Chapter IV: Catabolism of other organic compounds

Microorganisms are capable of using a wide variety of organic compounds beyond carbohydrates as sources of energy and carbon. These include lipids, proteins, and aromatic compounds, which are metabolized through specific enzymatic pathways that ultimately feed into central metabolic processes like glycolysis, the tricarboxylic acid (TCA) cycle, or the glyoxylate shunt.

1- Lipid catabolism

Lipids, primarily in the form of triglycerides and phospholipids, are a rich source of energy and carbon for microorganisms. The catabolism of lipids occurs in several coordinated steps, ultimately feeding into central metabolic pathways such as glycolysis and the TCA cycle (Parsons and Rock, 2013).

1-1- Hydrolysis of lipids

Triglycerides are hydrolyzed by lipases, releasing glycerol and fatty acids.

Phospholipids are degraded by phospholipases, generating glycerol, fatty acids, and phosphate-containing head groups.

These initial hydrolytic steps occur either extracellularly or in the cytoplasm depending on the microorganism.

1-2- Metabolism of glycerol

Glycerol is converted into dihydroxyacetone phosphate (dhap) via the sequential actions of glycerol kinase (phosphorylation to glycerol-3-phosphate) and glycerol-3-phosphate dehydrogenase (oxidation to dhap).

dhap enters glycolysis, contributing to the production of pyruvate, NADH, and ATP.

1-3- β-Oxidation of fatty acids

Fatty acids are activated to fatty acyl-CoA by acyl-CoA synthetase, consuming one ATP per fatty acid.

Activated fatty acids undergo β-oxidation, a cyclic process involving:

- **Dehydrogenation** by acyl-CoA dehydrogenase, producing $FADH_2$.

- **Hydration** of the double bond by enoyl-CoA hydratase.
- **Oxidation** of the hydroxyl group by β-hydroxyacyl-CoA dehydrogenase, producing NADH.
- **Thiolytic cleavage** by β-ketothiolase, releasing **acetyl-CoA** and a shortened acyl-CoA chain.

Each cycle shortens the fatty acid by two carbons, generating one acetyl-CoA, one NADH, and one $FADH_2$ (Parsons and Rock, 2013).

1-4- Entry into central metabolism

Acetyl-CoA produced by β-oxidation enters the TCA cycle, contributing to the production of NADH, $FADH_2$, and ATP via oxidative phosphorylation.

The electrons carried by NADH and $FADH_2$ are transferred to the electron transport chain, generating a proton gradient and synthesizing ATP.

1-5- Energy yield

Lipid catabolism is highly exergonic; the complete oxidation of fatty acids yields more ATP per carbon than carbohydrates.

The exact ATP yield depends on fatty acid chain length and saturation.

1-6- Special considerations

Some microorganisms can metabolize odd-chain fatty acids, producing propionyl-CoA alongside acetyl-CoA, which enters the TCA cycle via the methylmalonyl-CoA pathway.

Lipid catabolism is tightly regulated to balance energy production and biosynthesis. Intermediates from β-oxidation can be diverted for biosynthesis of membrane lipids or secondary metabolites (Parsons and Rock, 2013).

2- Protein catabolism

2-1- Proteolysis: proteases and peptidases

Microorganisms produce a wide variety of proteases, which are generally extracellular and exhibit varying substrate specificity. Examples include collagenases and gelatinases. These enzymes act on both intact proteins and oligopeptides, cleaving them into smaller polypeptide fragments composed of only a few amino acids (McQuillen, 1958).

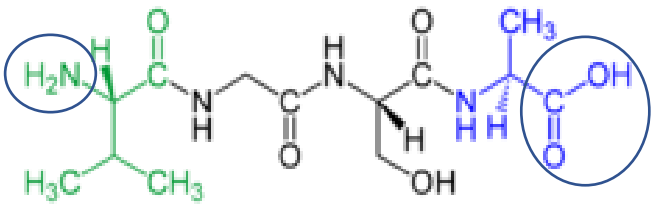
Prominent proteolytic microorganisms belong to bacterial genera such as Clostridium, Bacillus, Proteus, Streptomyces, Pseudomonas, Aeromonas, certain lactic acid bacteria, as well as numerous fungal genera.

Peptidases further hydrolyze polypeptides into their constituent amino acids, which can then be utilized by the cell. Small peptides, such as dipeptides and tripeptides in yeasts, can enter the cell directly. The uptake of free amino acids relies on diverse permease systems, which are abundant and highly specific, ensuring efficient transport into the cytoplasm.

Peptidases are classified into two main types based on their mode of action on the polypeptide chain:

- **Endopeptidases** – These enzymes cleave peptide bonds within the interior of the polypeptide chain.
- **Exopeptidases** – These enzymes act at the terminal ends of polypeptides and are further subdivided into two categories:
    - Aminopeptidases initiate hydrolysis at the free $–NH_2$ terminus of the polypeptide. Their activity often depends on the presence of metal ions.
    - Carboxypeptidases begin their action at the free –COOH terminus of the polypeptide.

The combined activity of these enzymes results in the release of dipeptides and tripeptides, which are subsequently hydrolyzed into individual amino acids for further metabolism (McQuillen, 1958).



2-2- Catabolism of free amino acids

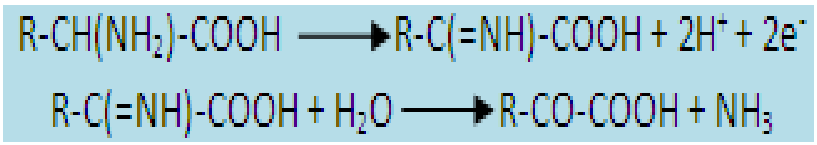
Two main pathways exist for amino acid catabolism: **deamination** and **decarboxylation** (Torres et al., 2023).

a. Deamination

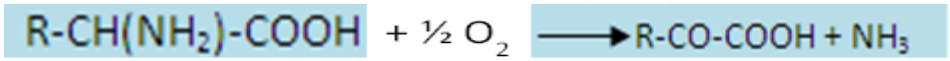
Amino acid deaminases in microorganisms can function as oxidative or non-oxidative enzymes.

✓ Oxidative deamination

This process leads to the transient formation of an amino acid intermediate, which is subsequently hydrolyzed into ammonia (NH_3) and an α-keto acid.



Oxidative deamination can involve a NADP-dependent dehydrogenase, which transfers electrons to the respiratory chain via a flavin coenzyme. The overall reaction can be summarized as follows (Torres et al., 2023).



✓ Non-oxidative deamination

Non-oxidative deamination can occur via several mechanisms:

- **Desaturative deamination**: Produces ammonia and a corresponding unsaturated acid. *Example*: Aspartate is converted into fumarate.

  $$R\text{-}CH{=}CH\text{-}COOH + NH_3$$

- **Reductive deamination**: Produces ammonia and a corresponding saturated acid.

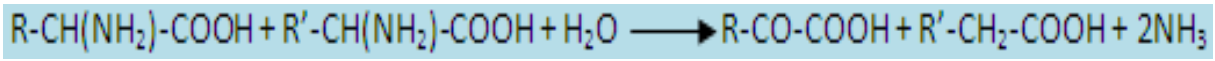
  $$R\text{-}CH_2\text{-}CH_2\text{-}COOH + NH_3$$

- **Dehydration-based deamination**: Specific to hydroxylated amino acids such as serine; this pathway is exclusively microbial. It results in the formation of ammonia and a keto acid.

  The degradation of cysteine proceeds via a similar reaction, but with the release of H_2S catalyzed by cysteine sulfhydrase.

- **Coupled deamination (Stickland reaction):** the Stickland reaction is a coupled oxidation-reduction process between two amino acids, in which one amino acid serves as a hydrogen acceptor. This reaction is carried out by many strictly anaerobic spore-

forming bacteria, particularly Clostridium species, and involves a NAD-dependent coenzyme (Torres et al., 2023).



b. Decarboxylation

Decarboxylases act on amino acids to produce carbon dioxide (CO_2) and an amine.

This reaction is carried out by a wide range of microorganisms, whether proteolytic or not, including Enterobacteriaceae and certain *Clostridium* species (Torres et al., 2023).

The amine formed can be further transformed into other biologically active compounds. The most important reactions include:

- Histidine → histamine
- Lysine → cadaverine
- Arginine → putrescine → spermidine → spermine
- Tyrosine → tyramine → dopamine → norepinephrine
- Tryptophan → tryptamine

The way a microorganism degrades amino acids is partially controlled by the pH of the environment:

- **Acidic conditions** promote the production of **decarboxylases**.
- **Alkaline conditions** promote the production of **deaminases**.

3- Urea catabolism

The enzymatic breakdown of urea is catalyzed by urease. Initially, the substrate and enzyme establish an equilibrium, forming an enzyme–substrate complex. In a subsequent step, this complex is rapidly converted into the degradation products (Hausinger, 2004).

$CO(NH_2)_2 + H_2O$ ---> $CO_2 + 2NH_3$

4- Ethanol catabolim

Ethanol can be completely oxidized to CO_2 and H_2O, as observed in certain yeasts, or it can be partially oxidized to acetic acid, as in *Acetobacter* and *Gluconobacter* species. In both cases, the first step involves the formation of acetaldehyde (Salaspuro, 1997).

5- Catabolism of other alcohols

The catabolism of glycerol has been studied in *Enterobacteriaceae, Lactobacillus* species, and *Clostridium butyricum*. Glycerol can be degraded via two metabolic pathways**.**

Acetobacter suboxydans, which lacks a complete Krebs cycle, can still metabolize glycerol. This bacterium is employed for the production of dihydroxyacetone, an intermediate in glycerol degradation, which is widely used in the cosmetics industry.

In Enterobacteriaceae, glycerol is catabolized either into dihydroxyacetone or glyceraldehyde-3-phosphate, which are then further metabolized through the glycolytic pathway. This process occurs exclusively via fermentation. In *Escherichia coli*, glycerol catabolism involves a glycerol kinase, which phosphorylates glycerol to produce α-glycerophosphate; this compound is subsequently converted into dihydroxyacetone phosphate**,** entering glycolysis (Gonçalves et al., 2019).

Yeasts, in contrast, are capable of respiring glycerol**.**

Mannitol is metabolized via two main pathways:

1. **Phosphorylation pathway**: Mannitol is first phosphorylated and subsequently converted into **fructose-6-phosphate**, which then enters the **glycolytic pathway**.
2. **Dehydrogenation pathway**: Mannitol is oxidized to **fructose-6-phosphate**, which is also degraded through glycolysis.

The catabolism of sorbitol is very similar to that of mannitol. In this case, D-sorbitol is oxidized to D-fructose, which subsequently enters glycolysis for further metabolism (Gonçalves et al., 2019).

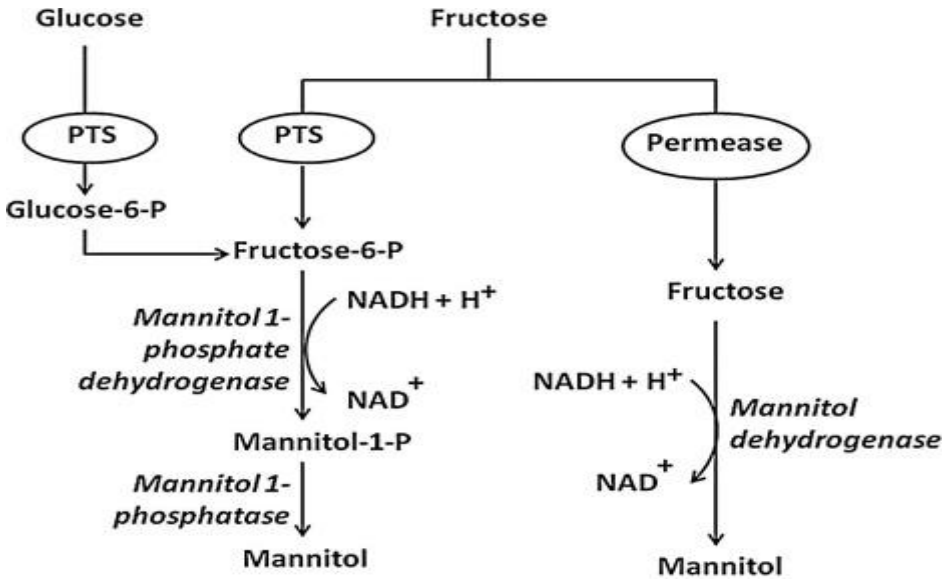


Figure 1. Mannitol production pathway in case of lactic acid bacteria (Gonçalves et al., 2019)

6- Specificity according to sugar type

Fructose and mannose are catabolized similarly to glucose, with mannose requiring the action of an isomerase.

Sucrose is degraded by invertase into glucose and fructose.

Lactose is hydrolyzed by β-galactosidase into glucose and galactose.

Galactose is often metabolized via the Leloir pathway, yielding glucose-1-phosphate.

Maltose can be degraded according to the following reaction (Ehrmann and Vogel, 1998):

$$\textbf{Maltose + dextran}_{(n-1)} \rightarrow \textbf{glucose + dextran}_{(n)}$$

Pentoses are typically catabolized through the pentose phosphate pathway (hexose monophosphate pathway), generally under aerobic conditions.

In bacteria, xylose is converted into xylulose by xylose isomerase, and subsequently phosphorylated to xylulose-5-phosphate.

In yeasts, there is no xylose isomerase; instead, xylose is first reduced to xylitol by xylose reductase, followed by oxidation of xylitol to xylulose via xylitol dehydrogenase.

Some microorganisms ferment pentoses into lactic acid (e.g., *Lactobacillus*), ethanol (certain yeasts), or other products (e.g., *Escherichia coli*).

6-1- Galactose metabolism (Leloir pathway)

In the Leloir pathway, galactose is first phosphorylated to galactose-1-phosphate by galactokinase. Galactose-1-phosphate then reacts with UDP-glucose via galactose-1-phosphate uridylyltransferase, producing UDP-galactose and glucose-1-phosphate.

UDP-galactose can be converted back to UDP-glucose by UDP-galactose 4-epimerase, completing the cycle. Glucose-1-phosphate can subsequently enter glycolysis after conversion to glucose-6-phosphate by phosphoglucomutase, linking galactose catabolism to central energy metabolism.

This pathway allows cells to efficiently utilize galactose as a carbon and energy source (Daenzer et al., 2012).

Phosphorylation of galactose to galactose-1-phosphate

Like other hexoses, galactose must be phosphorylated before it can be metabolized. Most organisms possess a specific enzyme for this purpose, galactokinase. Galactose is phosphorylated by galactokinase to form galactose-1-phosphate, with ATP serving as the phosphate donor.

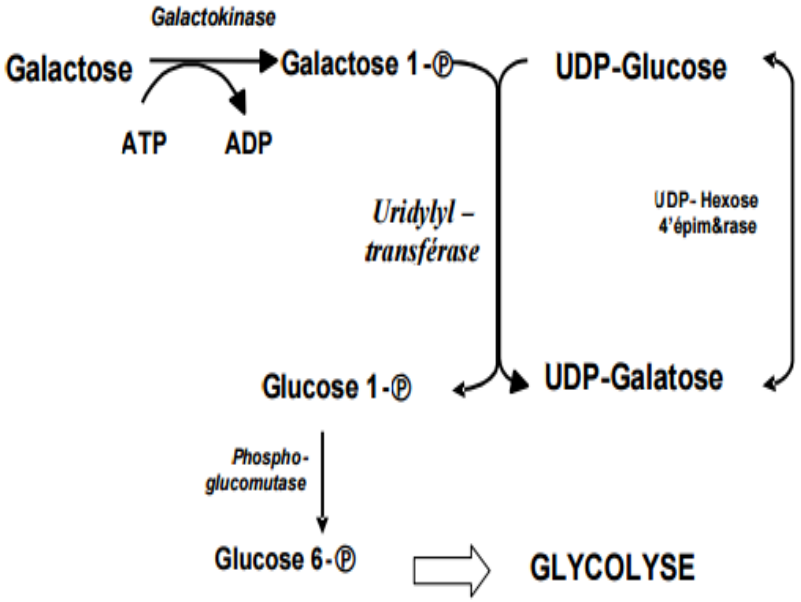


Figure 2. Galactose metabolism (Leloir pathway) (Daenzer et al., 2012)

Formation of UDP-galactose and conversion to UDP-glucose:

Galactose-1-phosphate cannot directly enter the glycolytic pathway. To do so, it must be converted into glucose-1-phosphate. This conversion occurs through a sequence of reactions

that generate an important intermediate, UDP-galactose. UDP-galactose is formed by the transfer of the UDP moiety from UDP-glucose to galactose-1-phosphate, releasing glucose-1-phosphate. This reaction is catalyzed by UDP-glucose: galactose-1-phosphate uridylyltransferase (Daenzer et al., 2012).

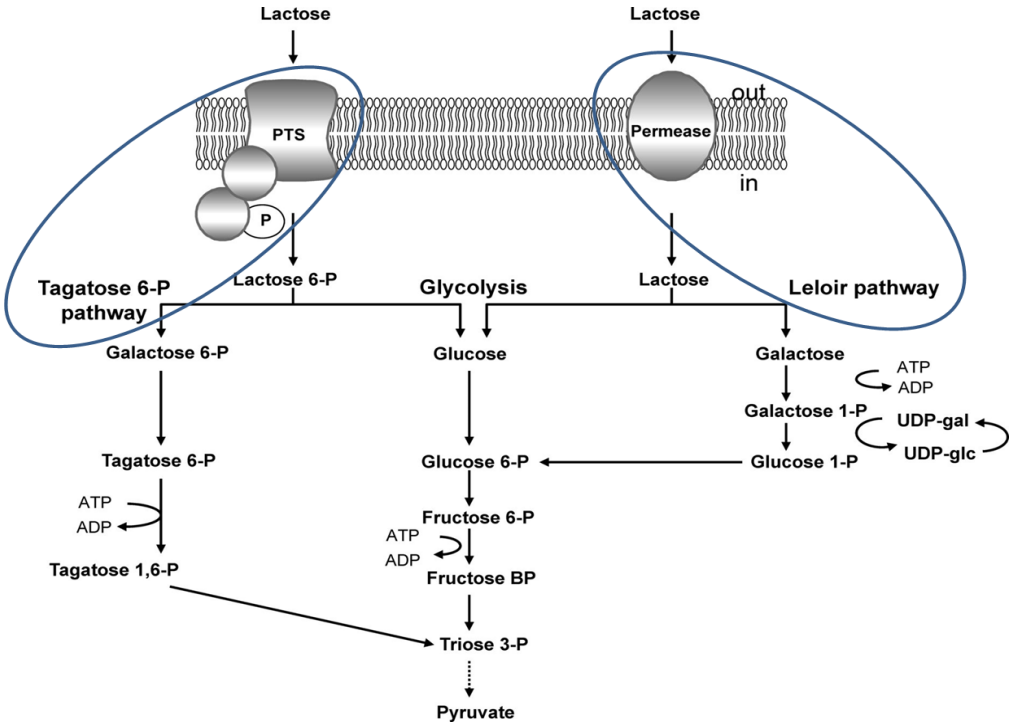


Figure 3. Galactose catabolism by both pathways, Leloir (right) and Tagatose (left) (Neves et al., 2010)

6-2- Citrate catabolism

Citrate can be metabolized through two distinct pathways**:**

1. **Oxidative pathway** – In this route, citrate is catabolized via the Krebs cycle**,** passing through oxaloacetate, which is subsequently decarboxylated to form pyruvate**.**
2. **Fermentative pathway** – This aerobic or anaerobic route begins with the cleavage of citrate into acetate and oxaloacetate. The oxaloacetate is then decarboxylated to yield pyruvate and CO_2**.** This pathway is commonly observed in lactic acid bacteria (Sánchez et al., 2008).

CITRATE CATABOLISM