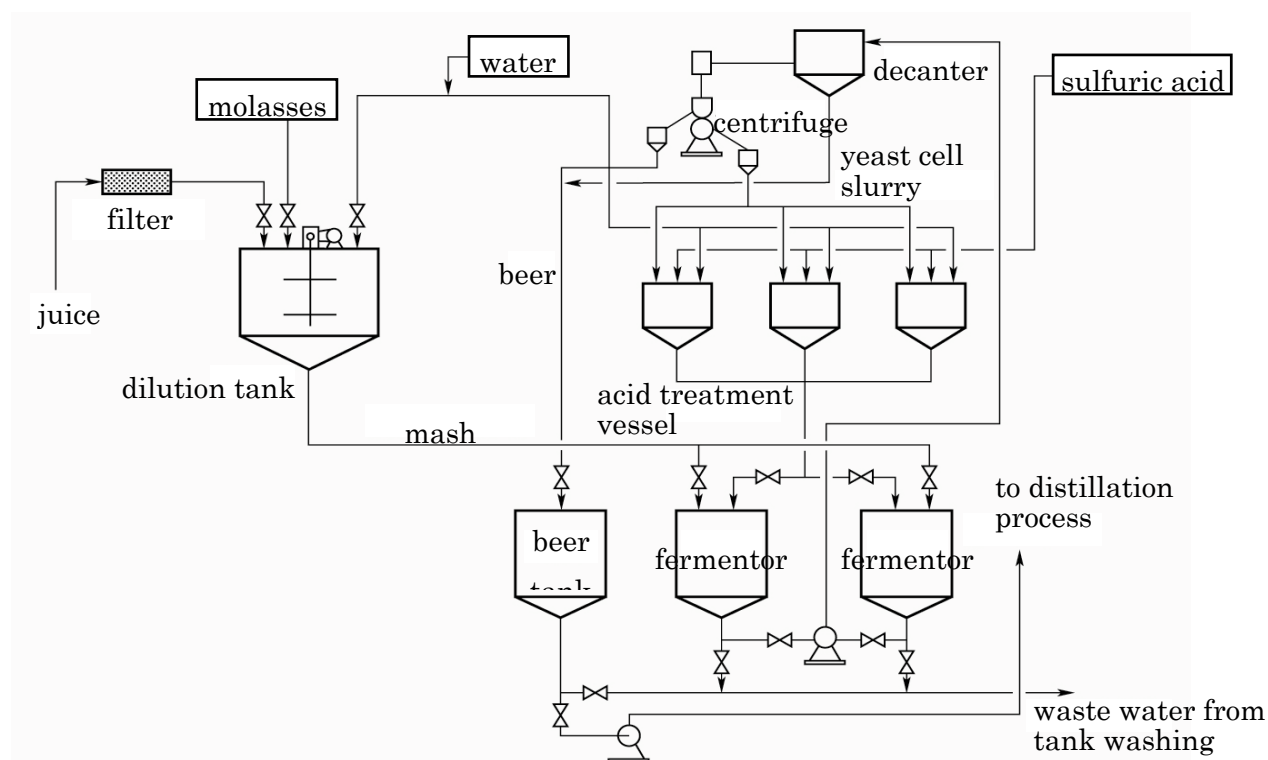
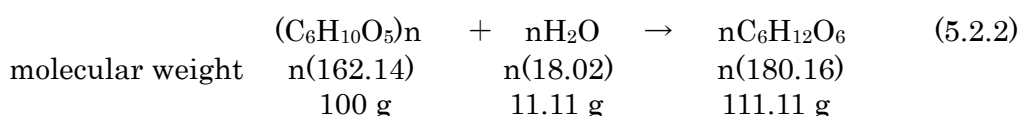


Fig. 5.2.1. Ethanol Fermentation by Melle-Boinot Process (Saiki, 2007).

### 5.2.3 Ethanol fermentation of starch

Starch is a polymer of glucose in which glucose units are linked with each other via  $\alpha$ -1,4 and  $\alpha$ -1,6 linkages. Starchy materials are first hydrolyzed to glucose using amylase enzymes (Eq. (5.2.2)).



Starchy materials are first cooked at temperatures between 100 and 130°C and then hydrolyzed to glucose by using  $\alpha$ -amylase and gluco-amylase.

A large amount of ethanol is produced from corn in USA and from sweet potato in China.

Low temperature cooking procedure for ethanol production from sweet potato which was the practice in Japan until 1990's is described below. Raw sweet potato is first crushed by hammer-mill, cooked at 80-90 °C for 60min, added with  $\alpha$ -amylase to liquefy starch and to reduce viscosity, and then cooled to temperature of about 58°C. Liquefied starch is hydrolyzed to glucose in two hours of hydrolysis by gluco-amylase. Glucose concentration of mash is adjusted at about 15%. Fermented beer of about 8 vol% ethanol is obtained after four days of batch-wise fermentation at 30-34°C. When starch value of raw sweet potato is 24.3% (27% glucose equivalent), and fermentation yield is 92%, the amount of raw sweet potato required to produce 1 m<sup>3</sup> (kL) of 95% ethanol is 6.03 t-wet.

Fuel ethanol is produced mostly from corn in USA. In a wet mill process corn is immersed in dilute sulfite solution, fractionated into starch, germ, gluten, and fiber. Starch fraction is hydrolyzed to glucose by the amylases after cooking and then fermented by yeast. One of the popular fermentation process is a continuous fermentation process with several fermentation tanks connected in series in which yeast cells are recycled via centrifuge resulting in high fermentation rate. Traditional batch-wise process is also the practice in some of ethanol plants. Final ethanol concentration of fermented beer is 8 to 11 vol% on average. Wet-mill process of corn to ethanol conversion is indicated in Figure 5.2.2. When starch value is 63% (70% glucose equivalent), and fermentation yield 90%, the amount of corn necessary to produce 1 m<sup>3</sup> (kL) of 95% ethanol is about 2.4 t-wet.

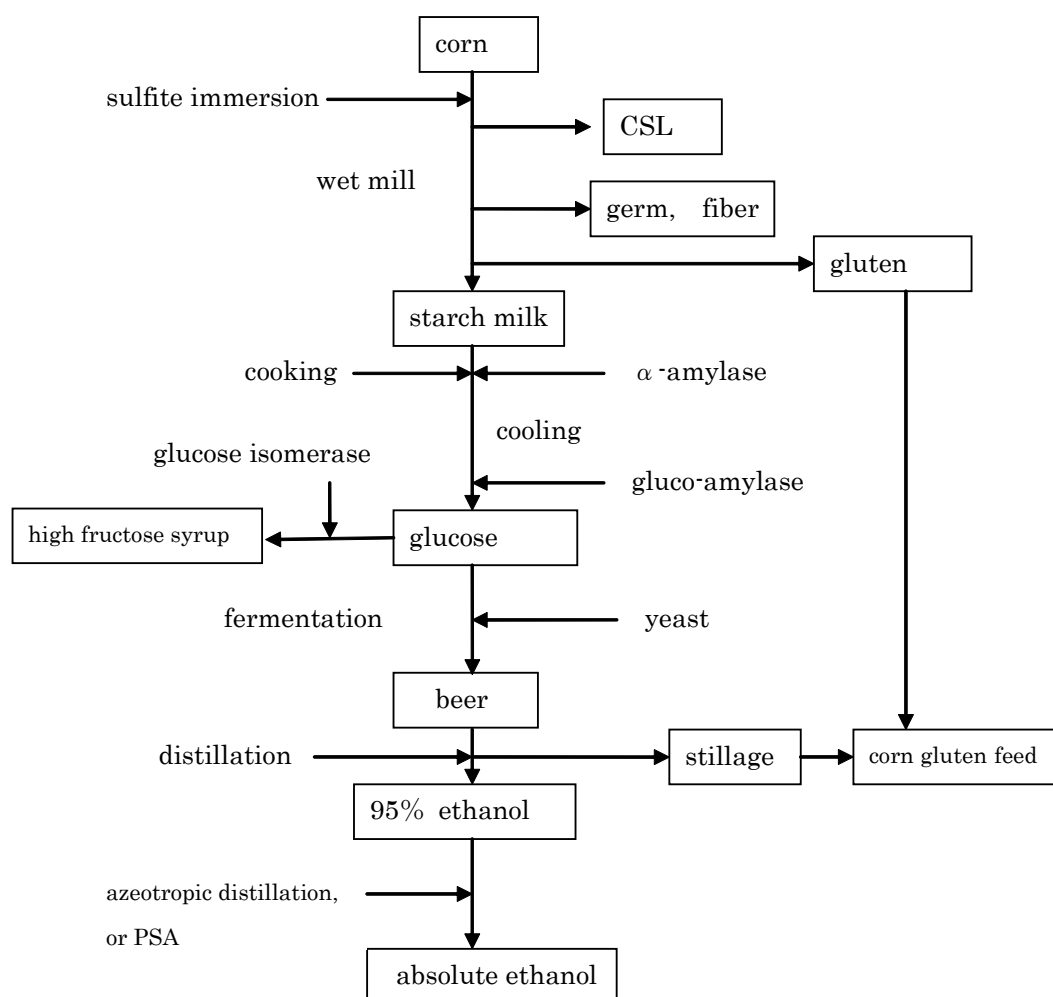


Fig. 5.2.2. Production process of ethanol and high fructose syrup from corn.  
(Modified from (Elander, 1996))

#### 5.2.4 Ethanol fermentation of lignocellulosics

Lignocellulosic biomass is generally composed of cellulose, hemicellulose and lignin (Table 5.2.1). Before ethanol fermentation, biomass has to be pre-treated with acid or alkali, and/or with cellulases to be hydrolyzed into sugar solution.

Table 5.2.1. Compositions of Various Biomass (%).

	cellulose	hemicellulose	lignin
Soft wood	43	28	29
Hard wood	43	35	22
Rice straw	38	25	12
Office paper	69	2	11



### **(a) Concentrated Sulfuric Acid Process**

The process of Arkenol Co. Ltd. (USA) was modified and improved in NEDO project between 2001 and 2005. Concentrated sulfuric acid is sprayed on to wood chips (moisture of about 15%) which is then kneaded well at room temperature. During the kneading the structure of cellulose is decrystallized. Concentration of sulfuric acid is then adjusted at 20 to 30% by adding water, and the wood material is kept at around 90°C for 10 to 15 min for hydrolysis. After solid fraction is removed through filtration, sugar components are separated from the acid on ion-exchange chromatography. Sugar solution containing hexoses and pentoses are fermented by yeast or *Zymomonas* strains genetically modified. Sulfuric acid is concentrated again to reuse.

### **(b) Dilute Sulfuric Acid Process**

Hemicellulose fraction is degraded into sugars composed of mainly pentose and some hexoses through first dilute sulfuric acid (0.5-1.0%) treatment at 150-180°C, and at about 1 MPa (10 atm). Residual fraction containing cellulose and lignin is again treated with similar concentration of the dilute acid at 230-250°C and at 3-5 MPa (30-50 atm) to produce glucose. Sugar yield in first and secondary treatments are reported to be about 90%, and 50-60%, respectively. In the ethanol plant of “Bioethanol Japan Kansai” (Osaka, Japan), which has been operated since January, 2007, sugar solution only from hemicellulose fraction of wood is reported to be converted to ethanol through fermentation by genetically modified *E. coli*.

In the United States, research groups including NREL have been challenging to improve cellulase activity for industrial use in dilute sulfuric acid process. Their target is, reportedly, to start production of bioethanol from biomass like corn stover in 2013.

### **Further information**

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## 5.3 Acetone-Butanol Fermentation

### 5.3.1 What is acetone-butanol fermentation?

Acetone-butanol fermentation is a reaction in which acetone and butanol are produced from glucose using *Clostridium*, a strictly anaerobic bacterium. Further, ethanol is also produced. Therefore, acetone-butanol fermentation is also called as ABE fermentation. *Clostridium* is widely distributed in soil, and secretes amylase, xylanase, protease and lipase out of cells. There are two types of strain in acetone-butanol fermentation. One is Weizmann-type which produces butanol from starch, and the other is Saccaro-type which produces butanol from sucrose.

### 5.3.2 Characteristics of acetone-butanol fermentation

Acetone-butanol fermentation has a long history and is industrialized technology. Acetone-butanol fermentation was utilized to produce acetone as a raw material of smokeless powder in WWI and butanol for fuels of fighter planes in WWII. After WWII, acetone-butanol fermentation fell out of use due to development of petroleum chemistry. Recently, butanol is reviewed as biofuel.

### 5.3.3 Reactions of acetone-butanol fermentation

The strains of bacteria utilized for industrial production of butanol were acetone-butanol producing bacteria and butanol-isopropanol producing bacteria which produce butanol and isopropanol, a reduced product of acetone. The reaction pathway is shown in Fig. 5.3.1. Glucose is decomposed to pyruvate, acetyl-CoA, and acetoacetyl-CoA via EMP pathway, and finally acetone, butanol, isopropanol and ethanol are produced. The stoichiometrical equation of acetone-butanol fermentation is shown in Eq. 5.3.1.



In acetone-butanol fermentation, butanol is gradually accumulated and cause production

inhibition at more than 3 kg/m<sup>3</sup> (g/L) of the butanol concentration. When the production inhibition occurs, the growth of bacteria cells, consumption of substrates, and accumulation of products are suppressed. Final concentration of butanol reaches about 30 kg/m<sup>3</sup> (g/L). After fermentation, the culture solution is distilled and products are separated by the difference in their boiling points, e.g. acetone (BP 56.3°C), ethanol (BP 78.3°C) and butanol (BP 117°C).

#### **5.3.4 Energy efficiency of acetone-butanol fermentation.**

From Eq. 5.3.1, 60 mol butanol (170 MJ), 30 mol acetone (54 MJ), 10 mol ethanol (14 MJ) and 120 mol hydrogen (34 MJ) are produced from 95 mol glucose (273 MJ) in acetone-butanol fermentation. Almost all energy in glucose can be moved to butanol, acetone, ethanol and hydrogen.

#### **5.3.5 Products of acetone-butanol fermentation**

Acetone-butanol fermentation was industrialized for supply of raw materials of smokeless powder and fighter planes. Presently, acetone and butanol are synthesized in petroleum industry. Biofuels, renewable gasoline or diesel additives are now paid much attention throughout the world. Butanol can be added to both gasoline and diesel fuels and has more affinity for gasoline than ethanol. Therefore, butanol will be promising biofuel.

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## 5.4 Hydrogen Fermentation

### 5.4.1 What is hydrogen fermentation?

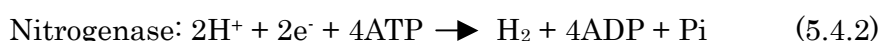
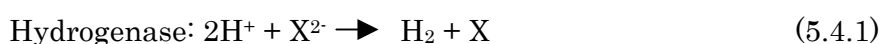
Anaerobic fermentation is a reaction in which anaerobic microorganisms oxidatively decompose organic materials to get energy under anaerobic conditions. We call fermentative reactions in which hydrogen is the final product as hydrogen fermentation. In hydrogen fermentation, some organic materials and alcohols are produced with hydrogen. Although final electron acceptor is oxygen or inorganic materials in respiration, oxidatively degraded organic materials and carbon dioxide etc. from substrate materials are final products in fermentation. For example, final products are ethanol and carbon dioxide from glucose during ethanol fermentation. While ATP synthesis is coupled with electron transfer chain in respiration, ATP is produced in reactions at substrate level in fermentation. Energy gained from fermentation is smaller than that from respiration for the same amount of substrate.

### 5.4.2 Characteristics of hydrogen fermentation

The role of hydrogen production is to regulate oxidation-reduction level in bacterial cells by converting excess reducing power to hydrogen. There are bacteria which can take in and utilize such hydrogen. In order to increase hydrogen yields, the reverse reactions consuming hydrogen should be suppressed. Generally, it is needed to treat waste water from hydrogen fermentation, since hydrogen fermentation includes the production of some organic materials.

### 5.4.3 Reactions of hydrogen fermentation

Hydrogen-producing bacteria are classified into 2 types by the difference in reaction enzymes. One is bacteria with hydrogenase, and the other with nitrogenase.



X: electron carrier, Pi: inorganic phosphate

As shown in above reactions, hydrogenase catalyzes a reversible reaction of evolution and uptake of hydrogen. On the other hand, the reaction by nitrogenase needs energy (ATP). In anaerobic fermentation, the reaction by hydrogenase is mainly investigated. Representative

hydrogen fermentation reactions are as follows:



Figure 5.4.1 shows the pathway of hydrogen fermentation. Hydrogen is formed from hydrogenase via both NADH and ferredoxin, via only ferredoxin, or via formate-lyase. In hydrogen fermentation, hydrogen is produced from oxidative decomposition of organic substrates. Therefore, hydrogen fermentation is utilized as treatment of wastes and waste water. In such cases, the following treatment such as methane fermentation or activated sludge method is needed, since hydrogen fermentation accompanies the production of organic acids. The reaction rate of hydrogen fermentation is fast compared with methane fermentation. It may be promising pre-treatment method of methane fermentation.

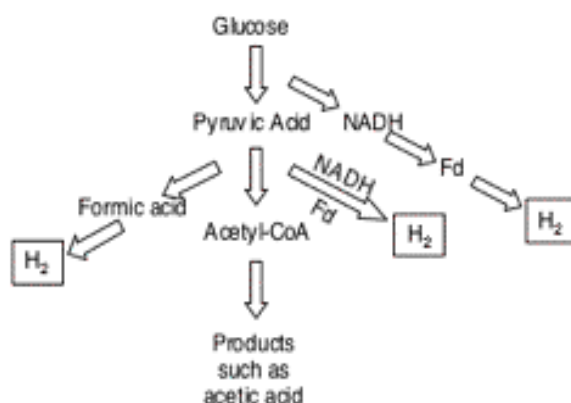


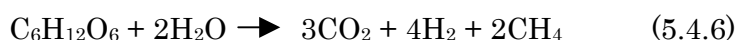
Fig.5.4.1. Pathway of hydrogen fermentation

#### 5.4.4 Energy efficiency of hydrogen fermentation

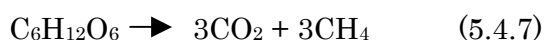
Since hydrogen fermentation accompanies organic acids production, it is needed to consider total system combined subsequent treatment methods such as methane fermentation. In hydrogen fermentation, 4 mol of hydrogen is theoretically produced from 1 mol of glucose (Eq. (5.4.3)). When subsequently formed acetate is utilized for methane fermentation and converted to methane, the reaction is shown as follows:



Total reaction of hydrogen-methane two-stage fermentation is shown as follows:



The sum of high heat value of these products is 2.924 MJ (2,924 kJ). On the other hand, in only methane fermentation, the reaction is shown as follows:



High heat value of the product from Eq. (5.4.7) is 2.671 MJ (2,671 kJ). It is obvious from these results that the energy yield of hydrogen-methane fermentation increases 10% compared with that of only methane fermentation.

#### 5.4.5 Products of hydrogen fermentation

The product gas evolved from hydrogen and methane fermentation might be utilized to fuel cells which have higher energy conversion efficiency than those of gas turbine and gas engine. Methane from methane fermentation has to be converted to hydrogen for fuel cells.



Since Eq. 5.4.8 is an endothermic reaction, supply of energy is needed to proceed the reaction. Generally, methane gas is converted to hydrogen gas under nickel catalysts at 650-750°C. On the other hand, in hydrogen fermentation, energy yield is higher than that of methane fermentation and the catalytic conversion of methane is not needed to provide hydrogen gas to fuel cells.

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## 5.5 Lactic Acid Fermentation

### 5.5.1 What is lactic acid fermentation?

Lactic acid has alcohol (OH) and carboxylic (COOH) sites inside the molecule. Since it includes chiral carbon, it has two chiral isomers, D-lactic acid and L-lactic acid. Recently, demand of poly-lactate, a biomass plastic, is increasing, and demand of lactic acid is also increasing as a raw material of poly-lactate. Then the lactic acid with almost 100% of the optical purity is strongly requested. Generally, lactic acid is produced by chemical syntheses or by microbial fermentations. In the chemical syntheses, a method using hydrolysis of lacto-nitrile is usually adopted, yielding D-lactic acid and L-lactic acid half by half of which the optical purity is nul. Thus lactic acid for the production of poly-lactate is always produced by fermentation. Lactic acid can be produced by either bacteria or fungi. Here the lactic acid fermentation with bacteria is focused on.

### 5.5.2 Lactic acid bacteria

Lactic acid bacteria produce a lot of lactic acid from several types of sugars. They are gram-positive rod-type or spherical bacteria which can grow under an anaerobic condition. They show no mobility and negative in a catalatic reaction. They form no spores. They use only sugars as an energy source to yield lactic acid, and convert more than 50% of the consumed sugars. There are the following four groups in the bacterial species to satisfy the above-mentioned conditions: *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus*. Lactic acid bacteria can grow with higher growth rates and produce lactic acid with higher productivities. Since they request a lot of nutrients including amino acids and vitamins, the composition of the fermentation broth is not simple. We can classify lactic acid fermentation into two groups, homo-lactic acid fermentation and hetero-lactic acid fermentation. In the



homo fermentation two moles of lactic acid and two moles of ATP can be produced from one mole of mono-saccharides with almost 100% of the lactic yield. On the other hand, in the hetero fermentation, lactic acid and other compounds are produced; it is classified into two groups: 1) one yielding lactic acid, ethanol and carbon dioxide. 2) one yielding one mole of lactic acid and 1.5 moles of acetic acid from one mol of mono-saccharides. Lactic acid bacteria possess both D type and L type or either D type or L type of lactate-dehydrogenases. Thus D-lactic acid and (or) L-lactic acid can be produced by the bacteria. Most of lactic acid bacteria has enzymes which racemize the produced lactic acid, affecting the chiral quality of lactic acid. *Lactobacillus rhamnosus* can produce only L-lactic acid with almost 100% of the optical purity, which is used as a raw material for the poly-lactate production.

### 5.5.3 Biomass resources for lactic acid fermentation

Glucose is a major substrate for lactic acid fermentation, which is usually obtained by hydrolysis of starch. Starch is now obtained from crops. However, we sometimes worry about competition between energy or materials and food, as claimed in the ethanol production from biomass resources. Thus soft cellulosic biomass like rice husk which has been currently not used is expected for biomass resources. Anyhow such unused biomass has less quality and that is why it has not yet been used. Then some conditions must be considered for its utilization in fermentation. First, constant and stable supply of the biomass is required. Next, sugars should be obtained easily with energy as least as possible. Of course, more effective and sophisticated technology for the fermentation is needed, and moreover, it is also important to solve the issues accompanied by transportation and storage of biomass in terms of energy, cost, etc.

### 5.5.4 Utilization of unused biomass from palm oil industry

Palm oil is one the three major vegetable oils in the world. It can be harvested throughout a year only around the equator. In the mill operation to produce oil, more than 10 Tg (Mt) of unused biomass is obtained as business as usual. Since the palm oil mills are very large, more than a few tens Gg (thousand tones) of uniform unused biomass can be collected constantly throughout a year. Moreover, the replanting season of oil palm trees is arriving currently and a large amount of oil palm trunks shall be wasted. Recently, it was found that there exists a lot of glucose inside the trunks and that glucose syrup can be easily obtained by pressing them like sugarcane processing.

### 5.5.5 Lactic acid fermentation from kitchen garbage

Japan is a narrow country and has higher population density. That is why we cannot use a simple dumping method for the garbage treatment in Japan. Thus we have around two thousand incinerators for burning the garbage. Every day, garbage is collected in the incinerators and burned to get heat energy, part of which is used for power generation. Therefore, unused steam with lower pressure is available. Kitchen garbage comprises

30% in the total garbage in Japan. Especially, kitchen garbage from business sectors including supermarket and convenience stores can be easily separated from the others. Japanese kitchen garbage should be a good resource for sugars because half of the solid in the kitchen garbage is composed by starch, even though the compositions of the kitchen garbage should be changed every day. Then kitchen garbage contains a variety of nutrients including vitamins and it is good for the lactic acid fermentation. Fig.5.5.1 shows the lactic acid yield from kitchen garbage in the lactic acid fermentation after enzymatic treatment with glucoamylase using *Lactobacillus rhamnosus*. Generally, kitchen garbage contains 80% of moisture and indicating that around the 10% of the lactic acid yield shown in the figure should be quite high.

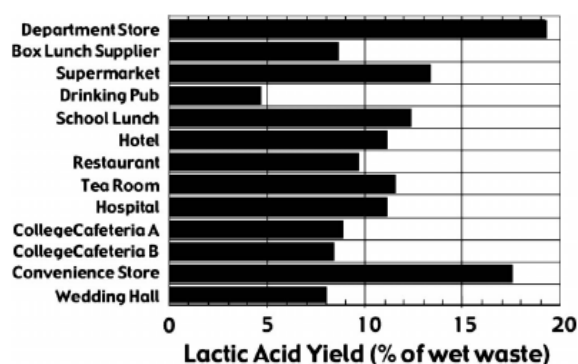


Fig.5.5.1 Lactic acid yield from lactic acid fermentation with kitchen garbage

### 5.5.6 Purification of lactic acid

Lactic acid for poly-lactate is requested to guarantee not only quite high optical purity but also its quite high purity as lactic acid. In lactic acid fermentation, any higher class purification technology to yield pure lactic acid is required because the fermentation broth contains a variety of compositions. Usually distillation technology is adopted for this purpose: In the purification of lactic acid from fermented kitchen garbage, butyl-lactate is separated by distillation after esterification of lactic acid with butanol. Moreover, ammonia can be recovered in the esterification reaction, which can be again used for pH adjustment in the fermentation. However, in this process more energy would be required to remove water to encourage the esterification reaction. When the energy would be supplied from fossil resources, it is neither ecologically nor economically suitable. Utilization of unused heat energy from incinerators should be an acceptable solution.

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## 5.6 Silage

### 5.6.1 What is silage?

**Silage** is now the most commonly preserved cattle and sheep feed in many countries. It is produced by the controlled fermentation of crops with high moisture. Silage is a fermented and stored process which ensilages with forage crops and grasses in a silo (Fig. 5.6.1). The types of silo in which the farmer may choose to ferment their crop are greatly varied. For convenience, commercial silos can be classified into the main categories: stack or clamp without retaining walls, tower, bunker, vacuum, plastic sausage and roll bale. Compared with the hay, the feed intake, digestibility and nutritive value of silage are excellent. Silage can be made of many other by-products of field crops and food, and other materials.



Fig. 5.6.1. Forage cutting (left) and stack silo (right).

### 5.6.2 Silage making

Silage originated in ancient Egypt. The silage research on the fermentation mechanism has been made rapid progress in the 20th century. Silage can be prepared from forage crops and grasses at the optimum stage of growth with suitable moisture content, about 50% to 70%. The forage material is collected, chopped into about 10- to 20-mm lengths and packed into silo. Current mechanical forage harvesters are used to collect and chop the forage material, and deposit it in trucks or wagons. These forage harvesters can either be tractor-drawn or self-propelled. Harvesters blow the silage into the wagon via a chute at the rear or a side of the machine. The inoculants of LAB were used for high quality silage making (Fig. 5.6.2).



Fig. 5.6.2. Cell form (left) and inoculant (right) of lactic acid bacteria “Chikuso 1”

### 5.6.3 Silage fermentation

The preservation of forage crops as silage depends upon the production of sufficient acids to inhibit the activity of undesirable microorganisms under anaerobic conditions. The epiphytic lactic acid bacteria (LAB) that naturally present on forage crops convey sugar into lactic acid in the ensiling process. It is well established that LAB play an important role in silage fermentation. LAB is a major component of the microbial flora which lives in various types of forage crops. The LAB commonly grow with other plant-associated microorganisms during silage fermentation, and they generally define the fermentation characteristics of silage. Moist dairy farm silage is based on natural lactic acid fermentation. The epiphytic LAB transform the water-soluble carbohydrates into organic acids during the ensiling process. As a result, the pH is reduced and the forage is preserved.

However, when the silo is opened and aerobic conditions prevail at feeding time, the silage is subject to aerobic microbial growth and is therefore potentially unstable. Furthermore, the deteriorated silage increases in dry matter loss and reduces in the nutritional value. Generally, the well-preserved silages are considered to be more liable to aerobic deterioration than poorly-

fermented silages and some aerobic microorganisms can be harmful to the health of livestock. Therefore, the prevention of aerobic deterioration is an important task in making silage.

#### 5.6.4 Roll bale silage

**Roll bale** is another form of stored forage. Grass is cut and baled while it's still fairly wet. If it is too wet, it can't be baled and stored the same as hay. Thus, the proper moisture content for roll bale silage making is around 60 to 70%. The bales are wrapped tightly with 6 layers of 0.025-mm thick plastic film on a bale wrapper. The material then goes through a limited fermentation in which short chain fatty acids are produced to protect and preserve the forage. This method has become popular on some farms. In Japan, the roll bale silage preparation method of fresh rice straw was developed (Fig. 5.6.3), and the animal feed production from biomass resources is expected to make the best use of roll bale technology.



Fig. 5.6.3 Roll bale silage making (left) and wrapping (right) of rice straw.

#### 5.6.5 Technological actuality

In recent years, the amount of silage conserved as roll bales has increased dramatically, and this system of making silage is now widely practiced in Japan and other countries. Today, the new silage inoculant of LAB and new types of chopped roll baler for silage making of corn and forage paddy rice were developed. The research of unused biomass resources for silage making of crops and food by-products are advanced in Japan.

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## 5.7 Composting

### 5.7.1 What is composting?

Compost is a mixture of biodegradable organic matter such as straw, husks, tree bark, animal waste products and organic animal/plant matter (excluding sludge and fish organs) that are accumulated or mixed, and decomposed by heat. However sludge and fish organs may be considered compost if properly processed.

### 5.7.2 Basic principles of composting

Composting is the process of accumulating, mixing and aerating organic matter to decompose them with aerobic bacteria inside the material, evaporate the moisture generated by the heat from decomposition, and sterilize or make the harmful microbes or weed seeds inactive, to render the compost mixture safe and hygienic. Figure 5.7.1 shows the composting process. Composting offers the advantages of: (1) being easy to handle by hygienically-minded users since it eliminates noxious odors and the sense of handling biological waste products, (2) producing nutrients including the correct amount of elements for safe and high quality fertilizer for soil and crops, and (3) contributing to create a society that recycles its resources.

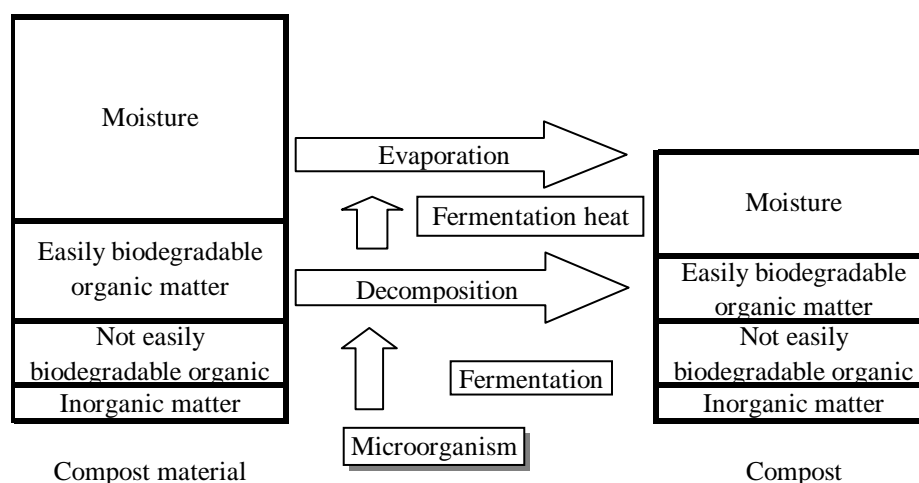


Fig. 5.7.1. Concept image of composting process.

### 5.7.3 Basic elements of composting

Composting is basically made up of (a) preprocessing, (a) fermentation processing, and (c)

product forming processing.

#### **(a) Preprocessing**

Preprocessing requires equipment for adjusting factors such as the moisture and organic matter, particle sizes, and aeration to make compost with the desired properties. When starting the composting, the moisture must usually be adjusted between 55 to 70 % and good aeration must be provided. This preprocessing includes additive methods (addition of adjustment materials such as shell, husks, sawdust, and chips), return methods (returning the product compost and mix with composting feedstock), and drying methods (drying using external energy).

#### **(b) Fermentation**

Fermentation requires a fermenting tank, aeration equipment and hydrolysis equipment. The fermentation tank decomposes organic matter and emits heat to raise the temperature of material accumulated in the fermentation tank so that conditions to produce safe and hygienic compost is attained by raising the temperature of the entire compost material to 65°C or higher and maintaining that temperature for 48 h or more. Fermentation methods are broadly grouped into accumulation methods and mechanical return methods. In the accumulation methods, materials such as compost, adjustment materials, and returned compost are accumulated on the floor and then repeatedly turned as needed with a shovel loader, etc. In the mechanical return method, an agitating device having a material loading slot and drainage slot for mixing the material is installed on the upper side wall of the fermentation tank. The aeration equipment maintains the material at a uniform aerobic state and provides ventilation to make the moisture evaporate from the material simultaneous with causing fermentation. The hydrolysis equipment supplies water to the material to ensure continued aerobic fermentation since microbial activity in the material ceases when the moisture in the material falls below 40%.

#### **(c) Product forming process**

The product forming process includes mechanical sorting and bagging/packing equipment for enhancing the product value and making the product compost easier to handle. Other facilities may include deodorizing equipment as part of environmental measures.

### ***5.7.4 Current composting technology***

Table 5.7.1 shows main materials usable in composting. Husk and woody materials have a low biodegradable material index so their decomposition takes time but are effective for

improving soil and can be used in combination with other materials to produce high quality compost. Raw garbage contains many materials impossible to ferment such as plastic, metal, and glass and also requires thorough sorting and the right preprocessing. Sludge may require special measures for dealing with heavy metals, etc.

Among the recycling technologies of composting, biogas, drying, carbonization, livestock feed, and incineration, composting can use various kinds of materials and offers significant advantages in terms of technology and distribution. However, amount and period of the product demand are limited, and some regions have excess compost stocks. Future production efforts will require elaborate quality control, composting of all material produced in a region, and regional consumption of the entire amount produced in those areas.

### Further information

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Table 5.7.1. Comparison of materials available for composting and other recycling technologies (Japan Organics Recycling Association 2004).

Material Name	Type	Composting	Biogas	Drying	Carbonization	Livestock feed	Incineration
Livestock	Cattle dung	◎	○				
	Cattle dung/urine	○	◎				
	Dairy cow dung	◎	○				
	Dairy cow dung/urine	△	◎				
	Pig dung	◎	○				
	Pig dung/urine	△	◎				
	Chicken dung	◎		◎	◎		◎
Garbage	Raw garbage	◎	◎		△	△	○
Sludge	Dehydrated sludge	◎	◎	◎	○		○
Crop residue	Rice husks	◎			◎	△	◎
	Paddy straw	◎				○	
Wood	Sawdust	○			△		◎
	Bark	◎					◎
	Pruning waste	○			◎		◎
	Chips	◎			◎		◎

Note: ◎: Matches category ○: Usable △: Usable after preprocessing