CHAPTER IV: CATABOLISM of other ORGANIC COMPOUNDS

1. Lipid catabolism

Microorganisms often use lipids as an energy source. Triglycerides or triacylglycerols, esters of glycerol and fatty acids, are common energy sources.

Triglycerides are hydrolyzed into fatty acids and glycerol, thanks to ***lipases*** or less specific ***esterases***, often extracellular. These lipases are found in molds (*Aspergillus, Penicillium, Rhizopus, Geotrichum,* …), yeasts (*Candida, Torulopsis, Saccharomyces, Saccharomycopsis,* …) and bacteria (*Serratia, Pseudomonas, Xanthomonas, Chromobacterium, Alcaligenes, Staphylococcus,* …).

Glycerol is phosphorylated and oxidized into dihydroxyacetone-P and degraded in glycolysis. Fatty acids, on the other hand, are catabolized by a process (cycle) called **β-oxidation** (Fig. 01 and 02). They are first activated by ATP in the presence of coenzyme A to form an acyl-CoA, which is oxidized into β-keto-acyl-CoA. After hydrolysis, acetyl-CoA and an acyl-CoA with two fewer carbons are formed. The oxidation reactions continue as much as necessary depending on the length of the carbon chain.

The acetyl-CoA formed can be incorporated into the Krebs cycle and the glyoxylate shunt. The NADH and $FADH_2$ produced respectively can be oxidized by the electron transport chain to produce ATP. Fatty acids constitute a rich energy source for microbial growth.

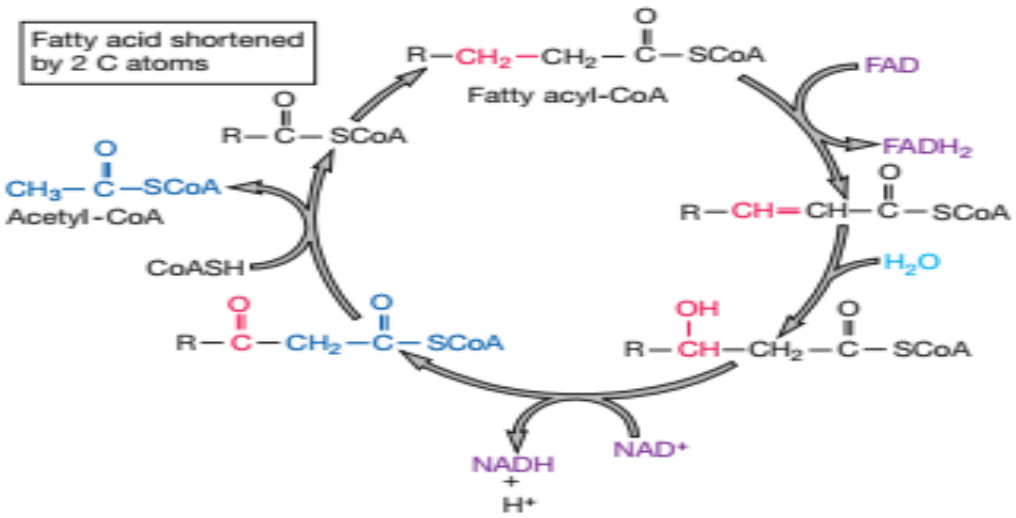


Figure 01: Fatty acid β-Oxidation.
[The portions of the fatty acid being modified are shown in red].

2. Protein catabolism

Proteins are high molecular weight organic compounds, composed of amino acids linked together by peptide bonds. Their degradation involves the following steps:

2.1. Proteolysis: Proteases and Peptidases

There are numerous microbial ***proteases*** (generally extracellular) more or less specific: collagenases, gelatinases, … They act on both proteins and oligopeptides. They split the protein molecule into polypeptide fragments, consisting of only a few amino acids. The best-known proteolytic species belong to bacterial genera *Clostridium, Bacillus, Proteus, Streptomyces, Pseudomonas, Aeromonas*, … as well as to many fungal genera.

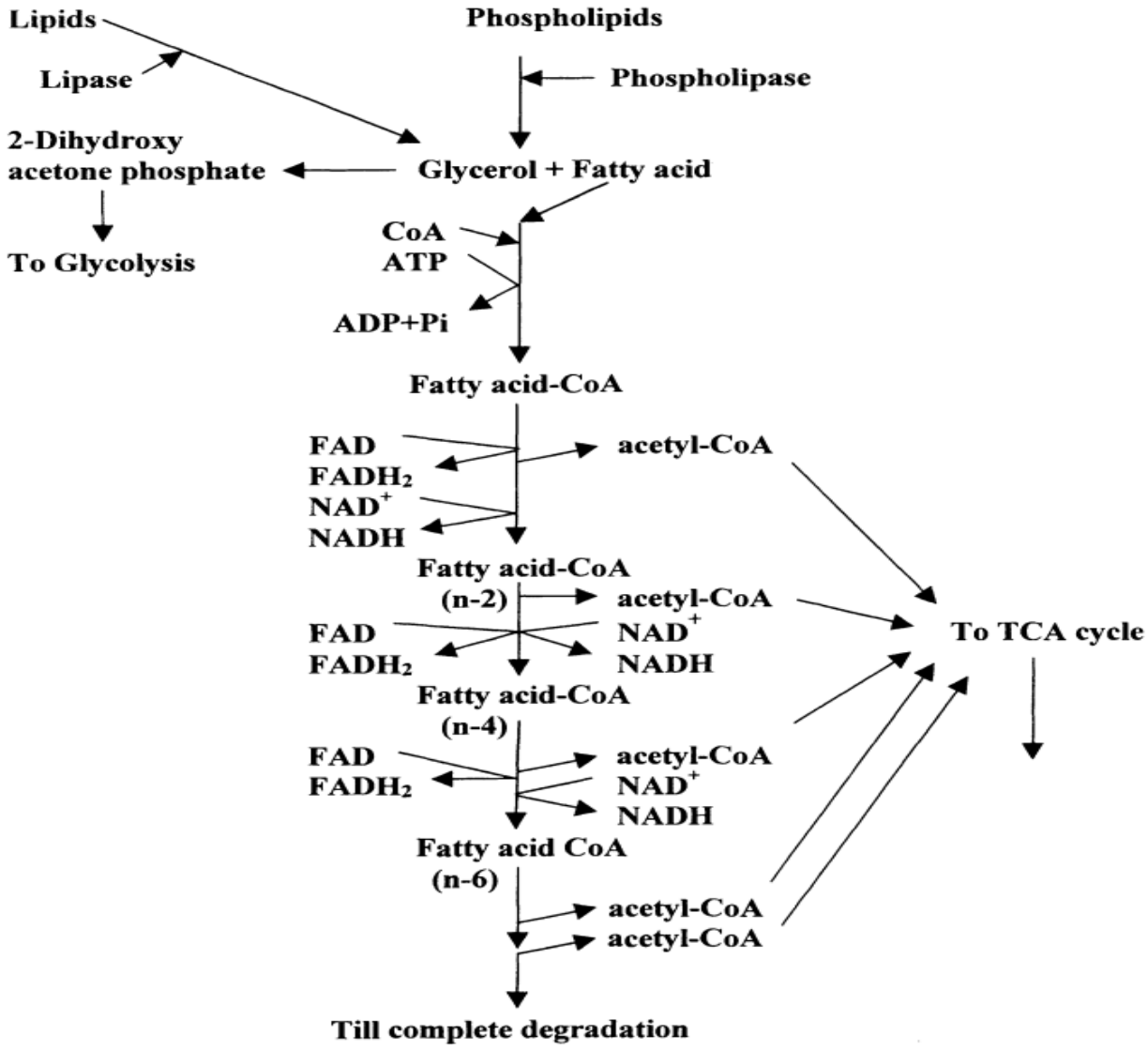


Figure 02: Simplified diagram of the utilization of lipids and phospholipids

Peptidases hydrolyze polypeptides and transform them into their constituent subunits, amino acids. Small polypeptides enter cells: in yeast, these are mainly di- and tripeptides. The entry of amino acids depends on the presence of numerous and varied '***permease***' systems.

Peptidases are of two types, ***endopeptidases*** and ***exopeptidases***, depending on their mode of attack on the polypeptide chain (Fig. 03). **Exopeptidases** are themselves subdivided into two categories:

- **Aminopeptidases** begin their action at the **free –NH_2 end** of the polypeptide and their activity often depends on the presence of metal ions.

- **Carboxypeptidases** begin their attack at the **free –COOH end** of the polypeptide.

The activity of these different enzymes leads to the release of di- and tripeptides which are then hydrolyzed into amino acids.

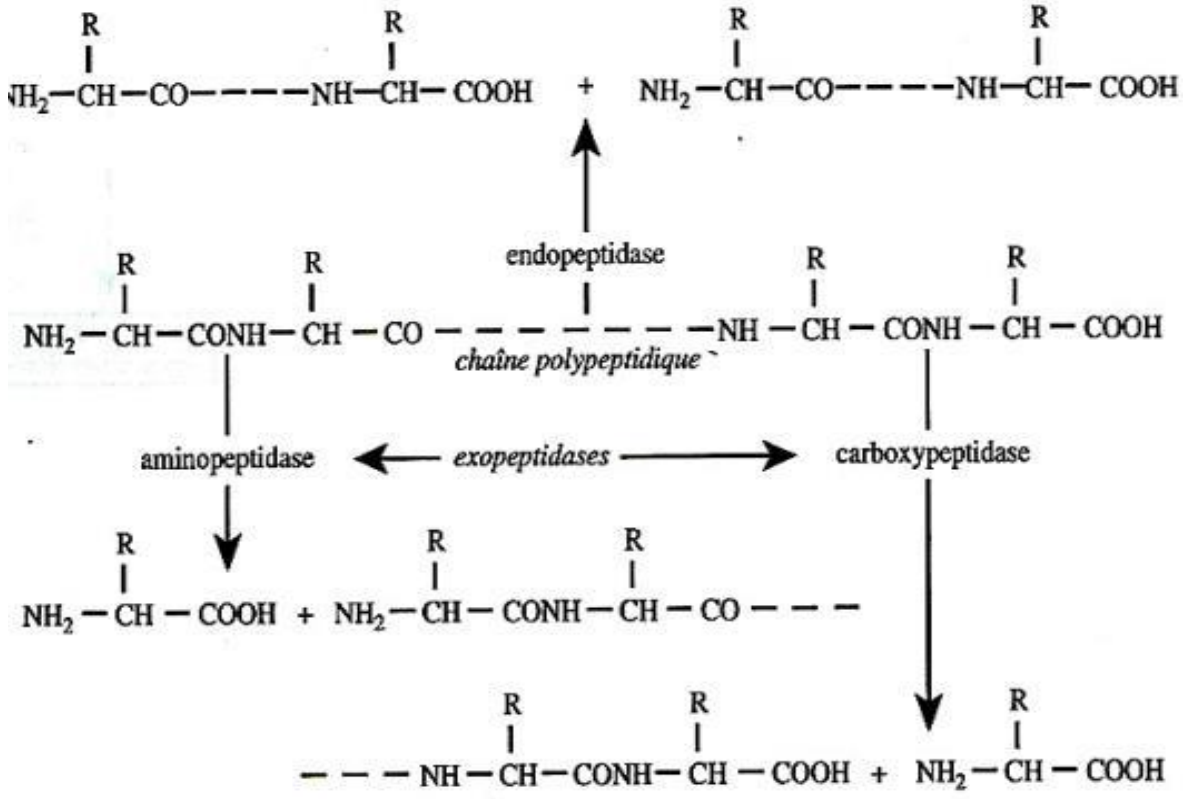


Figure 03: Mode of attack of the polypeptide chain.

2.2. Catabolism of released amino acids

There are two main pathways: **deamination** and **decarboxylation** (Fig. 04).

2.2.1. Deamination

a. Oxidative deamination leads to the formation of an **imino acid** (a molecule possessing both a functional group –COOH (carboxyl) and a functional group >C=N– (imine)) which is then hydrolyzed into **ammonia** and an α-keto acid: it involves flavin coenzymes (FAD).

$$R-CHNH_2-COOH + H_2O \rightarrow R-CO-COOH + NH_3 + 2e^- + 2H^+$$

b. Non-oxidative deamination which can be of three types:

- **Desaturating deamination** produces ammonia and an unsaturated acid (example: aspartate is transformed into fumarate).
- **Deamination by dehydration** is specific to hydroxylated amino acids (serine), it is exclusively microbial. There is formation of ammonia and a keto acid. The degradation of cysteine occurs by a similar reaction but there is release of H_2S (cysteine sulfhydrase).
- **Reductive deamination** consists of a reduction of the amino acid into the corresponding saturated acid, with formation of ammonia.

c. Coupled deamination (Stickland reaction) which is a coupled oxidation-reduction reaction between two amino acids, one playing the role of hydrogen acceptor, the other of donor:

$$R\text{-}CH(NH_2)\text{-}COOH + R'\text{-}CH(NH_2)\text{-}COOH + H_2O \longrightarrow R\text{-}CO\text{-}COOH + R'\text{-}CH_2\text{-}COOH + 2NH_3$$

It is carried out by a large number of strictly anaerobic spore-forming bacteria (*Clostridium*), it involves a coenzyme NAD. *Clostridium* that do not carry out this reaction degrade amino acids through a catalytic transamination process similar to that of higher animals.

The acids resulting from deamination enter carbohydrate metabolism pathways: pyruvate (alanine, glycine, serine, cysteine…), acetyl-CoA (leucine, isoleucine, lysine…), oxaloacetate (aspartate)…

2.2.2. Decarboxylation

Decarboxylases act on amino acids to form CO_2 and an amine:

$$R\text{-}CH(NH_2)\text{-}COOH \longrightarrow CO_2 + R\text{-}CH_2\text{-}NH_2$$

This reaction is carried out by a large number of proteolytic or non-proteolytic microorganisms. Amines are foul-smelling compounds, sometimes toxic (histamine).

The way an amino acid is degraded is partly controlled by the pH of the medium. An acidic medium favors the formation of decarboxylases whereas an alkaline medium stimulates that of deaminases.

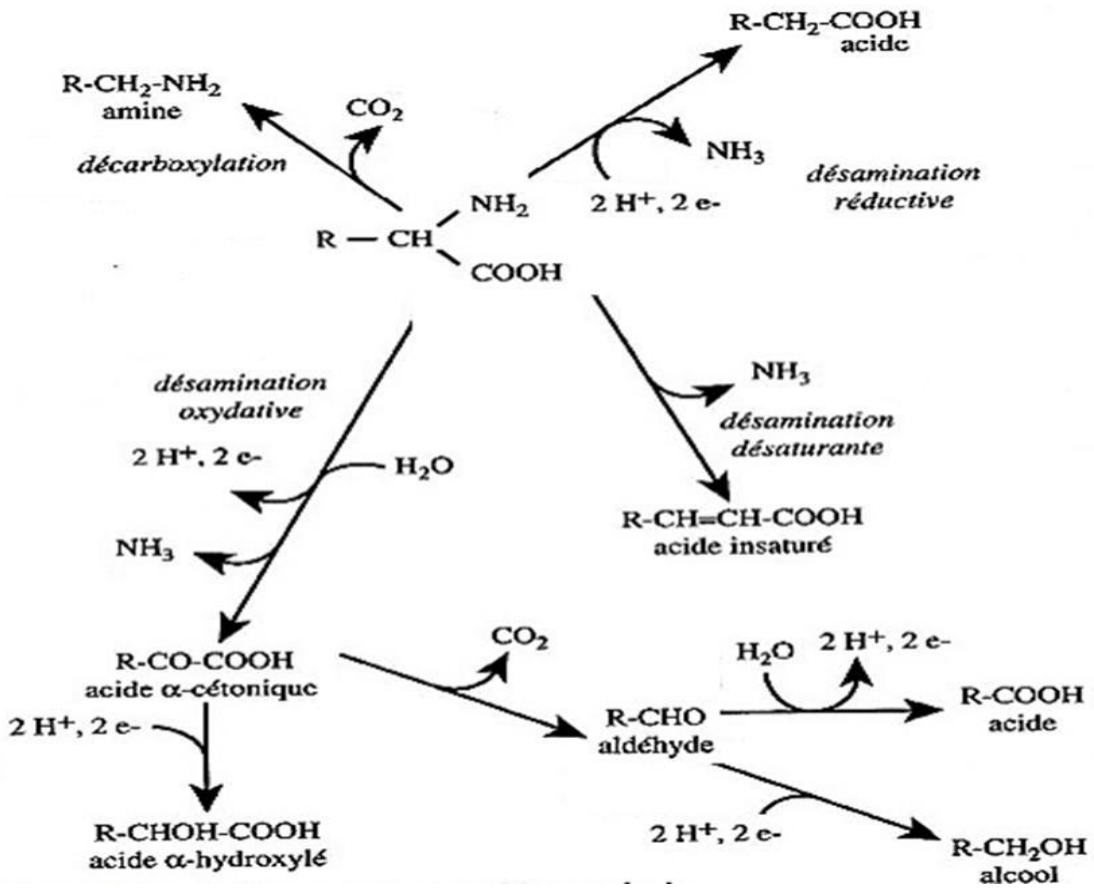


Figure 04: Amino acids degradation.

3. Catabolism of carbohydrates other than glucose

3.1. Pentoses

The degradation of pentoses has been well studied in Enterobacteria and Lactobacilli.

Whatever the pentose metabolized, its degradation leads to the formation of **D-xylulose-5P** (Fig. 06), which is then metabolized either by the ***Hexose Monophosphate Pathway*** (***Pentose cycle***) or by the pentose phosphate pathway (heterolactic bacteria pathway) with the involvement of phosphoketolase.

Depending on the starting pentose, isomerases, transketolases and transaldolases are involved before reaching xylulose-5P.

The assimilation of xylose in bacteria involves an isomerase:

Xylose →→→→ xylulose

Whereas in yeasts, there is an intermediate step:

Xylose →→→→ xylitol →→→→ xylulose

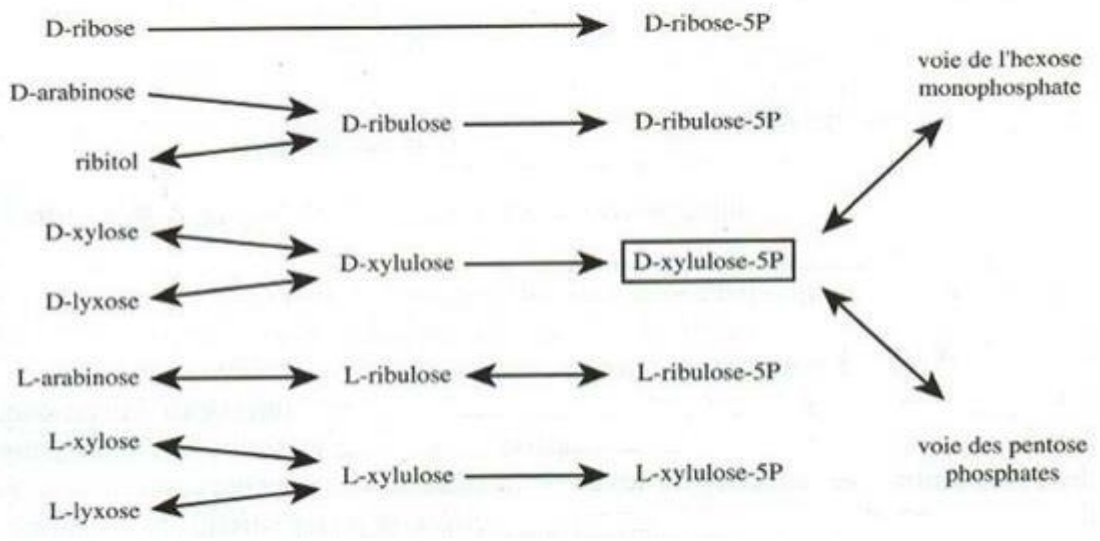


Figure 05: General scheme of pentose metabolism.

3.2. Fructose

Fructose can either be oxidized into 5-keto-D-fructose by D-fructose-NADP-5 oxidoreductase (*Acetobacter cerinus*, strictly aerobic bacterium), or phosphorylated into fructose-1P (*Escherichia coli*, *Zymomonas*, *Clostridium*) or more rarely into fructose-6P. The first phosphorylation is followed by a second which leads to fructose-1,6 diphosphate, which is then degraded via glycolysis.

3.3. Mannose

Mannose can be catabolized by two different mechanisms: a cyclic mechanism and a non-cyclic mechanism. Both mechanisms exist for the D isomer, whereas the L isomer seems to be catabolized only by the non-cyclic mechanism.

In the cyclic mechanism (*Aerobacter aerogenes*) (Fig. 06), D-mannose is phosphorylated into mannose-6P, which is then transformed into fructose-6P (then metabolized by glycolysis). The

phosphorylation of mannose occurs by transfer of phosphate from glucose-6P to mannose. Glucose-6P is then regenerated either by isomerization of mannose-6P into fructose-6P, or by direct phosphorylation of glucose, thanks to a ***glucokinase***.

The utilization of L-mannose involves the non-cyclic mechanism. L-mannose is first converted into L-fructose by an isomerase. There is then phosphorylation of fructose into fructose-1P, which is split into dihydroxyacetone phosphate and L-glyceraldehyde, whose metabolism proceeds via glycolysis.

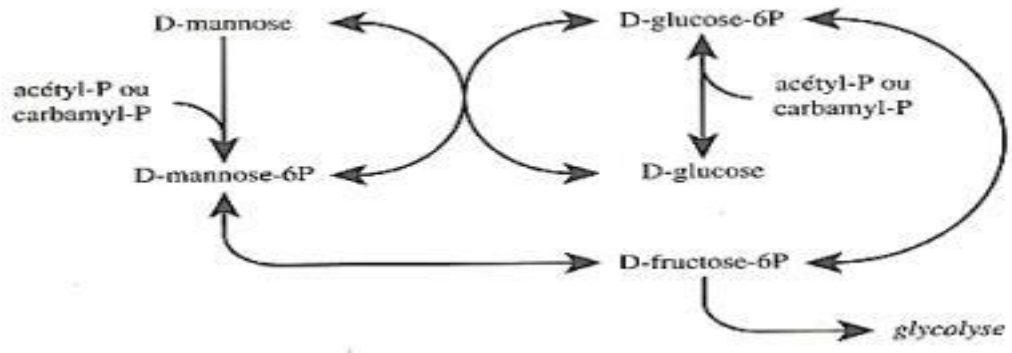


Figure 06: Cyclic Metabolism of Mannose

3.4. Sucrose

Sucrose is first hydrolyzed into **glucose and fructose** by ***invertase*** present in many yeasts (*Candida utilis, Saccharomyces cerevisiae*…), many molds (*Aspergillus niger, Penicillium chrysogenum*…) and many bacteria (*Clostridium pasteurianum, Streptococcus*…). Glucose and fructose are degraded by the pathways previously described.

Sucrose is hydrolyzed outside the cell in yeasts and molds. In many bacteria (lactic acid bacteria, *Bacillus subtilis*), sucrose is transported into the cell in the form of sucrose-P and then hydrolyzed into glucose-6P and fructose. In various bacteria (*Bacillus subtilis, Zymomonas*), there is also a levan sucrase that contributes to the synthesis of levans.

$$\text{Saccharose} + \text{levane(fructose)}_n \longrightarrow \text{glucose} + \text{levane(fructose)}_{n+1}$$

3.5. Lactose and Galactose

Many microorganisms possess a ***β-galactosidase***: yeasts (*Kluyveromyces, Candida*…), molds (*Aspergillus*…), bacteria (*E. coli, Lactobacillus, Bacillus*…).

After hydrolysis of **lactose**, the **glucose** formed is degraded by one of the previously described pathways. As for **galactose**, it is degraded, particularly in yeasts, by the ***Leloir–Kalckar pathway***. It is first phosphorylated and then transformed into glucose-1P, a metabolite directly usable by the cell after isomerization into glucose-6P. The isomerization reactions involve uridine diphosphoglucose (UDPG) and uridine diphosphogalactose (UDP-Gal).

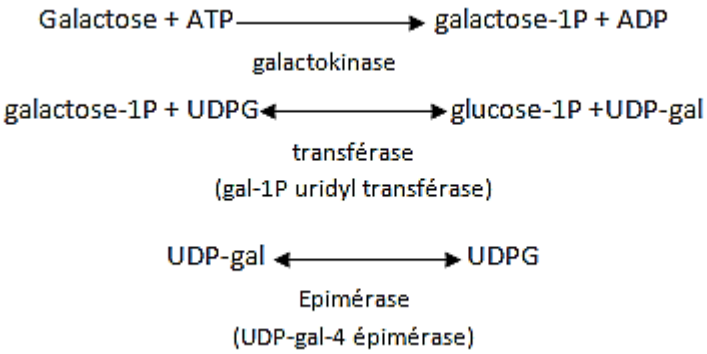
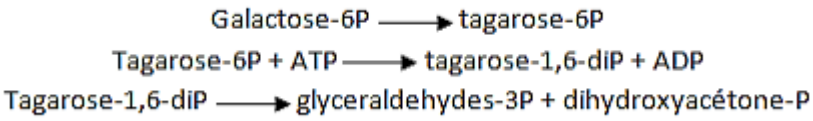


Figure 07: Galactose metabolism via the Leloir pathway.

In *Escherichia coli*, lactose metabolism depends on a specific permease and uses the Leloir pathway as in yeast.

In *Lactobacillus casei*, lactose is phosphorylated by a phosphotransferase system into **lactose-P**, which is split in the cell into **glucose and galactose-6P**; metabolism occurs via the ***tagatose pathway***.



The tagatose pathway is also used in *Staphylococcus aureus* for the metabolism of lactose and galactose.

3.6. Maltose

It is generally hydrolyzed into ***two molecules of glucose*** by a ***maltase*** (or ***glucoamylase***). In *E. coli*, it is metabolized with the involvement of a transglycosylation process.

$$\text{Maltose + maltodextrine (glucose)}_n \longrightarrow \text{glucose + maltodextrine (glucose)}_{n+1}$$

maltase

$$\text{maltodextrine (glucose)}_x + \text{ATP} \longrightarrow \text{maltodextrine(glucose)}_{x-1} + \text{ADP + glucose-1P}$$

phosphoamylase (ou maltodextrine phosphorylase)

$$\text{glucose-1P} \longrightarrow \text{glucose-6P}$$

phosphoglucomutase

4. Catabolism of monocarbon compounds: Ethanol and Glycerol

The catabolism of glycerol has been studied in Enterobacteria, lactobacilli, acetic bacteria and in *Clostridium butyricum*.

• **Glycerol** is degraded, particularly in acetic bacteria, by two pathways (Fig. 08). *Acetobacter suboxydans*, which does not possess a Krebs cycle, can nevertheless metabolize glycerol. This bacterium is used for the production of **dihydroxyacetone**, an intermediate in glycerol degradation.

Dihydroxyacetone is used as a tanning agent and in cosmetology. Enterobacteria catabolize glycerol by transforming it into **dihydroxyacetone** or **glyceraldehyde-3P**, which are then degraded via glycolysis. The process is exclusively fermentative.

The catabolism of glycerol in *Escherichia coli* involves a ***glycerol kinase*** which produces **α-glycerophosphate**, which is further transformed into dihydroxyacetone phosphate.

• **Ethanol** can be completely degraded into CO_2 and H_2O as in some yeasts (*Brettanomyces, Debaryomyces, Hansenula, Pichia*…), or it can be transformed into acetic acid (*Acetobacter, Gluconobacter*). In both cases, the first step leads to the formation of acetaldehyde.

$$CH_3\text{-}CH_2\text{-}OH + NAD^+ \longrightarrow CH_3\text{-}CHO + NADH,H^+$$

In the case of yeasts, acetaldehyde is incorporated into the Krebs cycle by oxidation into acetyl-CoA.

$$CH_3\text{-}CHO + CoA\text{-}SH + NAD^+ \longrightarrow CH_3\text{-}CO\text{-}SCoA + NADH,H^+$$

This degradation is aerobic. In the case of acetic bacteria, acetaldehyde is directly transformed into acetic acid.

$$CH_3\text{-}CHO + H_2O + NAD^+ \longrightarrow CH_3\text{-}COOH + NADH,H^+$$

This fermentation (basis of vinegar production) is aerobic. Some acetic bacteria can subsequently transform acetic acid into CO_2 and H_2O via acetyl-CoA.

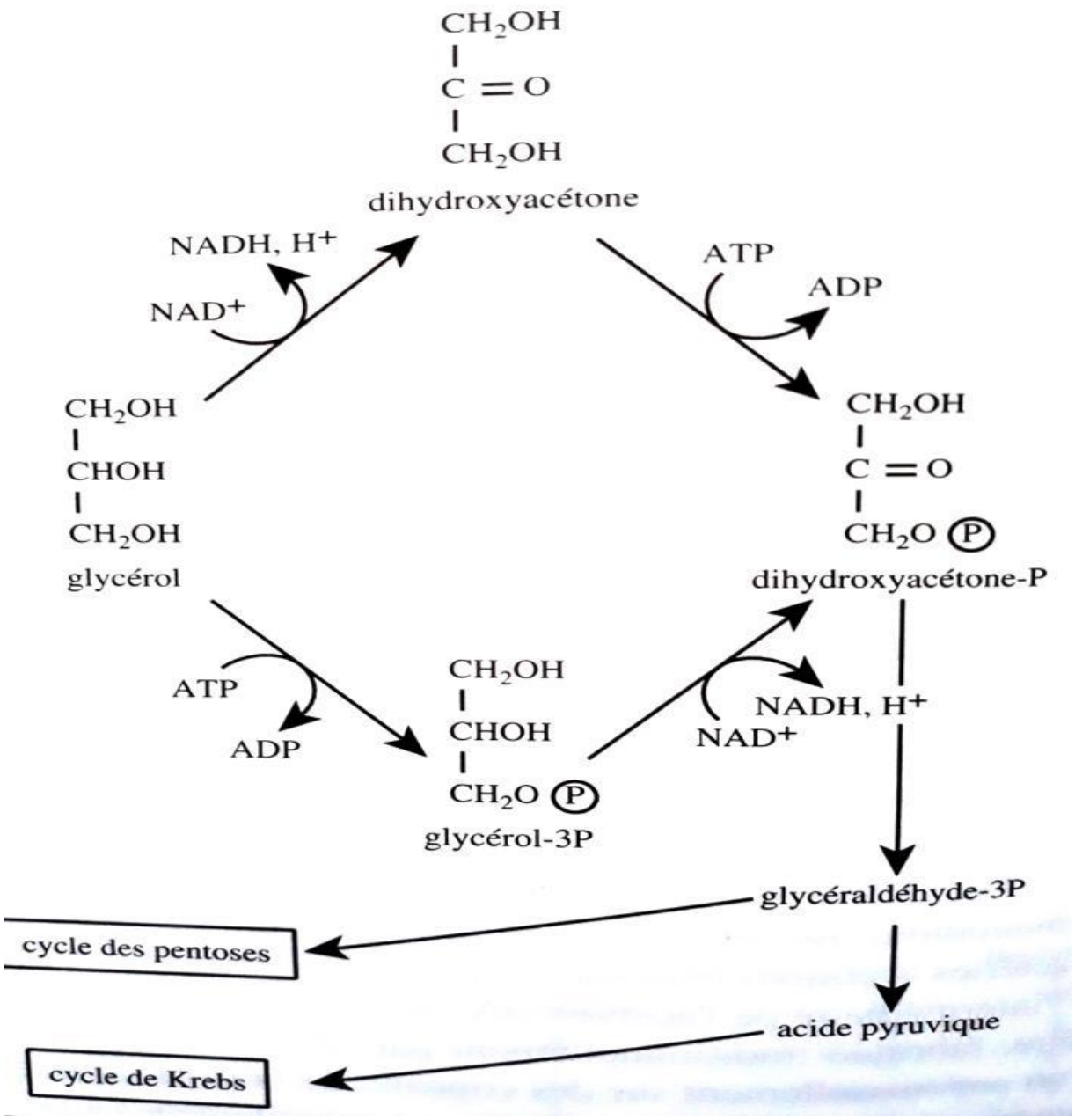


Figure 08: Glycerol catabolism.