

FE 411
FOOD BIOTECHNOLOGY

1-Biochemical Pathways

Lecturer: Dr. Derya Koçak Yanık

Dr. Ali Coşkun DALGIÇ

Exam/Lab	Weight
1.Midterm	20%
2.Midterm	20%
Laboratory	20%
Final	40%

Research Assistants: Hatice Neval Özbek

Reference book

- Food biotechnology Bhatia, S.C. Woodhead Publishing India Pvt Ltd
- Fundamentals of Food Biotechnology, Byong H. Lee, Publisher: Wiley-Blackwell; 2nd edition (December 1, 2014

CONTENT

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Lecture schedule

- Chapter 1: Biochemical Pathways
- Chapter 2: Microbial kinetics
- Chapter 3: Bioreactors
- Chapter 4: Sterilization
- Chapter 5: Agitation
- Chapter 6: Inoculum
- Chapter 7: Instrumentation
- Chapter 8: Downstream processing

1st Midterm

- Chapter 9: Beer
- Chapter 10:Wine
- Chapter 11: Spirit
- Chapter 12: Bread
- Chapter 13: Cheese
- Chapter 14: Cultured dairy products
- Chapter 15: Meat products

2nd Midterm

- Chapter 10: Pickling
- Chapter11: Vinegar
- Chapter 15: Tea
- Chapter 17: mushroom
- Chapter 18: Olive fermentation
- Chapter 19: Traditional fermented foods

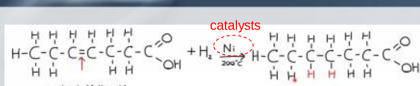
Final Exam

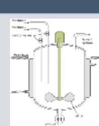
Biotechnology Branches Color Classification and Applications:

Colour	Industries
Red	Biomedicine, Biopharmaceutics, Diagnostics
Yellow	Food Biotechnology, Nutrition Science
Blue	Aquaculture, Coastal and Marine Biotechnology
Green	Agricultural Biotechnology, Bioenergetics (Biofuels), Biofertilizers, Bioremediation, Geomicrobiology
Brown	Arid Zone and Desert Biotechnology
Black	Bioterrorism, Biowarfare, Biocriminology, Anticrop Warfare
Violet	Patents, Publications, Inventions, Intellectual Property Rights (Legal, Ethical and Philosophic Issues)
White	Industrial Biotechnology
Gold	Bioinformatics, Nanobiotechnologies
Grey	Environmental (Ecological) Biotechnology

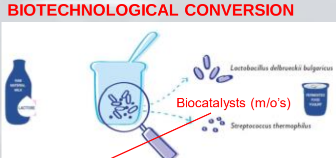
Composed by: [3; 4]

CHEMICAL REACTION





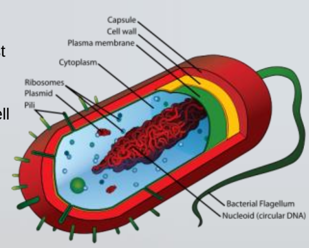
BIOTECHNOLOGICAL CONVERSION

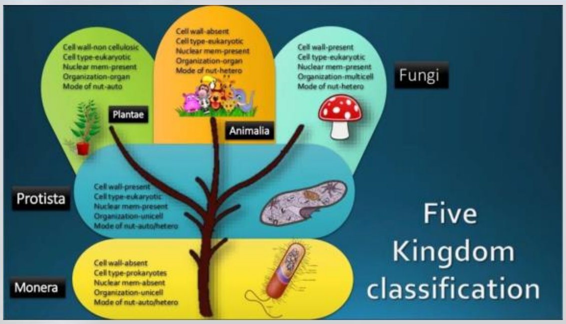


Living things (bacteria, mold, yeast..) so that reason fermentation rxn's are more complex than chemical reactions.

The Nature of Microorganisms

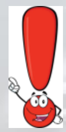
- Microorganisms can be found almost anywhere on the planet.
- Bacteria and archaea are almost always microscopic, while a number of eukaryotes are also microscopic, including most protists, some fungi, as well as some animals and plants.
- Viruses are generally regarded as not living and therefore are not microbes, although the field of microbiology also encompasses the study of viruses.





Microbial Growth Conditions

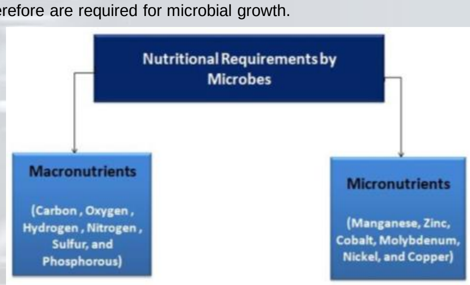
1. Macronutrients
2. Micronutrients
3. Growth factors
4. Environmental factors: T, pH, Oxygen ….



Microorganism require some elements in large quantities, because they are used to constract carbohydrates, lipids, proteins and nucleic acids. Some other elements are needed in very small amounts and are parts of enzymes and cofactors.

Nutritional Requirements: Micro and Macro Nutrients

Nutrients are substances used in biosynthesis and energy release and therefore are required for microbial growth.



Micro and Macro Nutrients

- The microbial cell is made up of several elements such as carbon, oxygen, hydrogen, nitrogen, sulfur, phosphorus, potassium, calcium, magnesium, and iron. These are also known as macro elements or macronutrients because these elements are required in high amounts by the microbes.Among these, C, H, O, N, S, and P are the major elements required for carbohydrates, lipids, proteins, and nucleic acids. Apart from these, the other macronutrients are found to have several biological functions. For example Potassium ions (K^+) involved in the activity of several enzymes, Calcium (Ca^{2+}) is an important element of bacterial endospores, Magnesium (Mg^{2+}) involved as cofactors of different enzymes, etc.

- On the other hand, several other elements are also required by the microbes on a small level which are known as microelements or micronutrients or traces elements. These nutrients include manganese, zinc, cobalt, molybdenum, nickel, and copper. These are not essential elements for the growth of the microbes but these are involved in biological functions in several ways. For example, zinc (Zn^{2+}) is present at the active site of several enzymes, manganese (Mn^{2+}) involved in catalysis of the transfer of phosphate group, Mo (Mo^{2+}) is essential for nitrogen fixation, etc.

Nutritional Requirements and Classification

All biological systems, from microorganisms to man, share a set of nutritional requirements, which are:

1. Sources of energy:

a. Phototrophs organisms which are capable of employing radiant energy.

b. Chemotrophs organisms which obtain the energy for their activities and self-synthesis from chemical reactions that can occur in the dark.

2. Sources of carbon:

a. Autotrophs organisms which can thrive on an entirely inorganic diet, using CO_2 or carbonates as a sole source of carbon.

b. Heterotrophs organisms which cannot use CO_2 as a sole source of carbon but require, in addition to minerals, one or more organic substances, such as glucose or amino acids, as sources of carbon.

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Sources of nitrogen:
Atmospheric nitrogen, inorganic nitrogen compounds, or other derived nitrogen.

Sources of sulfur and phosphorus:
Elementary sulfur, inorganic sulfur, or organic sulfur.

Sources of metallic elements:
Sodium, potassium, calcium, magnesium, manganese, iron, zinc, copper, and cobalt.

6. Sources of vitamins

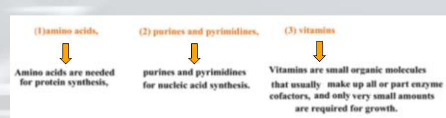
Nutritional Classification

Nutritional classification- based on how microorganism satisfy Carbon, Energy & Electron

Carbon source	
Autotrophs	CO2 sole or principal biosynthetic carbon source
Heterotrophs	Reduced, preformed, organic molecules from other organisms
Energy Sources	
Phototrophs	Light
Chemotrophs	Oxidation of organic or Inorganic compounds
Electron Sources	
Lithotrophs	Reduced inorganic molecules
Organotrophs	Organic molecules

Growth Factors

- Most of the organisms are capable of producing enzymes required for biochemical pathways by the presence of nutrients; however, there are several organisms that lack specific enzymes required by the microbes. Therefore, they must obtain these constituents or their precursors from the environment. Organic compounds that are essential cell components or precursors of such components but cannot be synthesized by the organism are called growth factors.
- There are three major types of growth factors such as Amino acids, purines (adenine and guanine), and pyrimidines (thymine and cytosine) and Vitamins.



Physical Conditions

- After determining the proper nutrients for the cultivation of bacteria, it is necessary to determine the physical environment in which the organisms will grow best. Three major physical factors to be taken into consideration are temperature, the gaseous environment, and pH.

Temperature requirements for growth of Microorganisms

All microorganisms are allocated to a specific group with respect to growth temperature.

- Obligate psychrophiles are defined as those organisms capable of growth at or near 0°C but not at 20°C. Such organisms usually have a maximum growth temperature of 15–17°C.
- Psychrotrophic organisms are capable of growth at or near 0°C but exhibit optimum growth at approximately 25°C and are frequently unable to grow at 30°C.
- Mesophiles exhibit growth from 20–45°C with an optimum growth temperature usually in the range of 30–35°C.
- Thermophiles exhibit growth in the range of 45–65°C.
- Hyperthermophiles are organisms from oceanic thermal vents and hot springs that are restricted to growth temperatures from 70–120°C. Hyperthermophiles have not yet been isolated from foods.

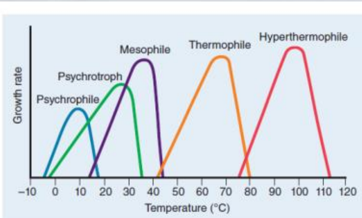


FIGURE 4.8 Temperature Requirements for Growth Microorganisms are commonly divided into five groups based on their optimum growth temperatures. This graph shows a typical example of each group. The optimum temperature, the point at which the growth rate is highest, is near the upper limit of the range.

Physical Conditions

- The principal gases in the cultivation of bacteria are oxygen and carbon dioxide. There are four types of bacteria, according to their response to oxygen:

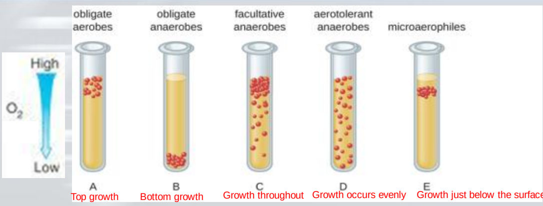
1. Aerobic bacteria grow in the presence of free oxygen.

2. Anaerobic bacteria grow in the absence of free oxygen.

3. Facultatively anaerobic bacteria grow in either the absence or the presence of free oxygen.

4. Microaerophilic bacteria grow in the presence of minute quantities of free oxygen.

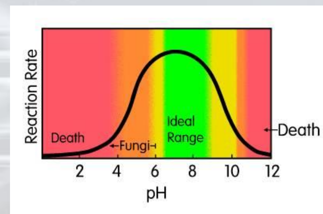
Classification according to their response to oxygen



The growth of bacteria with varying oxygen requirements in thioglycolate tubes is illustrated in **Figure 7.10**. In tube A, all the growth is seen at the top of the tube. The bacteria are **obligate (strict) aerobes** that cannot grow without an abundant supply of oxygen. Tube B looks like the opposite of tube A. Bacteria grow at the bottom of tube B. Those are **obligate anaerobes**, which are killed by oxygen. Tube C shows heavy growth at the top of the tube and growth throughout the tube, a typical result with **facultative anaerobe**s. Facultative anaerobes are organisms that thrive in the presence of oxygen but also grow in its absence by relying on fermentation or anaerobic respiration, if there is a suitable electron acceptor other than oxygen and the organism is able to perform anaerobic respiration. The **aerotolerant anaerobe**s in tube D are indifferent to the presence of oxygen. They do not use oxygen because they usually have a fermentative metabolism, but they are not harmed by the presence of oxygen as obligate anaerobes are. Tube E on the right shows a "Goldilocks" culture. The oxygen level has to be just right for growth, not too much and not too little. These **microaerophile**s are bacteria that require a minimum level of oxygen for growth, about 1%–10%, well below the 21% found in the atmosphere.

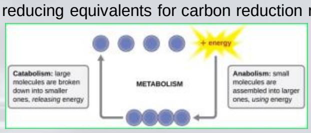
PH

For most bacteria the optimum pH for growth lies between 6.5 and 7.5. Although a few bacteria can grow at the extremes of the pH range, for most species the minimum and maximum limits fall somewhere between pH 4 and pH9.



Common Pathways of Energy Metabolism for Bacterial, Fungal, and Animal Cells

Catabolic pathways provide energy for biosynthesis and in most instances also provide reducing equivalents for carbon reduction reactions.



There are three major groups of compounds that link anabolic and catabolic pathways:

- adenosine phosphates (AMP, ADP, ATP), which link energy yielding and energy requiring reactions;
- nicotinamide adenine dinucleotide (NAD, NADH);
- nicotinamide adenine dinucleotide phosphate (NADP, NADPH).

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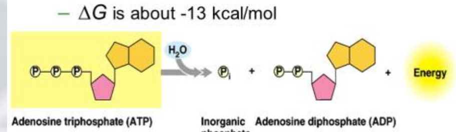
How does ATP release energy?

- The bonds between phosphate groups can be broken by hydrolysis.
  - Hydrolysis of the end phosphate group forms adenosine diphosphate [ATP -> ADP + P_i] and releases 7.3 kcal of energy per mole of ATP under standard conditions.
  - ΔG is about -13 kcal/mol



(b) Hydrolysis of ATP

Glycolysis and the Catabolism of Hexoses

The breakdown of glucose is central for energy and biosynthetic metabolism throughout all domains of life.

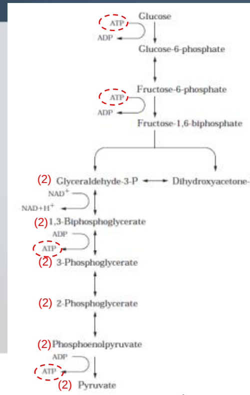
- **Glycolysis** is the metabolic pathway that converts glucose ($C_6H_{12}O_6$) into pyruvate (CH_3COCO_2H)
- The free energy released in this process is used to form high-energymolecules adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide (NADH).
- The Embden–Meyerhof–Parnas (EMP) pathway (glycolysis) and the oxidative pentose phosphate (OPP) pathway are the backbones of eukaryotic carbon and energy metabolism. They generate ATP, NAD(P)H, and biosynthetic precursors for amino acids, nucleotides, and fatty acids.
- Prokaryotes, in contrast, exhibit a broad diversity in sugar oxidation pathways. These routes differ in ATP yield, in the enzymes and cofactors involved, and in the chemical intermediates of the pathways. The most common glycolytic routes in prokaryotes are the *Embden–Meyerhof–Parnas* (EMP) , *Entner–Doudoroff pathway* (ED), and *oxidative pentose phosphate pathways* (OPP)

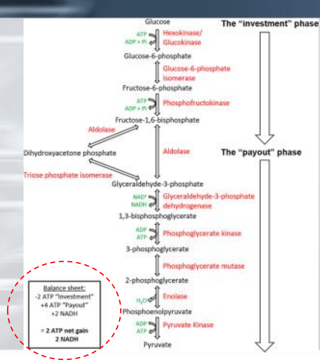
Embden–Meyerhof–Parnas pathway

- EMP pathway is the other name of glycolysis. It is named after the three scientists Gustav Embden, Otto Meyerhof, and J. Parnas, who gave the scheme of glycolysis.
- It is the pathway of glucose catabolism. It occurs in the cytoplasm of all living cells, aerobic as well as anaerobic.
- EMP pathway or glycolysis is the primary step of cellular respiration. 1 mol Glucose is partially oxidised to 2 mol pyruvate in this process.

In aerobic organisms, it is converted into acetyl-CoA followed by the Krebs cycle for the complete oxidation of glucose to CO_2 and water.

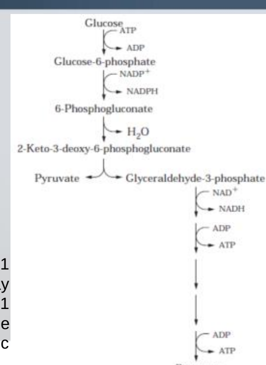
In anaerobic organisms, glycolysis is followed by fermentation to produce lactate.





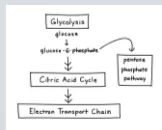
Entner–Doudoroff (ED) pathway

- The Entner–Doudoroff (ED) pathway is present in a number of bacteria where it can be a major pathway of glucose catabolism under aerobic conditions.
- Glucose is degraded in the absence of phosphofructokinase to pyruvate and glyceraldehyde-3-phosphate. The latter can be degraded further to pyruvate by the enzymes of glycolysis.
- This pathway is less efficient. From 1 molecule of glucose, the ED pathway produces 1 molecules of NADPH, 1 molecule of NADH/H+ and 1 molecule of ATP for use in cellular biosynthetic reactions.

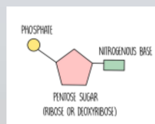


Pentose-phosphate pathway

- The pentose phosphate is a metabolic pathway parallel to glycolysis. It generates NADPH and pentoses (5-carbon sugars) as well as ribose 5-phosphate , a precursor for the synthesis of nucleotides that make up DNA and RNA.

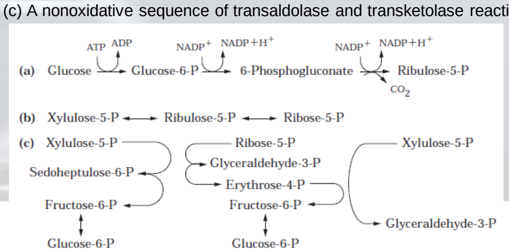


- While the pentose phosphate pathway does involve oxidation of glucose, its primary role is anabolic rather than catabolic. The pathway is especially important in red blood cells (erythrocytes).



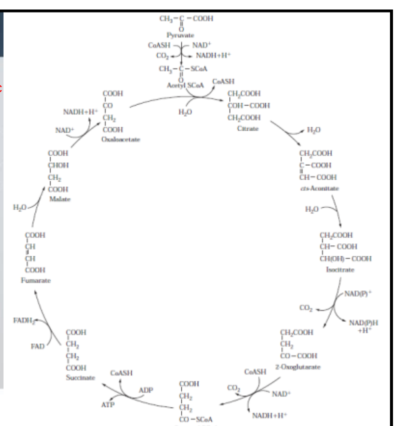
Pentose-phosphate pathway

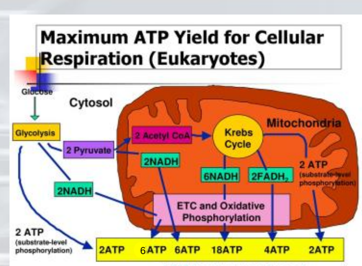
- This pathway is divided into three parts:
- (a) An oxidative sequence of reactions in which glucose is converted to the five-carbon, ribulose-5-phosphate.
- (b) Epimerization and isomerization reactions in which the important nucleic acid precursor, ribose-5-phosphate is formed.
- (c) A nonoxidative sequence of transaldolase and transketolase reactions.



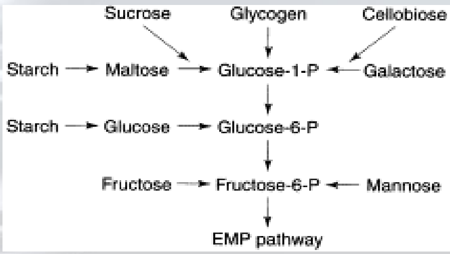
Tricarboxylic acid cycle

- Tricarboxylic acid (TCA) cycle also known as the Krebs or citric acid cycle is the main source of energy for cells and an important part of aerobic respiration. It operates in the mitochondria of eukaryotes.
- It allows the complete oxidation of pyruvate, which enters the cycle from glycolysis.
- The reduced coenzymes are reoxidized via the electron transport chain.
- The complete aerobic metabolism of glucose via glycolysis and the TCA cycle yields between 30 and 38 mol ATP/mol glucose, depending on the extent of the coupling of phosphorylation in the electron transport chain.





- Glucose is not the only carbohydrate that can be converted to pyruvate by glycolysis



Anaerobic Breakdown of Carbohydrates

- The terms glycolysis and fermentation have been applied to the anerobic decomposition of carbohydrate to the level of lactic acid.
- The final product in some organisms is lactic acid; in others, the lactic acid is further metabolized anaerobically to butyric acid, butyl alcohol, acetone and propionic acid.

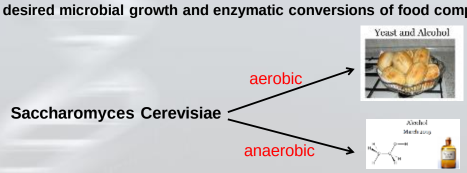
Fermentation is a general term denoting the anaerobic degradation of glucose or other organic nutrients into various products (characteristic for different organisms) to obtain energy in the form of ATP.

In a broader sense, Food Fermentation is microbial activities usually anaerobic in which a suitable substrate under controlled or non-controlled conditions results in the production of desirable foods or beverages which are characteristically more-stable, palatable and nutritious than raw substrate.

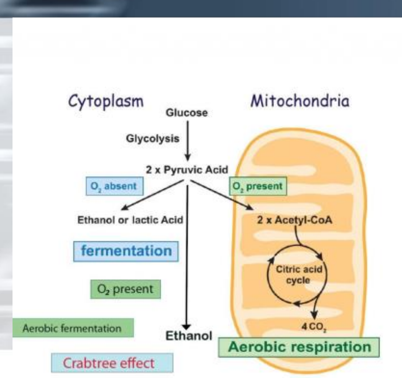
Food Fermentation

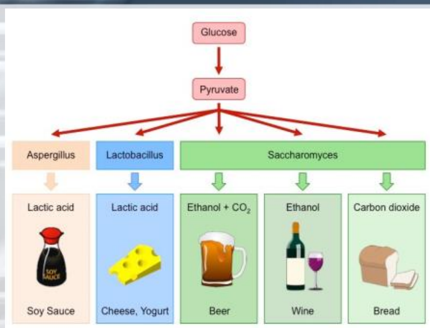
- In aerobic microbial activities the final electron acceptor is O_2.
- In anaerobic microbial activities final electron acceptor can be organic compounds.
- Food fermentations are generally an anaerobic fermentation.

Finally, fermented foods and beverages are defined as "foods made through desired microbial growth and enzymatic conversions of food components



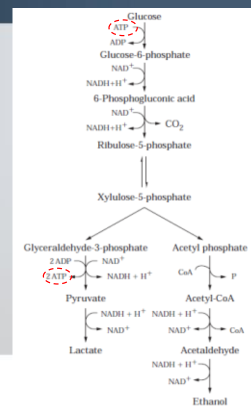
For thousands of years, people have used a fungus called baker's yeast, *Saccharomyces cerevisiae*, to ferment ethanol (drinking alcohol) and bake bread.





Fermentation

- The pentose phosphoketolase (PPK) pathway is a major route of glucose catabolism in heterolactic or heterofermentative lactic acid-producing bacteria
- The heterofermentative lactic acid-producing bacteria typically produce equimolar amounts of CO_2 plus lactate and ethanol or acetate from the catabolism of glucose.
- The key enzyme is phosphoketolase, which degrades the five-carbon xylulose into a two-carbon, acetyl and three-carbon, glyceraldehyde.
- Lactate and ethanol are carbon products of the pathway.
- The net energy gain is 1 mol ATP/mol glucose utilized.



- Homofermentative bacteria are a type of lactic acid bacteria that produce only lactic acid as a primary by-product in glucose fermentation. In biochemistry, homofermentative bacteria convert glucose molecules into two lactic acid molecules.

- Heterofermentative bacteria are a type of lactic acid bacteria that produce ethanol/acetic acid and CO_2 in addition to lactic acid as by-products in glucose fermentation.

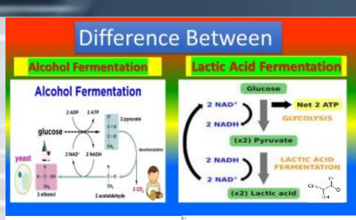
The two most common forms of fermentation are lactic and alcoholic.

Lactic Fermentation

- A familiar fermentation product is lactic acid, which is derived from the reduction of pyruvate with NADH to lactate by lactate dehydrogenase. A further means of energy conservation observed in several homolactic fermenting organisms, but which is also found in other organisms, is the generation of a proton motive force in association with the excretion of lactic acid out of the cells via a secondary transport mechanism.

Alcoholic Fermentation

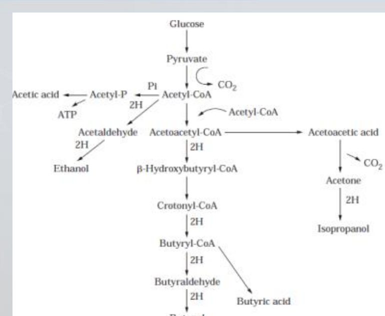
- The formation of ethanol as the sole organic fermentation product is a two-step process. The first step is the decarboxylation of pyruvate to acetaldehyde plus CO_2 by pyruvate decarboxylase. NAD is recycled in the next step, the NADH-dependent reduction of acetaldehyde by alcohol dehydrogenase. Organisms possessing pyruvate decarboxylase are capable of producing ethanol as a major fermentation product.

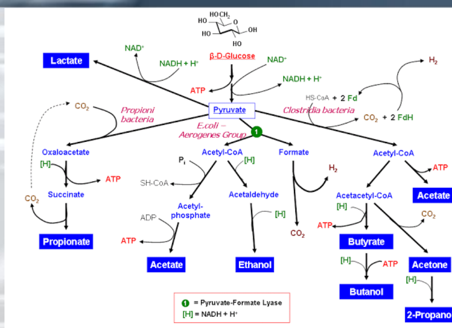


Alcoholic fermentation refers to a metabolic process by which glucose is converted into ethanol and carbon dioxide	Lactic acid fermentation refers to a metabolic process by which glucose is converted into the metabolite: lactate and cellular energy
Occurs in yeast and other microorganisms	Occurs in *Lactobacillus* spps, yeast, and muscle cells
Produces ethanol and carbon dioxide from the pyruvate molecule	Produces lactic acid molecules from the pyruvate molecule
Involves alcohol dehydrogenase and pyruvate decarboxylase	Involves lactate dehydrogenase and pyruvate decarboxylase
Used in the production of bread, beer, wine, and vinegar	Used in the production of yogurt and cheese

Butanol/acetone fermentation

- This anaerobic metabolism is typical of the genus Clostridium. Various fermentation products are formed by reduction using NADH derived from glycolysis.
- The proportion of each product formed is dependent on the fermentation conditions.





Bacterial fermentation products of pyruvate