IDHAYA COLLEGE FOR WOMEN, KUMBAKONAM

DEPARTMENT OF MICROBIOLOGY

CLASS : **I B.Sc MICROBIOLOGY**

SUBJECT NAME : **MICROBIAL METABOLISM**

SUBJECT CODE : **16SCCMB2**

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UNIT-V

ANAEROBIC RESPIRATION

Anaerobic respiration is respiration using electron acceptors other than molecular oxygen (O_2). Although oxygen is not the final electron acceptor, the process still uses a respiratory electron transport chain.

In aerobic organisms undergoing respiration, electrons are shuttled to an electron transport chain, and the final electron acceptor is oxygen. Molecular oxygen is a high-energy oxidizing agent and, therefore, is an excellent electron acceptor. In anaerobes, other less-oxidizing substances such as nitrate (NO_3^-), fumarate, sulphate (SO_4^{2-}), or sulphur (S) are used. These terminal electron acceptors have smaller reduction potentials than O_2, meaning that less energy is released per oxidized molecule. Therefore, anaerobic respiration is less efficient than aerobic.

SULFATE REDUCTION

Dissimilatory sulfate reduction is a form of anaerobic respiration that uses sulfate as the terminal electron acceptor. This metabolism is found in some types of bacteria and archaea which are often termed sulfate-reducing organisms.

Sulfate reduction is a type of anaerobic respiration that utilizes sulfate as a terminal electron acceptor in the electron transport chain. Compared to aerobic respiration, sulfate reduction is a relatively energetically poor process, though it is a vital mechanism for bacteria and archaea living in oxygen-depleted, sulfate-rich environments.

Many sulfate reducers are organotrophic, using carbon compounds, such as lactate and pyruvate (among many others) as electron donors, while others are lithotrophic, and use hydrogen gas (H_2) as an electron donor. Some unusual autotrophic sulfate-reducing bacteria (e.g., *Desulfotignum phosphitoxidans*) can use phosphite (HPO^{3-}) as an electron donor, whereas others (e.g., *Desulfovibrio sulfodismutans, Desulfocapsa thiozymogenes,* and *Desulfocapsa sulfoexigens*) are capable of sulfur disproportionation (splitting one compound into two different compounds, in this case an electron donor and an electron acceptor) using elemental sulfur (S0), sulfite (SO_3^{2-}), and thiosulfate ($S_2O_3^{2-}$) to produce both hydrogen sulfide (H_2S) and sulfate (SO_4^{2-}).

Many bacteria reduce small amounts of sulfates in order to synthesize sulfur-containing cell components; this is known as assimilatory sulfate reduction. By contrast, sulfate-reducing bacteria reduce sulfate in large amounts to obtain energy and expel the resulting sulfide as waste; this is known as "dissimilatory sulfate reduction. " Most sulfate-reducing bacteria can also reduce other oxidized inorganic sulfur compounds, such as sulfite, thiosulfate, or elemental sulfur (which is reduced to sulfide as hydrogen sulfide).

NITRATE REDUCTION

In anaerobic respiration, denitrification utilizes nitrate (NO_3^-) as a terminal electron acceptor in the respiratory electron transport chain. Denitrification is a widely used process; many facultative anaerobes use denitrification because nitrate, like oxygen, has a high reduction potential

Denitrification is a microbially facilitated process involving the stepwise reduction of nitrate to nitrite (NO_2^-) nitric oxide (NO), nitrous oxide (N_2O), and, eventually, to dinitrogen (N_2) by the enzymes nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase. The complete denitrification process can be expressed as a redox reaction: $2\ NO^{3-} + 10\ e^- + 12\ H^+ \rightarrow N_2 + 6\ H_2O$.

Protons are transported across the membrane by the initial NADH reductase, quinones and nitrous oxide reductase to produce the electrochemical gradient critical for respiration. Some organisms (e.g. *E. coli*) only produce nitrate reductase and therefore can accomplish only the first reduction leading to the accumulation of nitrite. Others (e.g. *Paracoccus denitrificans* or *Pseudomonas stutzeri*) reduce nitrate completely. Complete denitrification is an environmentally significant process because some intermediates of denitrification (nitric oxide and nitrous oxide) are significant greenhouse gases that react with sunlight and ozone to produce nitric acid, a component of acid rain. Denitrification is also important in biological wastewater treatment, where it can be used to reduce the amount of nitrogen released into the environment, thereby reducing eutrophication.

METHANOGENESIS

Methanogenesis or biomethanation is the formation of methane by microbes known as methanogens. Organisms capable of producing methane have been identified only from the domain Archaea, a group phylogenetically distinct from both eukaryotes and bacteria, although many live in close association with anaerobic bacteria. The production of methane is an important and widespread form of microbial metabolism. In anoxic environments, it is the final step in the decomposition of biomass. Methanogenesis is responsible for significant amounts of natural gas accumulations

Methanogenesis in microbes is a form of anaerobic respiration. Methanogens do not use oxygen to respire; in fact, oxygen inhibits the growth of methanogens. The terminal electron acceptor in methanogenesis is not oxygen, but carbon. The carbon can occur in a small number of organic compounds, all with low molecular weights. The two best described pathways involve the use of acetic acid or inorganic carbon dioxide as terminal electron acceptors:

$$CO_2 + 4\ H_2 \rightarrow CH_4 + 2\ H_2O$$

$$CH_3COOH \rightarrow CH_4 + CO_2$$

Methanogenesis is the final step in the decay of organic matter. During the decay process, electron acceptors (such as oxygen, ferric iron, sulfate, and nitrate) become depleted, while hydrogen (H_2) and carbon dioxide accumulate. Light organics produced by fermentation also accumulate. During advanced stages of organic decay, all electron acceptors become depleted except carbon dioxide. Carbon dioxide is a product of most catabolic processes, so it is not depleted like other potential electron acceptors.

ANAEROBIC FERMENTATION

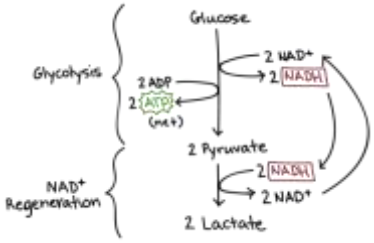
Fermentation is another anaerobic (non-oxygen-requiring) pathway for breaking down glucose, one that's performed by many types of organisms and cells. In fermentation, the only energy extraction pathway is glycolysis, with one or two extra reactions tacked on at the end.

Fermentation and cellular respiration begin the same way, with glycolysis. In fermentation, however, the pyruvate made in glycolysis does not continue through oxidation and the citric acid cycle, and the electron transport chain does not run. Because the electron transport chain isn't

functional, the NADH made in glycolysis cannot drop its electrons off there to turn back into NAD+

LACTIC ACID FERMENTATION

In lactic acid fermentation,NADH transfers its electrons directly to pyruvate, generating lactate as a byproduct. Lactate, which is just the deprotonated form of lactic acid, gives the process its name. The bacteria that make yogurt carry out lactic acid fermentation, as do the red blood cells in your body, which don't have mitochondria and thus can't perform cellular respiration.



ALCOHOL FERMENTATION

Another familiar fermentation process is alcohol fermentation, in which NADH donates its electrons to a derivative of pyruvate, producing ethanol.

Going from pyruvate to ethanol is a two-step process. In the first step, a carboxyl group is removed from pyruvate and released in as carbon dioxide, producing a two-carbon molecule called acetaldehyde. In the second step, NADH passes its electrons to acetaldehyde, regenerating NAD and forming ethanol.

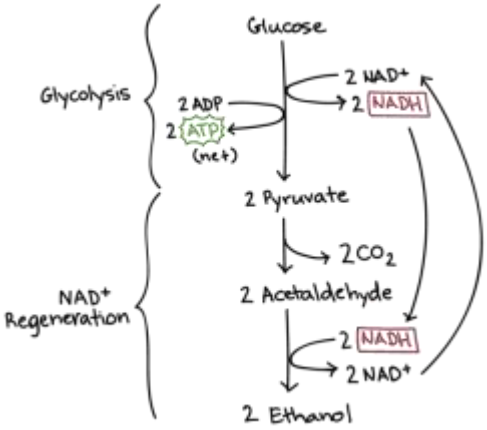


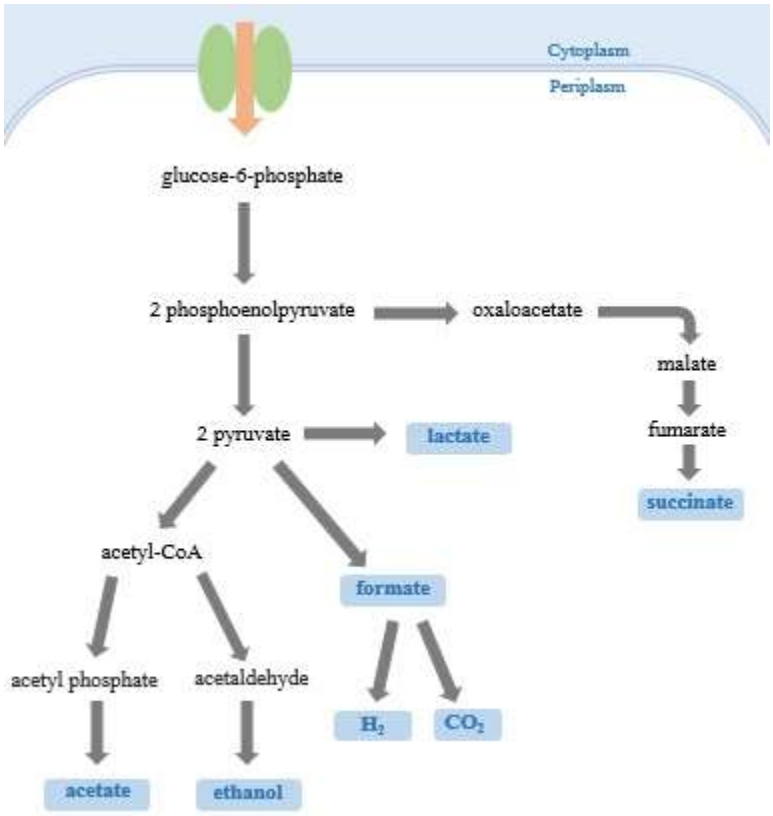
Diagram of alcohol fermentation. Alcohol fermentation has two steps: glycolysis and NADH regeneration.

During glycolysis, one glucose molecule is converted to two pyruvate molecules, producing two net ATP and two NADH.During NADH regeneration, the two pyruvate molecules are first converted to two acetaldehyde molecules, releasing two carbon dioxide molecules in the process. The two NADH then donate electrons and hydrogen atoms to the two acetaldehyde molecules, producing two ethanol molecules and regenerating NAD+.Alcohol fermentation by yeast produces the ethanol found in alcoholic drinks like beer and wine. However, alcohol is toxic to yeasts in large quantities (just as it is to humans), which puts an upper limit on the percentage alcohol in these drinks. Ethanol tolerance of yeast ranges from about 5 percent to 21percent, depending on the yeast strain and environmental conditions.

MIXED ACID FERMENTATION

Mixed acid fermentation is the biological process by which a six-carbon sugar e.g. glucose is converted into a complex and variable mixture of acids. It is an anaerobic fermentation reaction that is common in bacteria

The mixture of end products produced by mixed acid fermentation includes lactate, acetate, succinate, formate, ethanol and the gases H_2 and CO_2. The formation of these end products depends on the presence of certain key enzymes in the bacterium. The proportion in which they are formed varies between different bacterial species



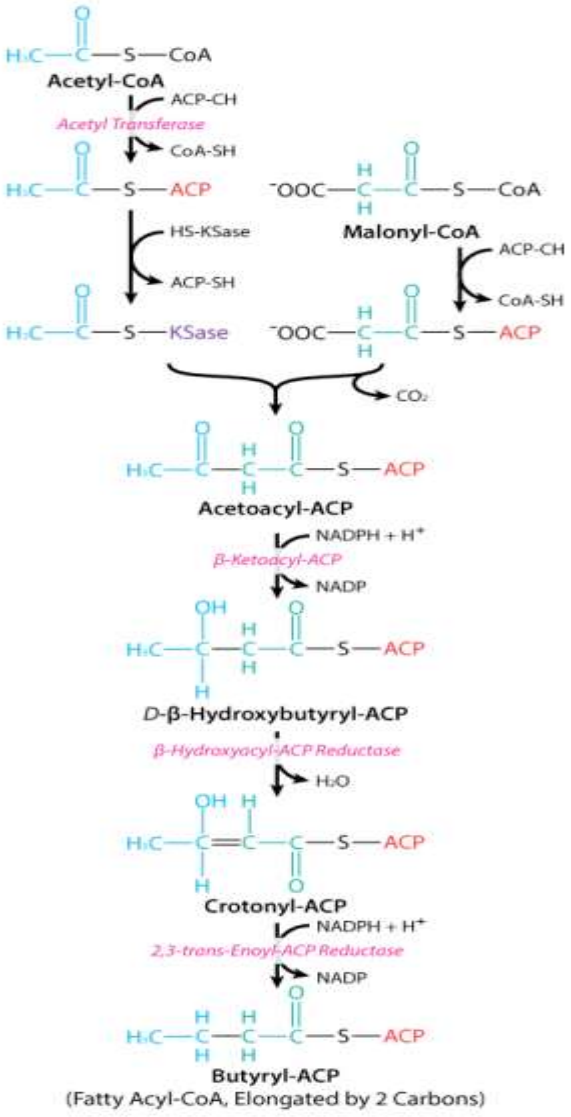
LIPID METABOLISM

Lipid metabolism is the synthesis and degradation of lipids in cells, involving the breakdown or storage of fats for energy and the synthesis of structural and functional lipids, such as those involved in the construction of cell membranes

FATTY ACID SYNTHESIS

Fatty acid synthesis is the creation of fatty acids from acetyl-CoA and NADPH through the action of enzymes called fatty acid synthases. This process takes place in the cytoplasm of the cell. Most of the acetyl-CoA which is converted into fatty acids is derived from carbohydrates via the glycolytic pathway. The glycolytic pathway also provides the glycerol with which three fatty acids can combine (by means of ester bonds) to form triglycerides (also known as "triacylglycerols" – to distinguish them from fatty "acids" – or simply as "fat"), the final product of the lipogenic process. When only two fatty acids combine with glycerol and the third alcohol group is phosphorylated with a group such as phosphatidylcholine, a phospholipid is formed. Phospholipids form the bulk of the lipid

bilayers that make up cell membranes and surround the organelles within the cells (e.g. the cell nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus etc.)



Starting with two acetyl-CoA, one is converted to malonyl-CoA by carboxylation catalyzed by the enzyme acetyl-CoA carboxylase (ACC), the only regulatory enzyme of fatty acid synthesis . Next, both molecules have their CoA portions replaced by a carrier protein known as ACP (acyl-carrier protein) to form acetyl-ACP and malonyl-ACP. Joining of a fatty acyl-ACP (in this case, acetyl-ACP) with malonyl-ACP splits out the carboxyl that was added .

BIOSYNTHESIS OF CHOLESTEROL

Cholesterol is an extremely important biological molecule that has roles in membrane structure as well as being a precursor for the synthesis of the steroid hormones, the bile acids, and vitamin D. Both dietary cholesterol, and that synthesized *de novo*, are transported through the circulation in lipoprotein particles. The same is true of cholesteryl esters, the form in which cholesterol is stored in cells. Due to its important role in membrane function, all cells express the enzymes of cholesterol biosynthesis. The initial steps in the pathway of cholesterol biosynthesis are collectively called the mevalonate pathway

The process of cholesterol synthesis can be considered to be composed of five major steps where the reactions that culminate in the synthesis of isopentenyl pyrophosphate, and its isomeric form dimethylallyl pyrophosphate, are commonly referred to as the mevlonate pathway:

1. Acetyl-CoAs are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)
2. HMG-CoA is converted to mevalonate
3. Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP)
4. IPP molecules are converted to squalene
5. Squalene is converted to cholesterol.

BETA OXIDATION

n biochemistry and metabolism, beta-oxidation is the catabolic process by which fatty acid molecules are broken down in the cytosol in prokaryotes and in the mitochondria in eukaryotes to generate acetyl-CoA, which enters the citric acid cycle, and NADH and $FADH_2$, which are co-enzymes used in the electron transport chain. It is named as such because the beta carbon of the fatty acid undergoes oxidation to a carbonyl group. Beta-oxidation is primarily facilitated by the mitochondrial trifunctional protein, an enzyme complex associated with the inner mitochondrial membrane, although very long chain fatty acids are oxidized in peroxisomes.

The overall reaction for one cycle of beta oxidation is:

C_n-acyl-CoA+FAD+ NAD^+ + H_2O +CoA→C_{n-2}-acyl-CoA+ $FADH_2$ +NADH+ H^+ + acetyl-CoA

Once the fatty acid is inside the mitochondrial matrix, beta-oxidation occurs by cleaving two carbons every cycle to form acetyl-CoA. The process consists of 4 steps.

1. A long-chain fatty acid is dehydrogenated to create a trans double bond between C2 and C3. This is catalyzed by acyl CoA dehydrogenase to produce trans-delta 2-enoyl CoA. It uses FAD as an electron acceptor and it is reduced to $FADH_2$.
2. Trans-delta2-enoyl CoA is hydrated at the double bond to produce L-3-hydroxyacyl CoA by enoyl-CoA hydratase.
3. L-3-hydroxyacyl CoA is dehydrogenated again to create 3-ketoacyl CoA by 3-hydroxyacyl CoA dehydrogenase. This enzyme uses NAD as an electron acceptor.
4. Thiolysis occurs between C2 and C3 (alpha and beta carbons) of 3-ketoacyl CoA. Thiolase enzyme catalyzes the reaction when a new molecule of coenzyme A breaks the bond by nucleophilic attack on C3. This releases the first two carbon units, as acetyl CoA, and a fatty acyl CoA minus two carbons. The process continues until all of the carbons in the fatty acid are turned into acetyl CoA.
