Biological Waste Treatment Biological

Unit 1

Microbiological Fundamentals

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1 Microbiological fundamentals

1.1 Introduction

1.1.1 Life - Definition

Since the time of Virchows famous sentence 'Omnis cellula e cellula' (1885), which freely translated means that a cell can only be generated from another cell, it is obvious that the phenomenon of life are inseparably connected to cells. Cells are the basic unit for the most important characteristics of 'life' which consist, simply explained, of metabolism and reproduction.

To maintain their vital functions all living organisms have to undergo a continuous metabolic process using substrates. Substrates are the original material for metabolic transactions. The end of these metabolic transactions means death for the living organism. Metabolism can also be measured in seeds, even if those do not seem to take up nutrition. The metabolism of the microorganisms leads in technical systems, e.g. composting plants or sewage plants, to a degradation or conversion of substrates (see **metabolism**).

Viruses take an exceptional position, as they possess only the information for certain things, but - similar to computers - need living cells ('hardware') for the transformation (e.g. reproduction).

The reproduction of living organisms enables on the one hand the evolutionary process, i.e. the adaptation of the species to the living conditions. Microorganisms which essentially exist of one cell react quickly to favourable living conditions by reproduction through cell division. The enormous rates of division enable bacteria to populate a substrate, like e.g. biowaste, very quickly and transform it into compost through their metabolic activity.

1.1.2 Three areas of biology

A great systematic difference exists between bacteria on the one hand and animals, plants and fungi on the other hand:

Bacteria are prokaryotes, which contrary to the eukaryotes (animals, plants and fungi (see *Chapter 1.3.2 Structure of the eukaryotic cells*).

The cells of the prokaryotes are built up in an essentially simpler way than those of the eukaryotes (see *Chapter 1.3.1 Prokaryotic cells - bacteria*)

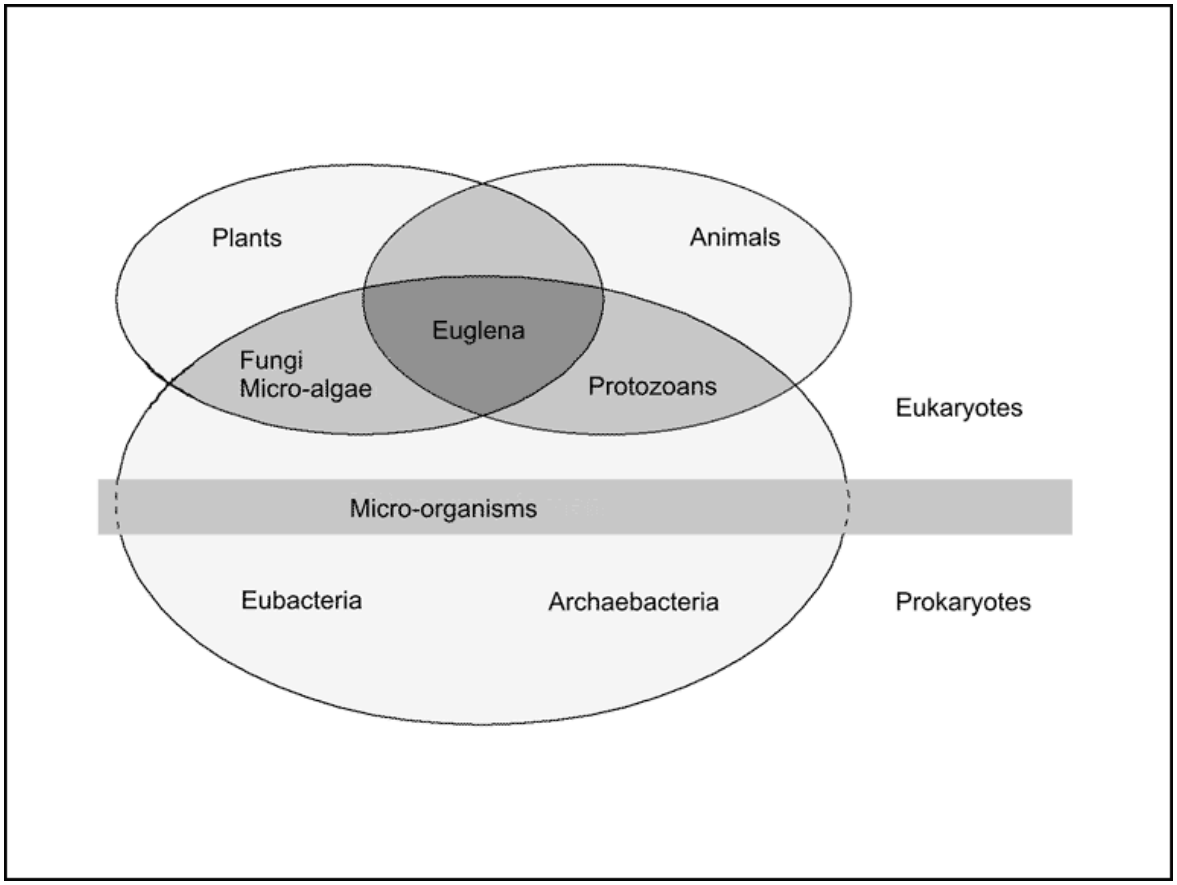

Fig. 0.1: Three areas of biology

As it can be seen in *Figure 1.1* the three areas plants, animals and bacteria are over-lapping. Furthermore bacteria are classified in eu- and archaebacteria.

The archaebacteria existed already in pre-historic times on the earth when there was nearly no oxygen in the atmosphere. Today this group populates special locations, which distinguish themselves by e.g. extremely high temperatures, low pH-values and the lack of oxygen (see also *Chapter 1.2.2 Position of the microorganisms in the animated nature*).

The methanogenic bacteria, which transform organic substrates like biowaste or surplus sludge in biogas plants or fermentation towers into biogas, belong to this group. On account of these reasons the methanogenic bacteria are also sensitive towards oxygen (see *1.3.2 Structure of the eukaryotic cells*)

1.2 Importance of microbiology

1.2.1 General properties of microorganisms

The collective characterisation of the microorganisms is their small size. The term micro-organism is not a systematic term in this context. Microorganisms are:

- bacteria,
- animals (e.g. unicellular paramecium),
- plants (e.g. unicellular green algae) and
- fungi (e.g. yeast)

The term micro-organism stands for a collective term which means unicellular eukaryotic and prokaryotic organisms (*Figure 1.1*, *Chapter 1.3.1 Prokaryotic cells bacteria*, *Chapter 1.3.2 Structure of the eukaryotic cells*).

The major efficiency in the range of the environmental technology is made by **bacteria**. Bacteria have a size about 1 mm, most of the other microorganisms are under 10 mm Compared with their surface is the volume of these small organisms extraordinarily high. If one would divide a cube with an edge length of 1 cm in the cubes of the size of a bacteria, one would receive 10^{12} equally sized cubes ($10^4 \times 10^4 \times 10^4 = 10^{12}$). The surface of the sum of the 10^{12} cubes is with 6×10^{12} mm^2 ($10^{12} \times 6$ mm^2 = 6×10^{12} mm^2) about 10000 times higher than the one of the compact cube, which has a surface of 6×10^8 mm.

The large surface-/volume ratio of microorganisms is responsible for the high metabolic activity of many microorganisms as the metabolism is not proportional to the mass but to the surface. Correspondingly high are the growth rates of the microorganisms, as shown in *Figure 1.2*, where the efficiency of 500 kg beef and 500 kg yeast cells are compared.

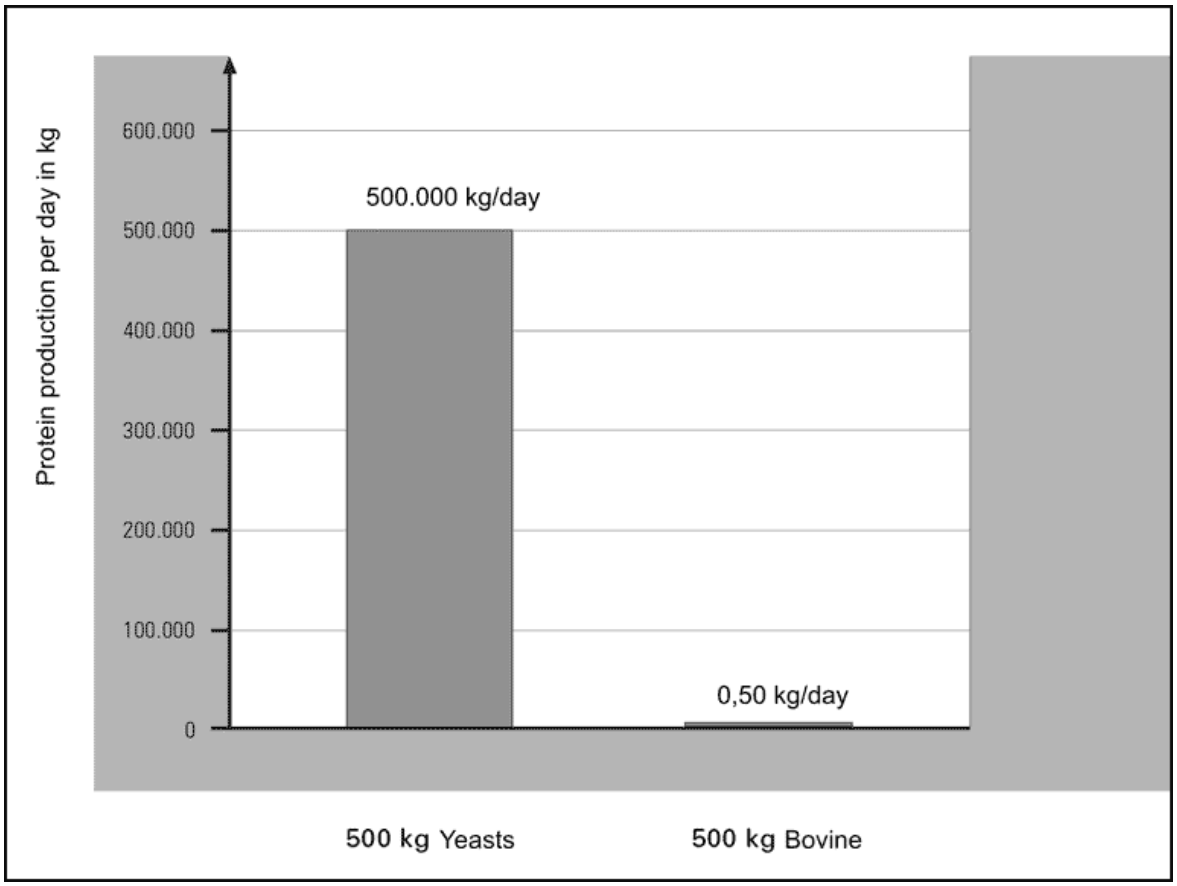

Fig. 0.2: Comparison of the growth rates of a culture from yeast cells with those of beef

1.2.2 Position of the microorganisms in the animated nature

Microorganisms are omnipresent. They can be found in the regions of the poles, the depth of the sea and in high atmospheric layers. They populate locations with extreme temperatures, pH-, salt- and pressure-amplitude. There are bacteria which exist at pH 1 and temperatures around 100 °C.

According to their role in the global ecosystem plants are allocated to the producers, animals to consumers and microorganisms to decomposers:

- producers: plants synthesize organic substance with sunlight and carbon dioxide,
- consumers: animals use this substance for their energy budget and material balance, and
- decomposers: microorganisms decompose the residues of both groups (mineralisation).

Source products (basically **carbon dioxide**) exist through mineralisation, which enter the loop again. If there were no decomposers the dioxide content of the atmosphere would be used up within some 40 years, the whole plant production would be disrupted. *Figure 1.3* shows the global loop of carbon dioxide.

Carbon loop

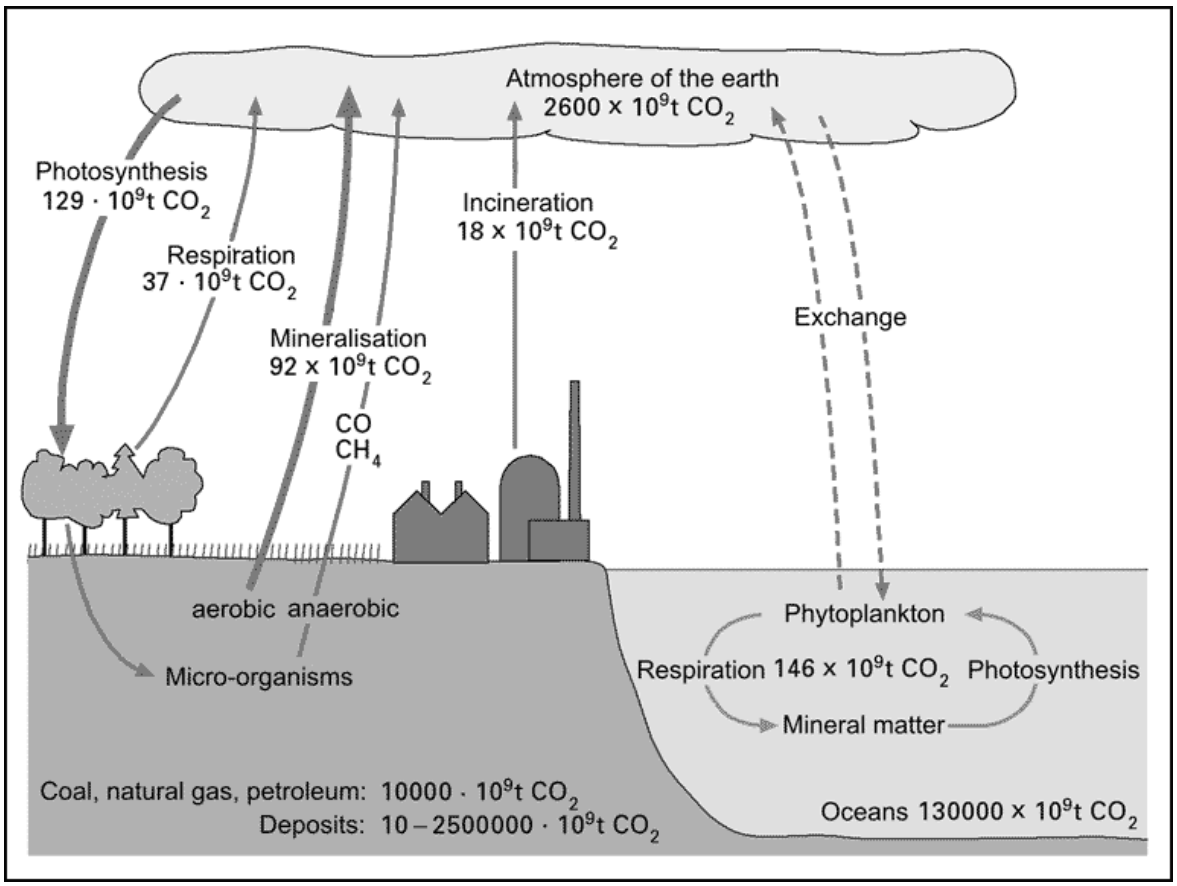

Fig. 0.3: Global carbon loop [7]

Besides the carbon loop the **nitrogen** loop is important. In the centre of this loop is **ammonium** the degradation product of proteins and amino acid. Proteins, as components of animals and plants, which are released during their degradation, will be decomposed to amino acids which represent the component of the proteins. Amino acids deliver the original substrate for a degradation chain, at the end of which is ammonium.

Ammonium oxidizes to nitrate by a specific bacteria group, the **nitrificants**, with the presence of atmospheric oxygen (nitrification).

Many bacteria can use bound oxygen in the nitrate in order to decompose carbon (**denitrification**). Atmospheric nitrogen is released by denitrification. Atmospheric nitrogen can be fixed with certain bacteria and thus contributes in a direct or indirect way to the fertilisation of plants. *Figure 1.4* shows the loop of nitrogen.

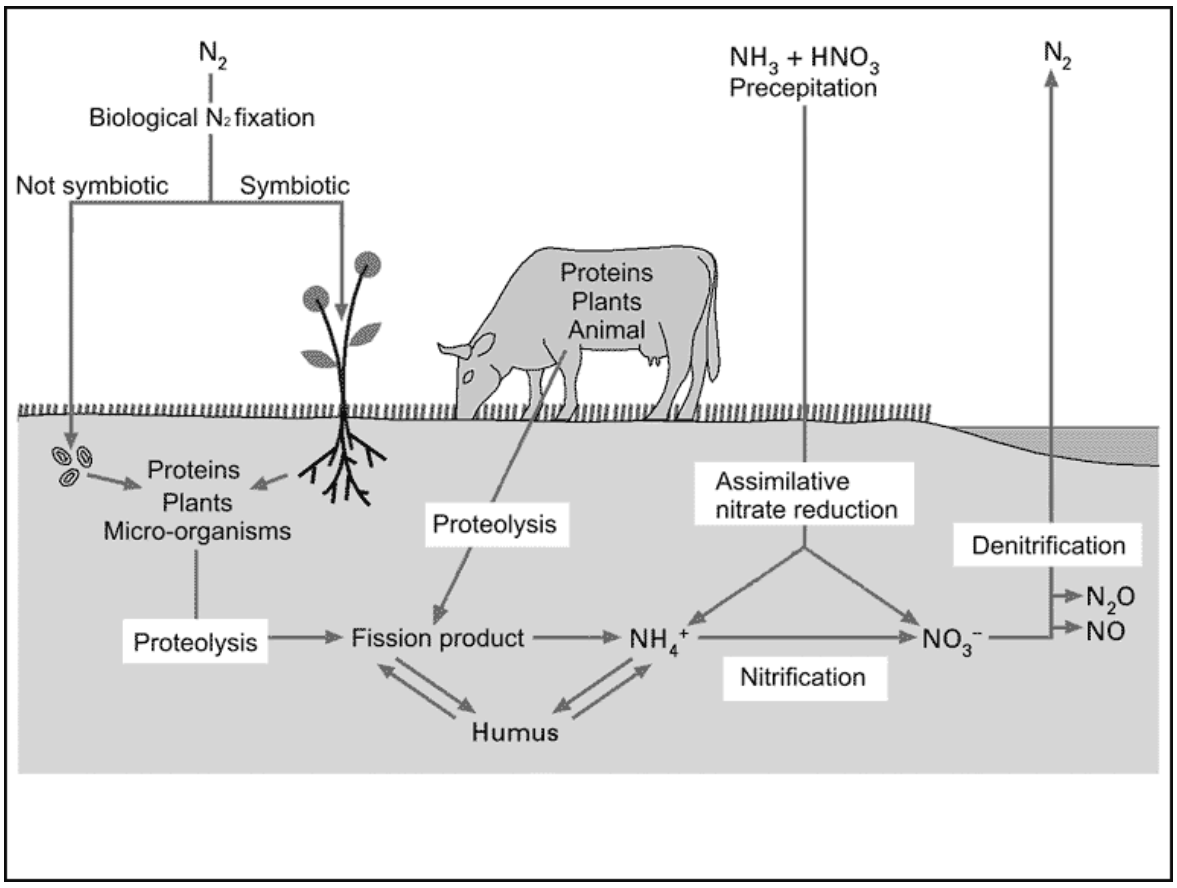

Fig. 0.4: Global nitrogen loop [7]

1.2.3 Importance of microbiology for humans

The importance of microorganisms can often not be registered before they damaged humans, animals and plants. This impression is wrong, as since a long time microorganisms have gained an important position in households, trade and industry where their metabolism activity is indispensable.

The example of beer and wine processing through yeasts, bread baking and the processing of milk products through lactic acid bacteria or the processing of vinegar by means of ethanoic acid proves that microorganisms belong to the oldest organisms domesticated by humans.

Beer has been brewed in Mesopotamia - the Iraq of today - already before 6.000 to 8.000 years, beer bread was then baked from germinated grains, the malt. Referring to this process the beer is known under the term 'liquid bread'.

On account of environmental and genetic engineering more and more new products are processed microbiologically. Since centuries the biological treatment of wastes is known and used, especially in India and China. *Table 1.1* shows some important areas where the technology of microbiology is used.

The advantage of microbial transformation processes contrary to chemical transformation processes lies in the high specificity and exploitation. Many materials like cellulose, lignin but also mineral oil and natural gas can only be decomposed and transformed by microorganisms whereby new and more 'precious' products arise.

Tab. 0.1: Range of application of microbiological technology

Application range	Product
Food production	Wine, beer, pasta, milk products, vine gar, citric acid, sugar
Pharmaceutical industry	Antibiotic agents, enzymes, antibodies
Environmental technology	Waste treatment, water preparation, waste water treatment, soil remediation
Agriculture	Silage

1.3 Structure and compound of cells and bacteria

1.3.1 Prokaryotic cells - bacteria

1.3.1.1 Structure prokaryotic cells

Mostly are bacteria cells rod shaped with a length of 1 mm. The cell content is scarcely differentiated, no cell organelle like mitochondrion or chloroplast exist.

However regions do exist in the cells, which contain e.g. the bacteria chromosome. The bacteria membrane is often turned into the cell interior in order to make surfaces available for certain metabolic processes. The bacteria become movable through flagellates. *Figure 1.5* shows a cross section of the bacteria cell. In the nucleoid is the genetic substance.

Polyphosphate granulate, polyhydroxy butyric acid and glycogenic particle serve *Energy storage* for storage.

- anaerobically living bacteria (without oxygen) store glycogen.
- aerobic bacteria store mostly polyhydroxy butyric acid.
- phosphate granulate can be found in nearly all bacteria species as phosphate and energy storage (compare *Chapter 1.5.1.1 ATP - Energy storage*)

Tylakoides enable a bacterial photosynthesis. The mesosomes enlarge the inner cell surface and serve certain metabolic processes.

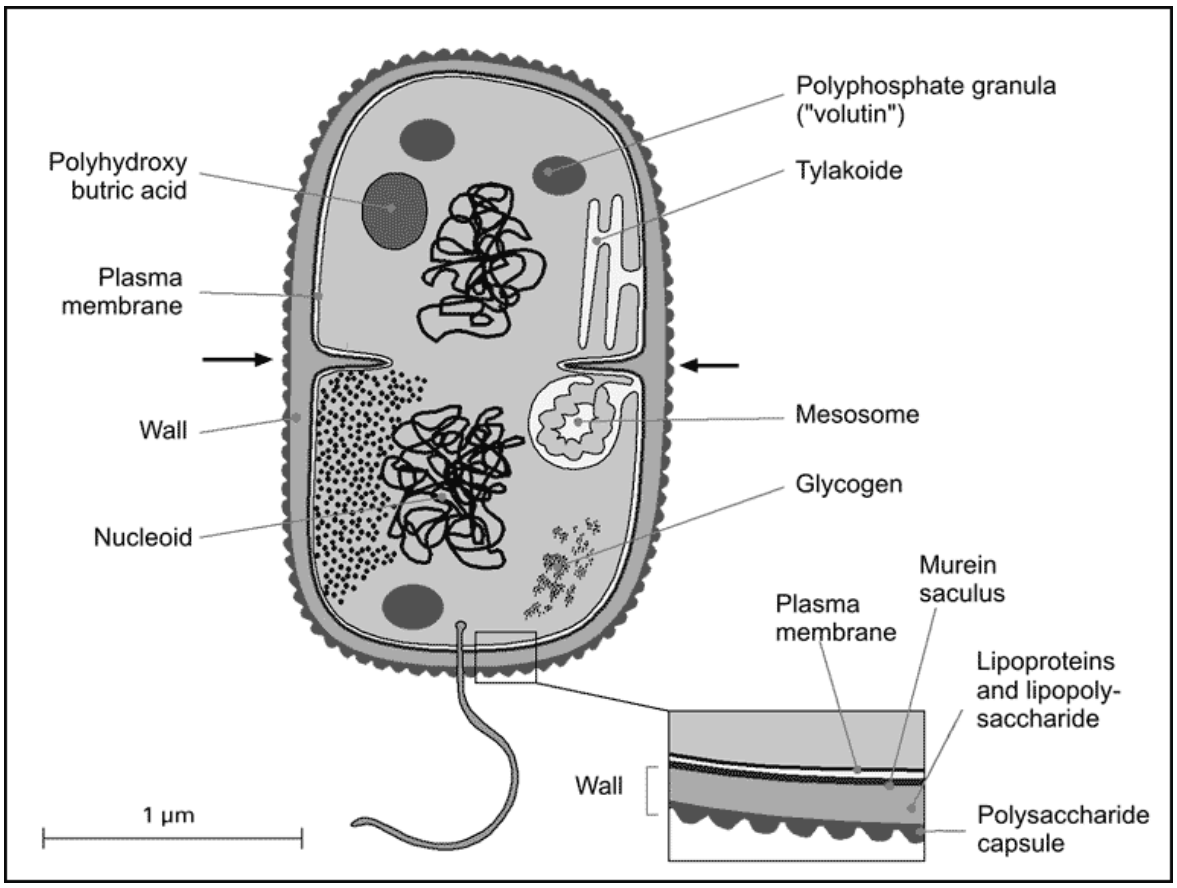

Fig. 0.5: Cross section through a bacteria cell [2]

Referring to their shape nearly all bacteria can be derived from the ball, the cylinder *Shapes* and the curved cylinder. *Figure 1.6* shows the most important shapes of bacteria.

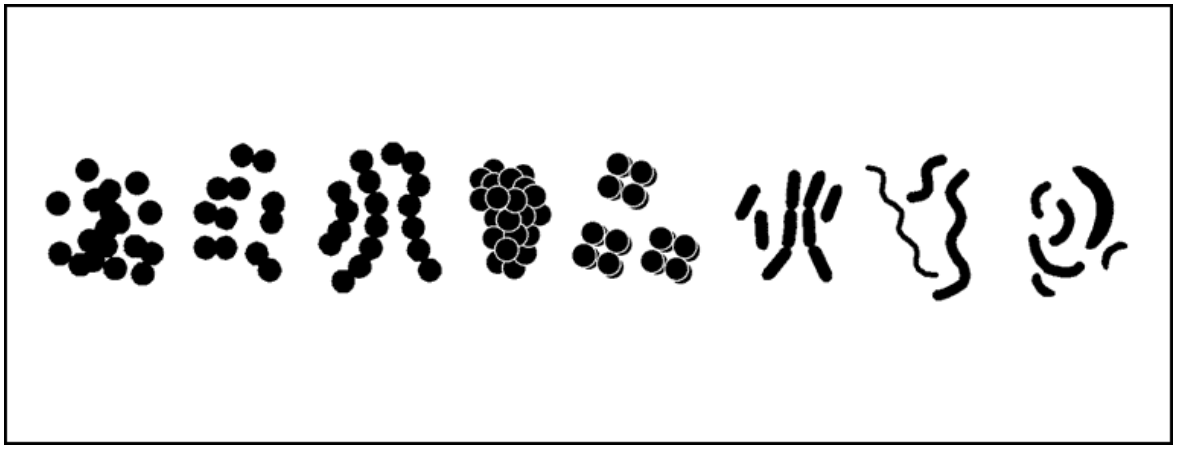

Fig. 0.6: Typical shapes of bacteria cells: micrococci, diplococci, streptococci, staphylococci, sarcinum, rod bacteria, spirilla, vibrio (from l. to r.) [7]

During cell division a septum is built on the cell walls, which divides the individual cell in the middle.

Contrary to eukaryotic cells (*Chapter 1.3.2 Structure of the eukaryotic cells*) cells of bacteria can only take up dissolved material. This is one of the reasons why, e.g., a sufficient water content must be available in compost piles, so that the material to be decomposed can be dissolved and via certain transport mechanisms absorbed by the bacteria.

1.3.1.2 Material structure of bacteria

The water content of the fresh cells lies between 70 and 85 %. If a high amount of reserve material exists the water content might be lower. The water content in the bacteria with permanent shapes, the spores, is distinctly lower. *Figure 1.7* shows the material structure of the dry matter of a bacteria cell.

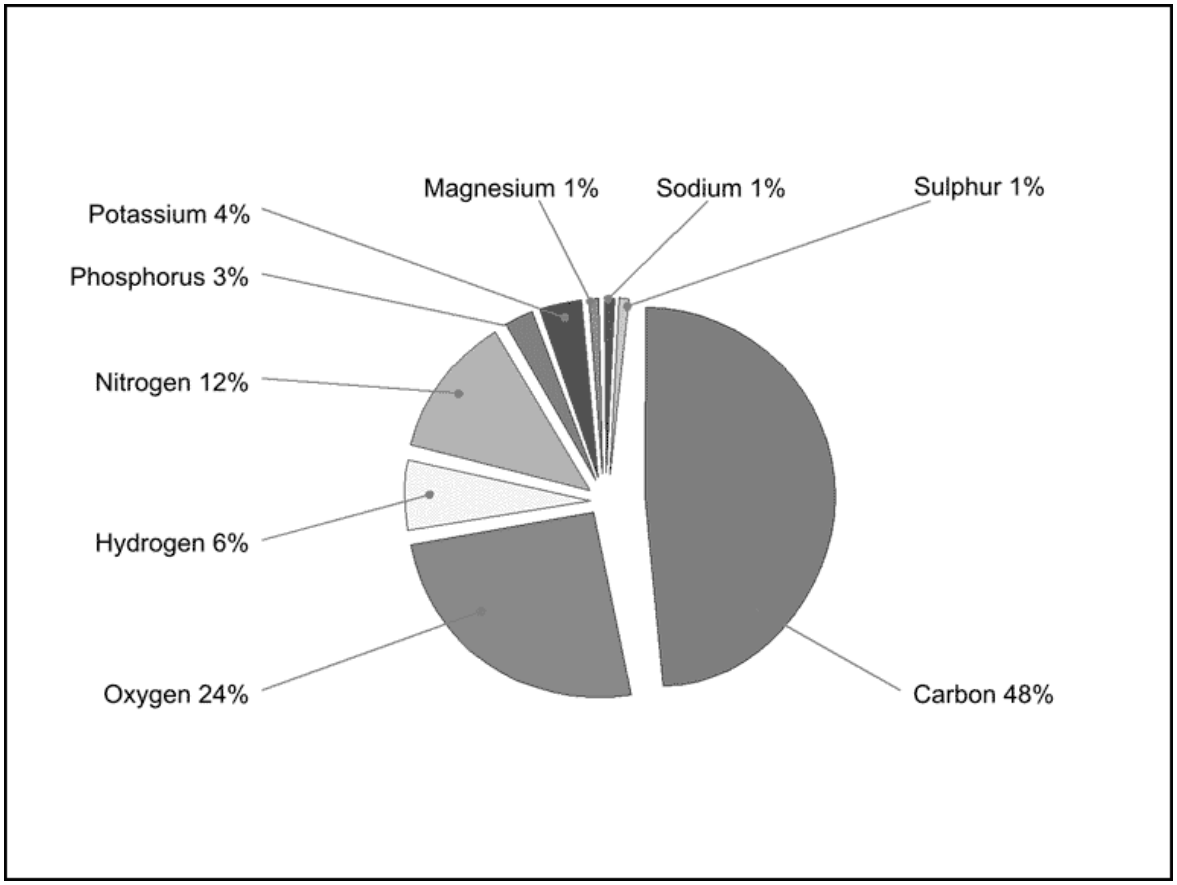

Fig. 0.7: Structure of the dry matter of a bacteria cell

In practical operation of biological waste treatment and waste water treatment the ratio of carbon/nitrogen/phosphorus in the source substrates plays an especial role.

- This ratio is 20 : 5 : 1 with biowaste and
- 100 : 5 : 1 with waste water

Furthermore bacteria need a multitude of elements which are usually sufficiently available in the wastes or waste waters to be treated.

1.3.2 Structure of the eukaryotic cells

Eukaryotic cells are essentially stronger differentiated through cell organelles like mitochondria or chloroplasts as prokaryotic cells. Reaction spaces are created through the cell organelles which maintain certain functions like energy supply or photosynthesis. Besides chloroplasts and mitochondria exist numerous other reaction spaces, (compartments) which are separated by membranes which divide the cell. Hereby are the compartments not rigid, but adjust themselves to the given conditions. *Figure 1.8* shows the cross section of a eukaryotic cell (explanations in *Table 1.2*).

Characteristic for a eukaryotic cell is the area named 'a'. Solid particles are picked up here by the cell. In the areas 'c' and 'd' material is released.

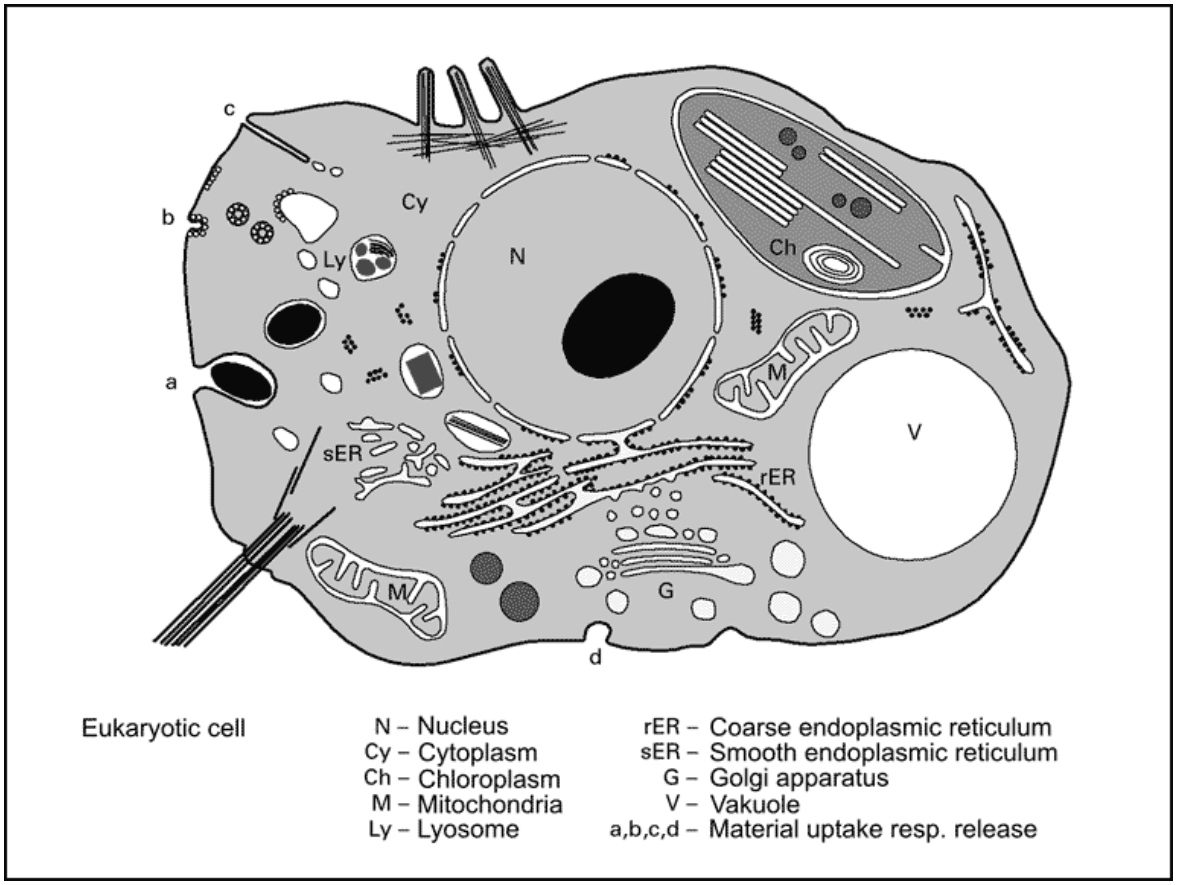

Fig. 0.8: Cross section of eukaryotic cells [4]

Tab. 0.2: Compartments of eukaryotic cells

Code	Compartment	Definition
N	Nucleus	Storage and reproduction of the hereditary property
Cy	Cytoplasm	Basic substance, location of the general metabolism
Ch	Chloroplasts	Photosynthesis
M	Mitochondria	Energy generation by respiration
rER	Coarse endoplasmic reticulum	Synthesis of lipids
sER	Smooth endoplasmic reticulum	Synthesis of lipids
G	Golgi-Apparatus	Sugar synthesis and transport

V	Vacuole	Storage and degradation of material (just plants)
Ly	Lysosomes	Degradation processes

1.4 Growth and metabolism of microorganisms

1.4.1 Growth phases

1.4.1.1 Heterotrophic metabolism

Microorganisms use the substrates for energy generation and for reproduction, i.e. for the composition of **biomass**.

- During the heterotrophic metabolism the carbon of the substrates is released in the first approximation by 50 % as CO_2 and is by 50 % built in the bacterial biomass.
- Furthermore H_2O and NH_3 (protein degradation) arises.

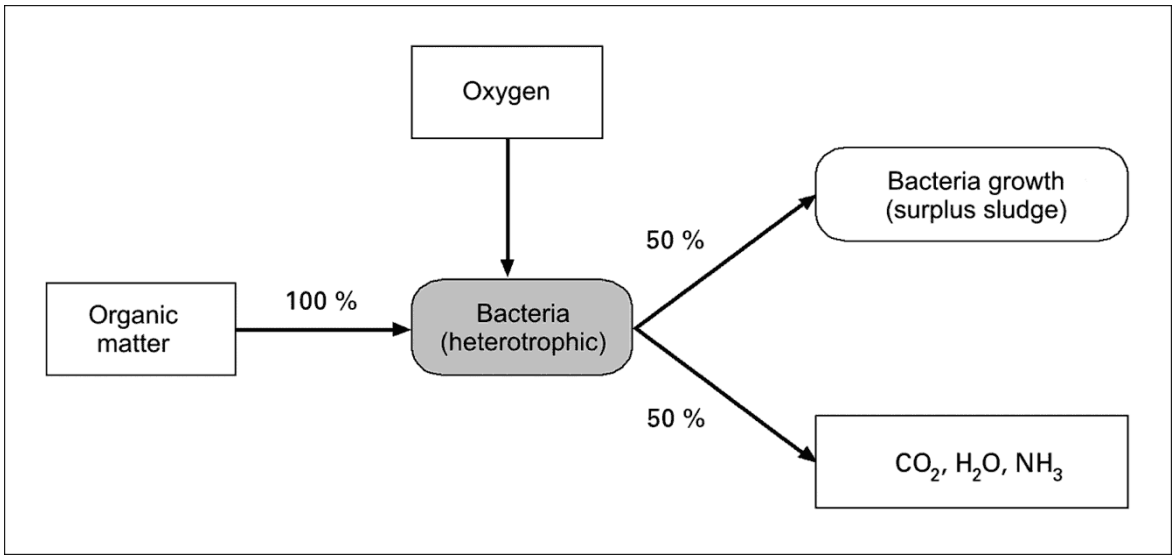

Fig. 0.9: Basic diagram of the heterotropic metabolism

1.4.1.2 Growth curve and growth phases

The reproduction of microorganisms corresponds to an exponential growth, whereby the period between two divisions lies between half an hour and several hours.

Formula 0.1: $N_t = N_0 \cdot 2^n$

N_t Cell number at the time t [-]

N_0 Cell number at the time t=0 [-]

n number of partitions [-]

Following from the rapid growth microorganisms can populate and decompose the substrate quickly. If e.g. bacteria are induced to grow in a closed vessel with a nutrient

solution, the cells divide themselves as far as the lack of at least one factor limits the growth. This static culture (batch culture) results in a characteristic growth curve which shows *Figure 1.10*.

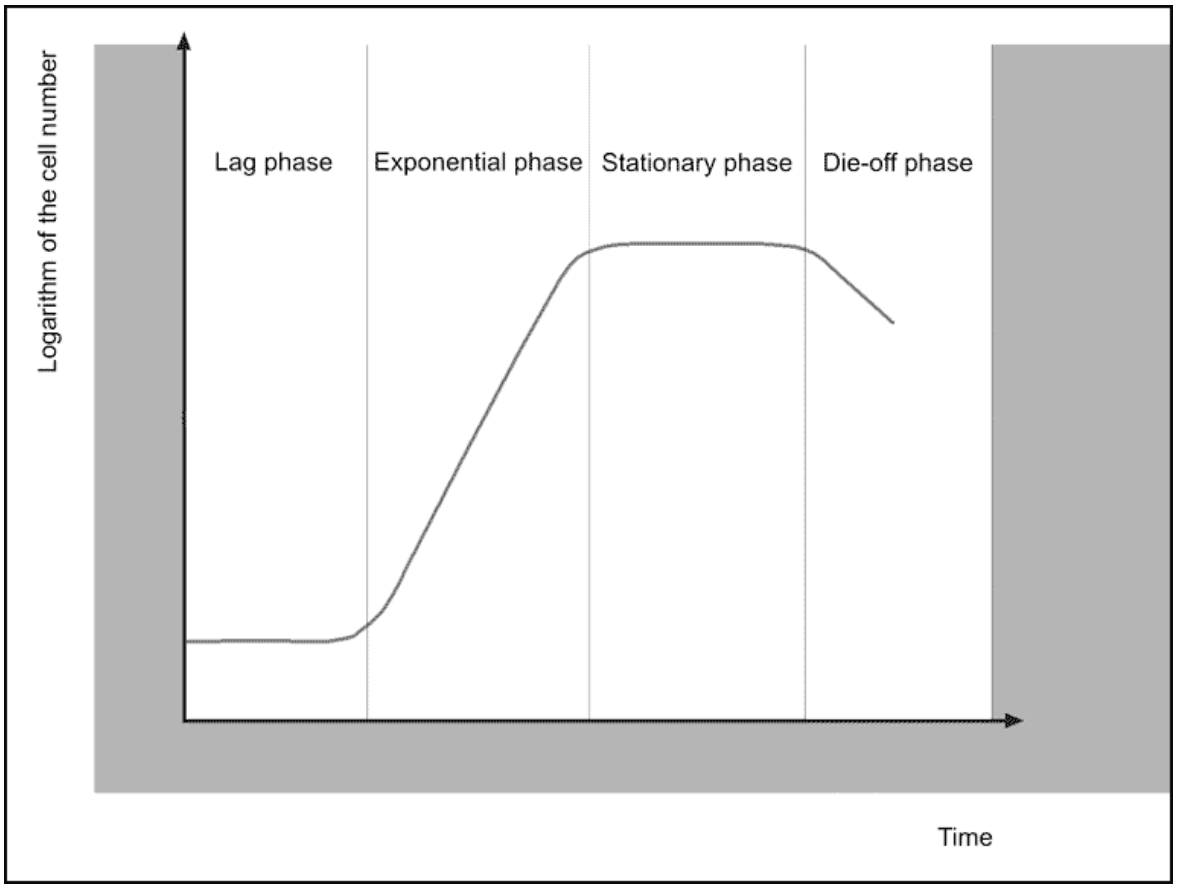

Fig. 0.10: Growth curve of a batch culture

The typical growth curve has a sigmoid shape and can be divided in several growth phases:

The lag-phase starts during inoculation of a substance with microorganisms and finishes with the maximum division rate of the cells. The period of the lag-phase depends on the inoculum (see inoculum). Inoculated e.g. can be a biogas fermenter with a liquid substance of another fermenter. A compost pile of biowaste can be inoculated e.g. with the substance of another compost pile by intensive mixing. In both cases the lag-phase can be reduced. Favourably effective is always an inoculum from a culture in which the cells are in an exponential phase. A lag-phase also occurs with substances which have not been inoculated (e.g. biowaste or a landfill). The bacteria need the phase to adjust e.g. to a determined substrate through synthesis of determined ferments. The synthesis of many enzymes starts economically not before the necessary substrates are available in the milieu (substrate induction of enzymes).

The time between two divisions in the exponential phase (lag-phase) is minimal. The degradation of the substrate is carried out with a maximum velocity. This phase is optimal for decomposition processes e.g. in compost piles or biogas plants.

The stationary phase starts when the concentration of the substrates decreases or other factors as e.g. oxygen, a high population density or final products from toxic metabolism reduce the growth rate. Some bacteria already die off in this phase; many others still live on the intracellular reserve substances. Transformation processes take place in the frame of the secondary metabolisms; the heat development in the compost piles is essentially lower than in the exponential phase.

The die off phase is researched only to a small extent. Acids which can accumulate as final products of metabolism are leading to an exponential decrease of the living cell number. Through autolysis (autodigestion) following from cellular-inherent enzymes the cells can die off, too.

1.4.2 Enzymes

1.4.2.1 Significance in microbiology

Organic substrates, like wood or alcohol, will hardly be degraded chemically in the air without the help of microorganisms, even after years, though this proceeds from the thermodynamic point of view automatically.

If activation energy is brought to the said substrates, e.g. in the form of heat, these incinerate very quickly and generate relatively high amounts of energy. Wood and alcohol are, like all organic compounds, metastable, i.e. they decompose without activation energy extremely slowly. This precondition is indispensable for all organisms as a spontaneous degradation of organic molecules would kill the highly organized structures and processes of the cells. A slow unorganised degradation is also unsuitable.

There must be molecules which enable a 'limited' and purposeful degradation and transformation of molecules. This is the work of the enzymes (*Chapter 1.4.2.2 Properties and efficiency of enzymes*). All the metabolic transformations in the cell are converted by enzymes (bio-catalysers).

1.4.2.2 Properties and efficiency of enzymes

Enzymes consist of proteins with the following properties:

- catalysation of definite reactions,
- specificity for definite substrates (substrate recognition),
- control and switch off capability
- induceability with the existence of corresponding substrates.

The velocity of a reaction induced by enzymes is about 10 dimensions higher than the one of a non-enzymatic reaction. So the half life period of a reaction is reduced by enzymes from 300 years to approximately one second.

Enzymes accelerate a reaction by reducing the activation energy. *Figure 1.11* shows that the usable energy (DG´) is not changed by enzymes. In fact the activation energy (the 'energy mountain' E_1 und E_2 in *Figure 1.11*) is reduced considerably.

The reduction of the activation energy happens through a more favourable positioning of the reactant or the reactants by enzymes. By a momentary bond the enzyme forces its substrate in a position where it can react extremely well. Without an enzyme the

substrate gets very rarely, or just through the use of a relatively high activation energy, in this favourable reaction position.

Enzymes are present within or outside of bacteria (exoenzymes). Outside of the cells exoenzymes split big molecules, like cellulose, lipids, lignin or proteins, which can only be taken up into the bacteria cell as small substances dissolved in a medium. Enzymes cannot be considered individually, but must be understood as parts of metabolic processes. (*Chapter 1.5.4 Decomposition of natural substances*).

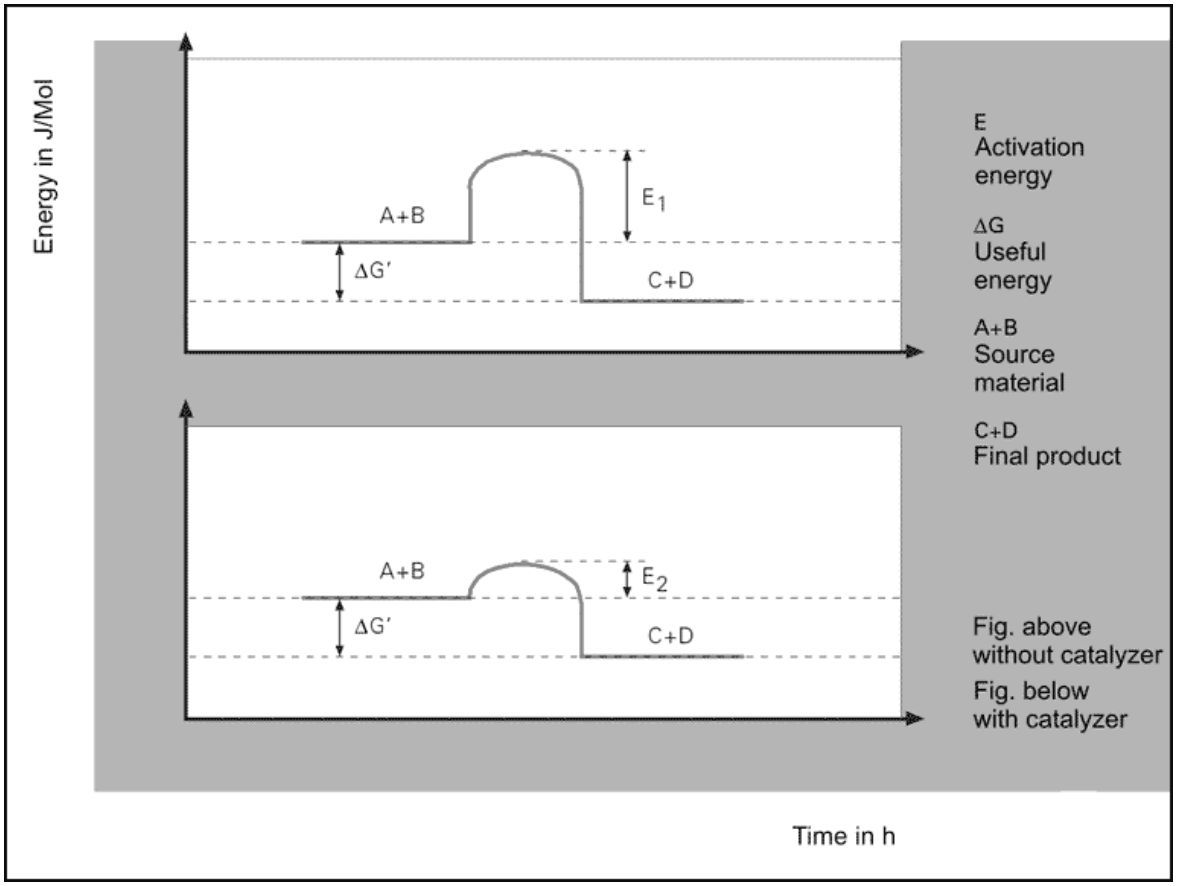

Fig. 0.11: Activation energy with and without enzymes

1.4.3 Monod-Kinetic

1.4.3.1 Monod-relation

The growth or Monod-Kinetic states the empiric connection between biomass *Growth rate* growth per time (growth rate) and the substrate concentration. Monod researched the influence of the concentration of a dissolved nutrient on the growth rate. He found out that the growth rate increases with the raise of the substrate concentration and gave the following coherence by means of a formula:

Formula 0.2: $$\mu_{obs} = \mu_{max} \cdot \frac{S}{K_S + S}$$

μ_{obs} Observed growth rate [h^{-1}], (current growth rate)

μ_{max} Maximal growth rate [h^{-1}]

S Substrate concentration [mg/l]

K_s Half saturation constant, K-value [mg/l]

1.4.3.2 Coherence between substrate concentration and growth rate

The Monod-Kinetic is similar to the one of Michaelis and Menten, is, however, based just on observations and cannot be interpreted mechanistically. (compare *Chapter 1.4.4 Michaelis-Menten-Kinetic*).

Regarding an increasing dilution rate following from an increased substrate addition, the bacteria react with increasing metabolic activity what entails an increasing substrate consumption. The increase of the substrate consumption occurs with a small time delay, which results in an increase of the growth rate. Corresponding to the Monod-Kinetic the growth rate of the bacteria increases (see *Figure 1.12* and *Formula 1.2*).

The bacterial substrate degradation is an enzyme-catalyzed reaction, which follows a saturation kinetic. As a result it can be stated that the growth rate, after achieving the maximal growth rate by adding more substrate, no longer increases. Is the maximal growth rate achieved and a further increase of the substrate is realised (dilution) the equilibrium breaks down on the washing out point, as the washing out rate cannot be compensated by the increasing bacteria amount (compare *Chapter 1.4.4 Mi-chaelis-Menten-Kinetic*, *Figure 1.13*).

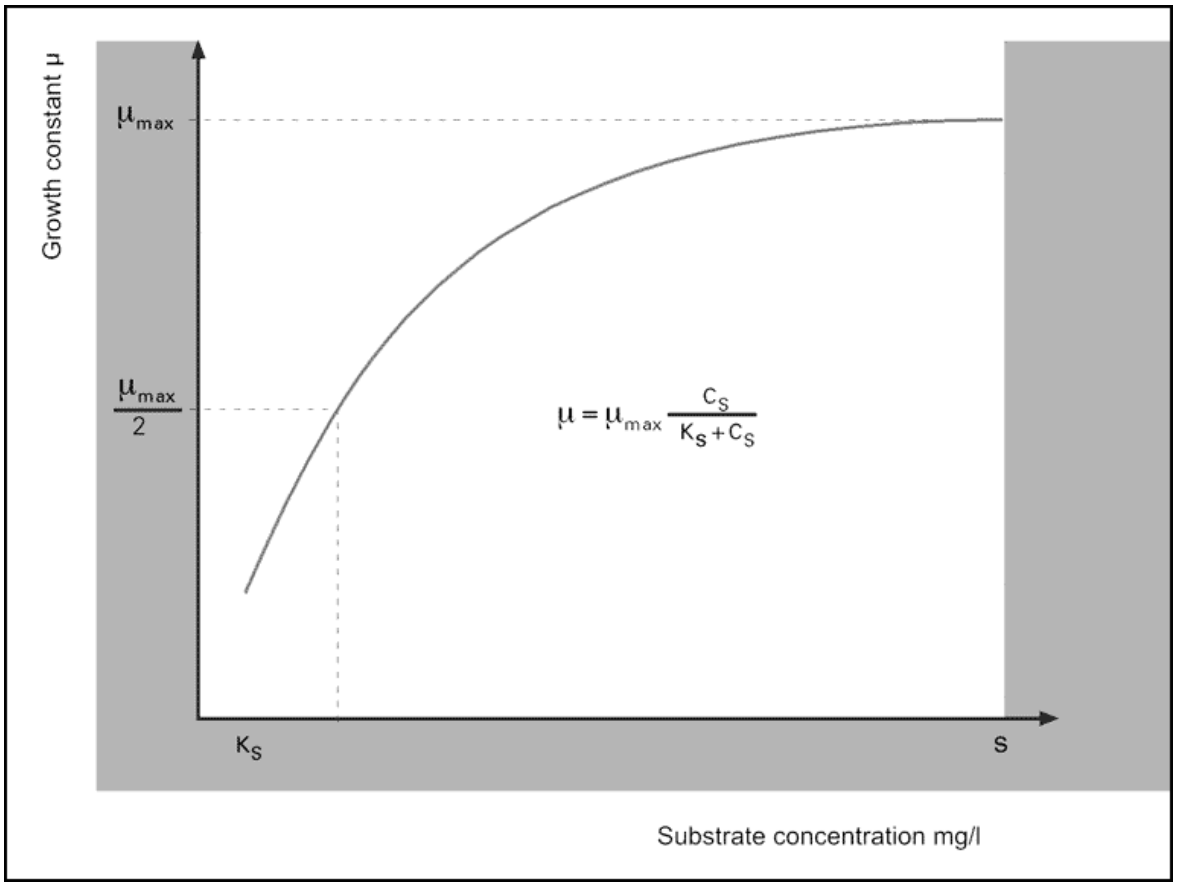

Fig. 0.12: Dependence of the growth rate on substrate concentration, Monod-relation [6]

Important findings considering the measuring and the operation of, e.g. biogas plants with liquid substrates result from the experience with continuously flowing-through fermentation reactors. Especially considered must be the alteration of the dry matter, which accompanies the fluctuation of the bacteria density.

The fluctuation of the bacteria density results from the velocity of the wash-out rate (-Dx) and the growth rate (μx).

Formula 0.3: $$\frac{dx}{dt} = \mu x - Dx$$

with

dx/dt	Fluctuation of the bacteria density [-]
μx	Rate of increment (growth rate) [-]
Dx	Wash-out rate [-]

The bacteria density is constant if the growth rate and the dilution rate (D) are constant. Is the growth rate smaller, the dilution rate must be correspondingly smaller too in order to maintain the equilibrium constant and not to wash out the bacteria of the solution.

An increasing dilution rate (accelerating flow rate) influences the bacteria density of a continuously flowed through reactor as follows:

- the water stream influences an exponential decrease of the bacteria density on account of the wash-out rate,
- the increasing nutrient fraction enables a growth of the bacteria yield,
- the raise of the nutrient concentration in the reactor induces an increase of the growth rate.

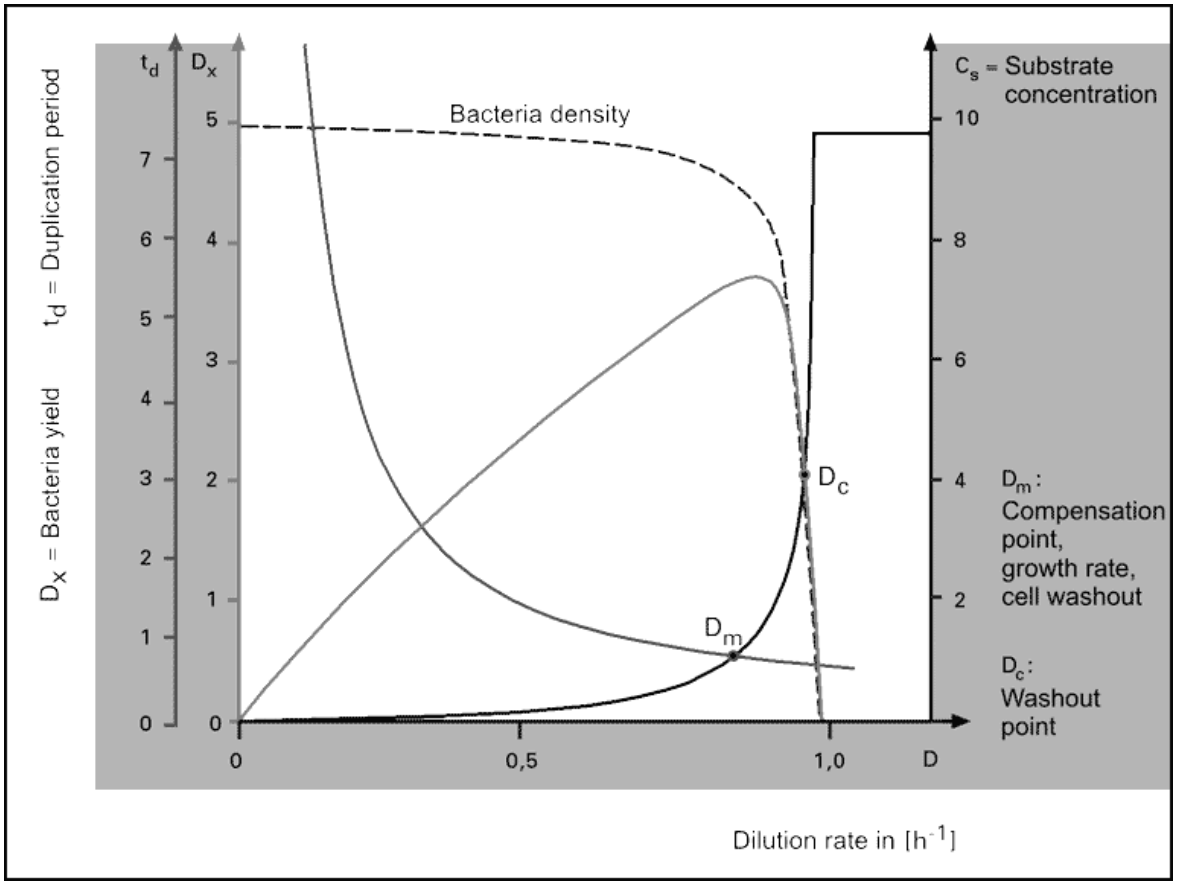

Fig. 0.13: Relation of the parameters in a continuously operated reactor (ideal reactor) [6]

1.4.3.3 Transfer into practical operation

Note:

- The higher the substrate concentration in a fermentation reactor, the higher the growth rate.
- The afflux and discharge in a fermentation reactor increases with the addition of more substrate. This action will wash-out more cells.
- The washing out of the cells will be compensated in a definite area by the increasing growth rate.
- If more cells are washed out than can be supplemented through cell division (growth rate), the wash out point will be surpassed and the system breaks down, i.e., the substrate leaves the system without a biological treatment.

The substrate concentration in many systems, e.g. in the inlet of a sewage plant, is
Conclusion relatively low, so that an optimal growth rate on account of the suboptimal

substrate concentrations cannot be reached. Therefore one must concentrate the bacteria substance, e.g. with a retaining system, or inoculate the substrate.

1.4.4 Michaelis-Menten-Kinetic

1.4.4.1 Relation between substrate concentration and transformation velocity

Biochemical transformation velocities depend on the concentration, i.e., the higher the concentration of the reaction of an involved substance, the higher is the transformation velocity, which finally determines the degradation velocity respectively degradation efficiency. This is only valid until a maximal transformation velocity, a later further increase of the concentration does not lead to a further increase of the velocity. Michaelis and Menten prepared the interrelationship listed below (*Formula 1.4*).

The transformation velocity of biological reactions can be described as follows:

Formula 0.4:
$$\frac{\Delta S}{\Delta t} = \frac{(K_m \cdot x) \cdot S}{S_m}$$

ΔS/Δt	Transformation velocity [mg/(lh)]
$K_m \cdot x$	Maximal transformation velocity [mg/(lh)]
S	Substrate concentration at the point of reaction [mg/l]
S_m	Substrate concentration at $K_m \cdot x/2$ [mg/l]
x	Constant [-]

1.4.4.2 Reactions 0. order and 1. order

In the range of high substrate concentrations 5S) the transformation velocity becomes practically independent of the substrate concentration.

Reactions of this kind are named reactions 0. order

Formula 0.5:
$$\frac{\Delta S}{\Delta t} = K_m \cdot x$$

ΔS/Δt	Transformation velocity [mg/(lh)]
$K_m \cdot x$	Maximal transformation velocity [mg/(lh)]

In the range of very low substrate concentrations becomes S+ SS. There exists a linear interrelationship between the transformation velocity and the substrate concentration. Reactions of this kind are named reaction 1. order.

Formula 0.6:
$$\frac{\Delta S}{\Delta t} = \frac{(K_m \cdot x) \cdot S}{S_m + S}$$

ΔS/Δt	Transformation velocity [mg/(lh)]
$K_m \cdot x$	Maximal transformation velocity [mg/(lh)]
S	Substrate concentration at the point of reaction [mg/l]

S_m Substrate concentration at $K_m \cdot x/2$ [mg/l]

If one measures the velocity of the enzymatic reaction and places it against the substrate concentration in a chart, the dependence shown in *Figure 1.14* will be achieved. Placed are, furthermore, the reactions 0. and 1. order. The K_m-value is defined as the substrate concentration at a half-maximal conversion velocity.

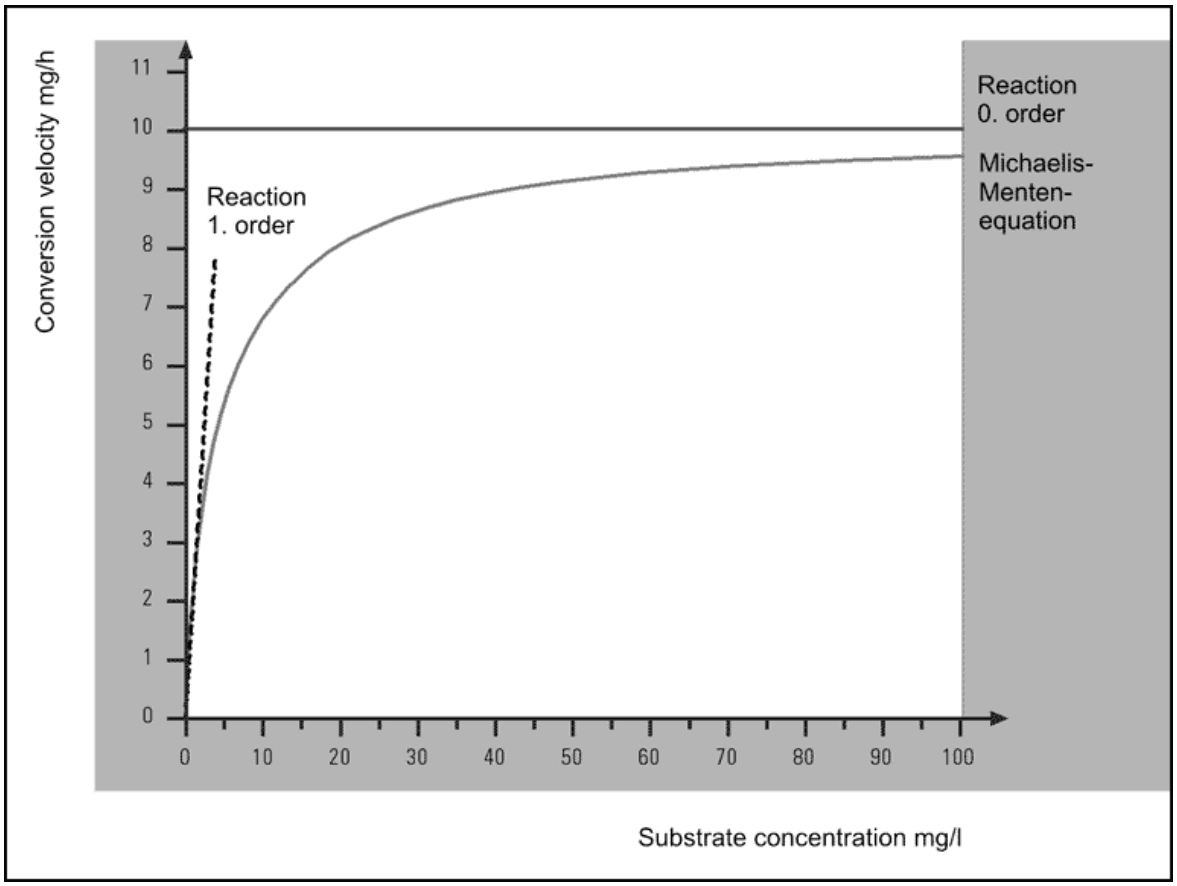

Fig. 0.14: Michaelis-Menten-Kinetic and reactions 0. and 1. order

The K_m-value corresponds in natural systems and in all organisms approximately to the substrate concentration of the reaction which is catalyzed. The demonstrated example has a K_m-value of 5 mg/l (*Figure 1.14*). Physiological substrate concentrations are present in the range of the K_m-value, the reaction runs optimal. The Km-value corresponds to the dissociation constant (transformation constant) of the enzyme-substrate-complex (ES complex). The higher the concentration of the ES-complex is, the lower is the K_m-value. Low K_m-values generally show that the enzyme binds the substrate relatively strong. This means that the half-maximal transformation velocity is already reached at relative low substrate concentrations.

Formula 0.7: $$K_m = \frac{|E| \cdot |S|}{|ES|}$$

K_m Michaelis-Menten-constant, substrate concentration at half maximal

conversion velocity or dissociation constant of the ES-complex [mg/l]

E Enzyme concentration [mg/l]

S Substrate concentration [mg/l]

ES Concentration of the enzyme-substrate-complex [mg/l]

The K_m-value is an enzyme-specific variable and is dependent on many factors, like e.g. the temperature and the pH-value.

In the range of

- lower substrate concentrations can a linearity be recognized between the transformation velocity and the substrate concentration (reaction 1. order).
- higher substrate concentrations becomes the transformation velocity nearly independent of the substrate concentration (reaction 0. order) (see *Figure 1.14*)

1.5 Metabolism and energy recovery

1.5.1 Introduction

The precondition for each metabolic activity is the recovery of bio chemically available energy. If biological energy is recovered, not only heat is released during degradation of the substrate like at a comparable chemical reaction. However, cells are able to store a large part of energy for operations like, e.g., synthesis of molecules or movement.

1.5.1.1 ATP - Energy storage

For the storage of energy all cells use the same molecule adenosine triphosphate (ATP). Therefore ATP is nominated as a universal energy currency. At all places where the cell needs energy it uses ATP.

Figure 1.15 shall explain the biochemical energy conservation by ATP. The 'voluntarily' proceeding reaction in (a) shall serve as analogue for the chemical oxidation of glucose to CO_2 and H_2O where only heat energy is generated (*Chapter 1.5.2.1 Aerobic respiration - degradation of carbohydrate, glycolisis, citric acid cycle*). The reaction in the cell (b) must be looked upon as being linked, whereby the synthesis of ATP can be put on the same level with the lifting power of the pail. The ATP is an energy form with a short-termed storage ability, with the help of which useful work for the cell can be achieved (c).

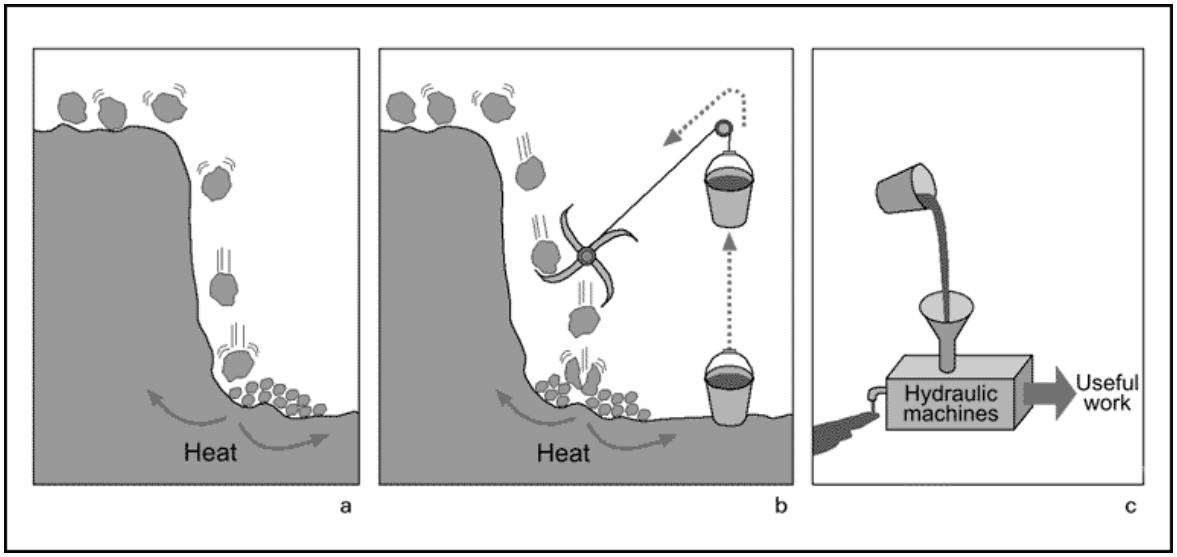

Fig. 0.15: Mechanical pattern for the biochemical energy conservation

1.5.1.2 Definitions

Similar to the global material loop (*Chapter 1.2.2 Position of the microorganisms in the animated nature*) the material loops in the cells exist too:

- built up and break down processes (anabolism and catabolism) and a
- metabolism which links the structure and degradation (amphibolism).

Both metabolic processes are changing gradually and serve to the recovery of energy and the performance of molecular modules for the synthesis of cell components.

Referring to anabolism one distinguishes between heterotrophic and autotrophic bacteria:

- The heterotrophics generate their carbon for the cell structure from organic components and play a central role at the decomposition of organics.
- The autotrophics are able to generate their carbon from CO_2. This being very energy consumptive autotrophic bacteria are growing distinctly slower than heterotrophic bacteria.

One distinguishes the varying metabolism of the microorganisms according to:

- energy sources
- hydrogen or electron donor and
- carbon source

The energy source can be:

- of chemical nature (chemotrophic) or
- from solar radiation (phototrophic).

Hydrogen donor is a collective term for organic and inorganic substances which release bound hydrogen for the oxidation in the frame of metabolism.

Carbon can be generated either from organic material or from CO_2 (compare *Chapter 1.5.2.3 Inorganic substances as hydrogen donors*).

Table 1.3 shows the great variety of the metabolic spectrum of the microorganisms, especially of the bacteria. Many bacteria can be allocated to several metabolic species. To simplify matters the terms:

- chemoorganoheterotrophic bacteria are called **heterotrophic** bacteria.
- nitrificants are called **autotrophic**
- animals, fungi and many bacteria are chemoorganoheterotroph, as shown in *Figure 1.16*

Tab. 0.3: Metabolic species of the organisms

Metabolic specie	Energy source	Hydrogen donor	Carbon source	Example
chemoorgano heterotroph	chemical energy organic compounds	organic compounds $C_6H_{12}O_6$	organic compounds	animals, fungi, bacteria
chemoorgano autotroph		organic compounds e.g. CH_3COOH	CO_2	methanogenic bacteria
chemolitho heterotroph	chemical energy inorganic compounds	inorganic compound e.g. H_2	organic compounds	oxyhydrogen gas bacteria, methanogenic bacteria
Chemolithoautotroph		inorganic compounds e.g. NH_4^+	CO_2	Nitrificants, sulphuroxidizer, oxyhydrogen gas bacteria, methanogenic bacteria
photoorgano heterotroph	radiation energy of the sunlight	organic compounds	organic compounds	green bacteria, purple bacteria
photoorganoautotroph		organic compounds	CO_2	green bacteria, purple bacteria
photolithoheterotroph		inorganic compounds e.g. H_2S	organic compounds	Sulphur purple bacteria
photolithoautotroph		inorganic compounds e.g. H_2O	CO_2	plants, algae, bacteria

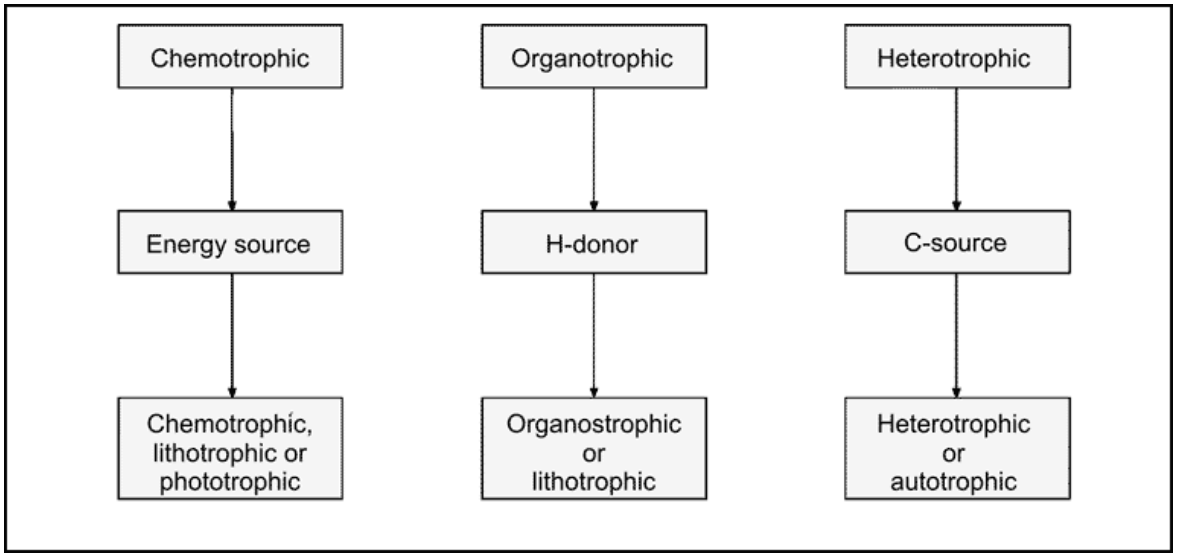

Fig. 0.16: Example metabolic type chemoorganoheterotroph (animals, fungi, bacteria)

1.5.2 Aerobic processes

1.5.2.1 Aerobic respiration - degradation of carbohydrate, glycolisis, citric acid cycle

To a high amount biowaste consist of carbohydrates coming first of all from vegetal wastes. The main fraction are complex components like starch and cellulose (see biowaste). By the splitting of those big molecules through enzymes which are released (exoenzymes) and which cannot be absorbed by the bacteria, glucose is generated which are dissolved and then can be absorbed by the cell.

The start substrate for metabolism is glucose, which is now gradually decomposed to CO_2 and H_2 (*Figure 1.17*). Glucose serves the heterotrophic cells for both, the generation of energy and the supply with source material for the anabolic metabolism.

At the start of the degradation process is glucose split in smaller components which are partly infiltrated into the constructional material metabolism (anabolism) of the cell and partly oxidise oxidized to CO_2. The splitting of glucose in two equal parts is called glycolysis and is one of the central reactions in the metabolism of nearly all organisms. The glycolyse reaction splits glucose in two equal fragments (pyruvates). Energy is generated in the glycolysis in form of 2 mol ATP per mol glucose. Further on reduction equivalents are generated (bound hydrogen).

The pyruvate is oxygenated further on, whereby CO_2 and bound hydrogen is generated. The product of this reaction is activated ethanoic acid.

The activated ethanoic acid is completely degraded through oxidation in the citric acid cycle to CO_2 and bound hydrogen.

The bound hydrogen with oxygen is stepwise oxygenated to H_2O in the respiration chain. Hereby the overwhelming part of the energy is generated in form of ATP. The oxidation of the bound hydrogen with oxygen to H_2O is called aerobe respiration.

The velocity of the decomposition of glucose depends on the metabolic rate of the

respiration chain, which can be measured by the oxygen demand. Therefore the supply of atmospheric oxygen during the operation of a composting plant is very important. The respiration chain consists of a cascade of enzymes which induce that the reaction between the bound hydrogen and oxygen runs step by step under the generation of energy.

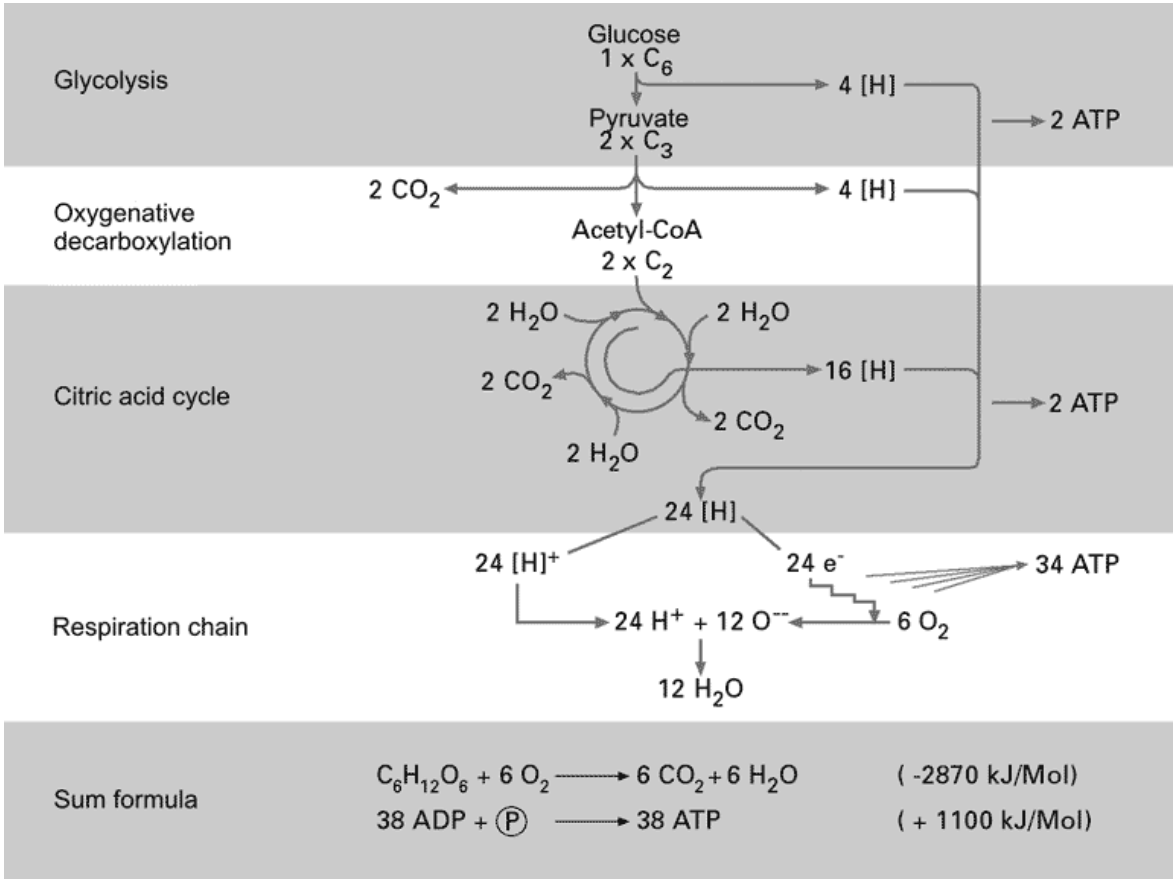

Fig. 0.17: Scheme of the aerobic glucose degradation [5]

1.5.2.2 Incomplete oxidation

There are many microorganisms, especially unicellular fungi, which under presence of atmospheric oxygen do not fully oxidize organic substrates, mostly sugar or alcohol.
The products of these incomplete oxidations range from

- simple ethanoic acid over
- citric acid and
- other acids to
- penicillin

Therefore is this metabolic type especially important for the microbial biotechnology.

1.5.2.3 Inorganic substances as hydrogen donors

The group of the aerobic chemolithotroph bacteria is capable of using:

- inorganic compounds or ions,
- elemental sulphur,
- hydrogen and
- carbon monoxide as hydrogen respectively electron donor.

Thus, energy recovery is realized through respiration with oxygen, whereby the oxygen oxidizes reduced inorganic substrates. *Table 1.4* shows the most important chemolithotrophs.

Tab. 0.4: Metabolic types of the chemolithotrophic bacteria

Term	Substrate	Final product	Example
Ammonia oxidants	NH_3	NO_2^-	Nitrosomonas
Nitrite oxidants	NO_2^-	NO_3^-	Nitrobacter
Sulphur oxidants	S, S^{2-}, $S_2O_3^{2}$	SO_4^{2}	Thiobacillus thiooxidans
Ferrous bacteria	Fe^{2+}	Fe^{3+}	Thiobacillus ferrooxidans
Oxyhydrogen bacteria	H_2	H_2O	Alcaligines eutrophus
Carboxide bacteria	CO	CO_2	Pseudomonas carboxidovorans

Most of the bacteria of the presented metabolic type grow with CO_2 as a sole carbon source. Therefore are they characterized as chemolithoautotroph (compare *Table 1.3*). As the fixation of CO_2 takes relatively much energy, those bacteria have a relatively slow growth.

Ammonium and nitrite oxidising bacteria together form the group of nitrificants, which are playing an essential role in waste water purification during the first step for the removal of nitrogen from the waste water (see nitrification). The basis for denitrification (nitrate respiration) is created by the oxidization of ammonium to nitrite and nitrate where nitrogen is released as N_2.

Denitrification is desired at waste water purification, however, leads to the loss of the valuable nitrogen fertiliser during the aerobic treatment of biowaste. The nitrogen loss in the compost can be avoided through an optimised aeration of the material. Indirectly involved are nitrificants in the destruction of limestone and hardened cement paste, as here the final product nitric acid affects the material.

Sulphur oxidants oxidize reduced sulphur compounds to sulphur acid, which can lead to corrosion at concrete surfaces.

1.5.3 Anaerobic Nutrient Chain

1.5.3.1 Fermentation

Fermenting microorganisms can be met everywhere, where organic compounds are present and oxygen is missing. In nature and in the anaerobic treatment of biowaste cellulose is mostly fermented. There is no possibility to bind the bound hydrogen with oxygen to H_2O during metabolism without oxygen. As during degradation bound hydrogen arises, it must be bound elsewhere (compare *Chapter 4.1.2 Stages of the anaerobic decomposition*).

During fermentation the substrate is split in the first instance via the glycolysis (fructosebiphosphate -path). A reduced product which takes up the activated hydrogen arises always and in addition an oxidised product, mostly CO_2. Thus, energy generation by fermentation is the result of an oxidation reaction. The activated hydrogen must be removed via the reduction of the split products as the deoxidation of hydrogen to H_2O cannot take place (*Figure 1.18*).

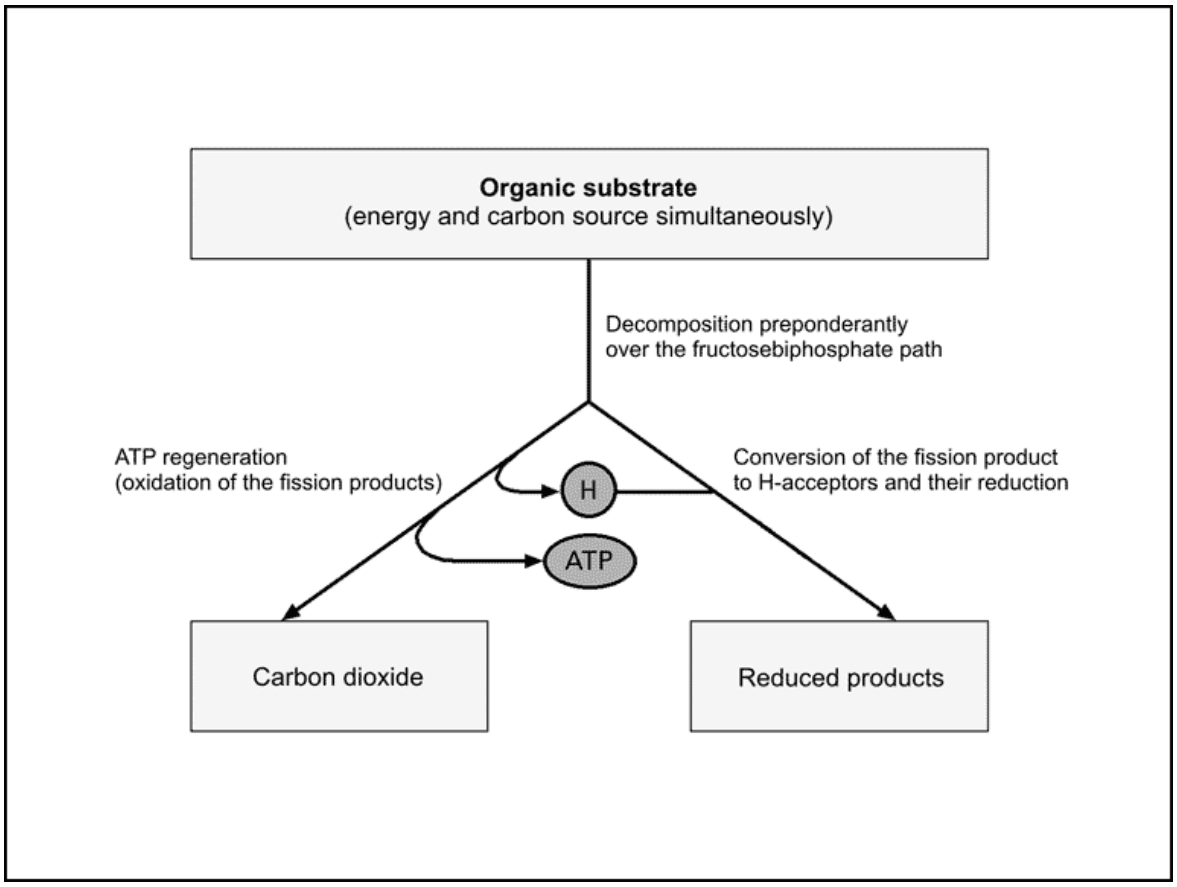

Fig. 0.18: Principle of fermentation

The energy recovery by fermentation is with 2 mol ATP per mol glucose essentially smaller as when glucose is aerobically decomposed (38 mol ATP/mol Glucose) (compare *Chapter 1.5.2.1 Aerobic respiration - degradation of carbohydrate, glycolisis, citric acid cycle*). On account of this reason many microorganisms are changing their metabolism to

aerobic decomposition as soon as oxygen is available. During operation of a fermentation plant it is always necessary to check that no oxygen enters the system.

When the reduced products enrich themselves in the medium of microorganisms, the fermentation stops acting. This is the case during fermentation on account of alcohol at about 10 % volume. The arising fermentation products, among them alcohol, organic acids, CO_2 and hydrogen are available for other bacteria which finally are forming methane through further specific fermentations. The fermentation of cellulose leads into the anaerobic nutrient chain (see *Chapter 1.5.3.2 Anaerobic respiration*).

1.5.3.2 Anaerobic respiration

In deposits of water bodies, where sufficient organics are available, are numerous bacteria which use the final products of fermenting bacteria as hydrogen donators and carbon source. Thus these organisms are at the end of the anaerobic nutrient chain. While during aerobic respiration the atmospheric oxygen is consumed, during anaerobic respiration the substrates named in *Table 1.5* can be respired.

Tab. 0.5: Anaerobic respiration

Term	Substrate	End product	Example
Nitrate-respira-tion	NO_3^-	NO_2^-, N_2O, N_2	aerobic and facultative anae-robic bacteria
Sulphate-respira-tion	SO_4^{2-}	S^{2-}	obligate anaerobic bacteria
Sulphur-respira-tion	S	S^{2-}	obligate und facultative anae-robic bacteria
Carbonate-respi-ration	CO_2, HCO_3^-	Ethanoic acid	acetogenic bacteria
Carbonate-respi-ration	CO_2, HCO_3^-	Methane	methanogenic bacteria
Fumarate-respi-ration	Fumarate	Succinate	succinogenic bacteria
Ferrous-respira-tion	Fe^{3+}	Fe^{2+}	alteromonas putrefaciens

The end product of the respective respiration gives it its name:

- The nitrate respiration corresponds to denitrification, which plays a role in the removal of nitrogen in the sewage plant.
- The products of the sulphate and sulphurous respiration can be recognised with the odour of the hydrogen sulphide (H_2S).
- The carbonate respiration plays an essential role at the anaerobic treatment of biowaste, as it synthesises methane.

Methanogenic bacteria are the final elements of an anaerobic nutrient chain, which is shown in *Figure 1.19*.

Oxygen stops the anaerobic respiration processes. Facultative anaerobic bacteria (see facultative anaerobies) are immediately changing to aerobic respiration what is energetically more favourable (compare *Chapter 1.5.2.1 Aerobic respiration - degradation of carbohydrate, glycolisis, citric acid cycle* and *Chapter 1.5.3.1 Fermentation*). Obligate anaerobic bacteria are killed when aerated.

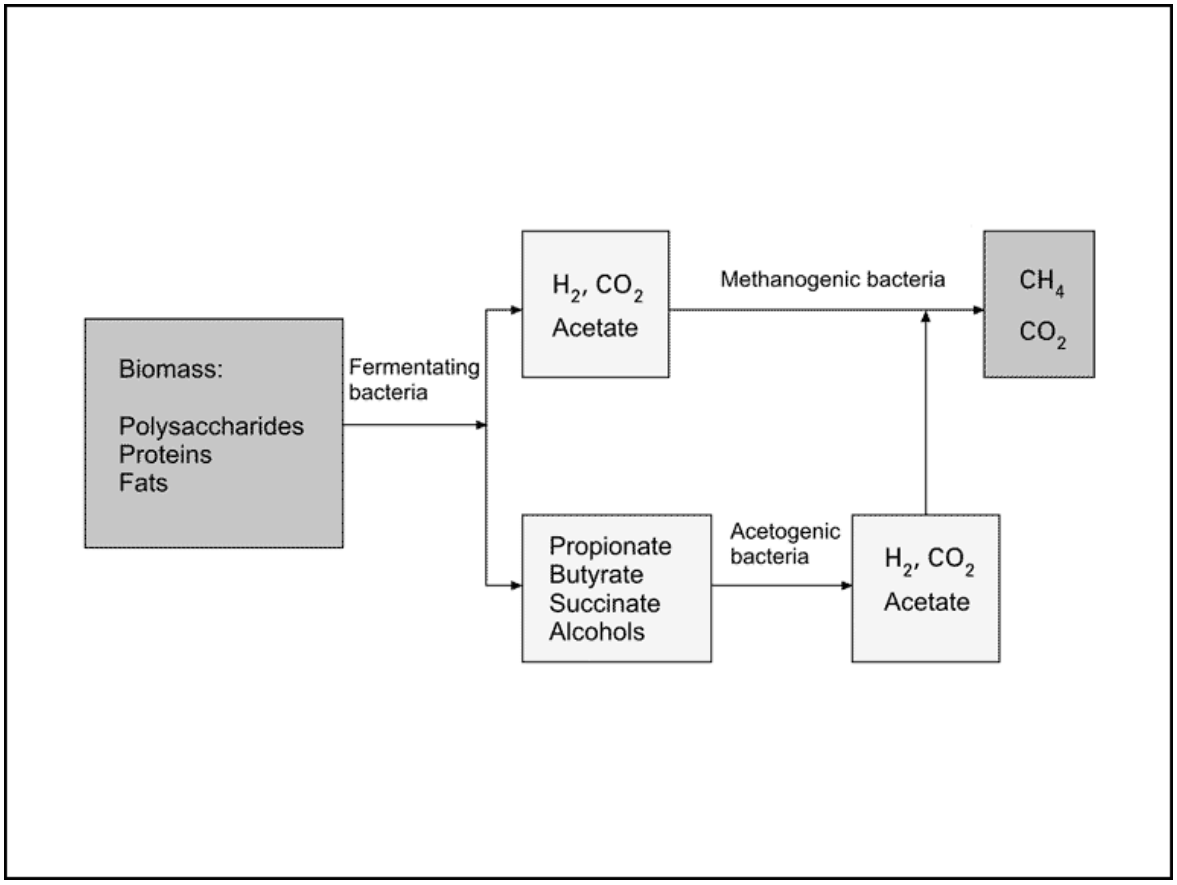

Fig. 0.19: Anaerobic nutrient chain [6]

1.5.4 Decomposition of natural substances

All biologically arising compounds can be decomposed under aerobic conditions. There is a micro-organism for each biochemical substance which can decompose the material partially or completely (see biological degradability). In this connection one also talks of

omnipotence. If this would not be so, a global carbon loop could not exist (see *Figure 1.3*), the world would rather quickly be drowned in the products of the producers (the plants).

In order to find microorganisms which decompose certain natural substances one takes so-called enrichment cultures (concentrates). These consist of a simple basic nutrient solution and the natural substance to be tested as energy source. After inoculation of the preparation, e.g. with soil material (see inoculation material) which contains the test substance, those microorganisms which are growing most rapidly are succeeding and can be isolated. By means of this method microorganisms can also be isolated which decompose xenobiotica, i.e. substances which are not of biological origin. This can be important for the biological remediation of the soil.

The majority of all natural substances are decomposed aerobically, the smaller part anaerobically. The deposits of water bodies, rice fields, swamps, landfills, fermentation containers and to a large part also the intestinal passage of a lot of animals belong to the anaerobic ecosystem. Beside the ruminant animals a considerable amount also belongs to insects like ants and termites, worms and other small animals.

1.5.4.1 Cellulose

Cellulose represents the main component of the plants and thus can be found to a great amount in the biowaste. Paper and cotton are to a large part of cellulose. This substance consists of branched chains, the links of which are sugar molecules (hexoses).

Cellulose is essentially decomposed by microbial fungi. As the microorganisms cannot take up the cellulose molecule, which consists partially of more than 10.000 sugar molecules, it must be split in sugar molecules out of the cell. This is catalyzed through exoenzymes (compare *Chapter 1.4.2 Enzymes*). Then the sugar molecules can be taken up and sluiced into the glycolysis.

The bacteria are dominating during the anaerobic decomposition. A great amount of the anaerobic decomposition of cellulose in the natural loop takes place in the rumen of the ruminants.

1.5.4.2 Hemicellulose (xylan)

Regarding the quantity hemicellulose is besides cellulose (xylan) the widest spread carbohydrate in nature. The hemicellulose is built-up with other sugars than the cellulose, on the whole from pentose, but also glucose, manose and galactose. The linkage form of sugars among each other also differs between hemicellulose and cellulose. The chain length is with 30 to 100 sugars essentially smaller than with the cellulose.

The degradation is similar to the one of cellulose (*Chapter 1.5.4.1 Cellulose*) yet more rapidly and with a larger amount of microorganisms.

1.5.4.3 Starch

Starch is the main storage substance of the plant. The substance consists of amylose and amylopectin. Amylose is built-up from not branched chains, amylopectin forms branches. Starch consists of less sugar molecules than cellulose. Similar to the cellulose the starch must be split in sugar molecules out of the cell before they can be taken up.

Starch can be decomposed

- aerobic by fungi and bacteria and
- anaerobic predominantly by bacteria

1.5.4.4 Lignin

Besides cellulose lignin is, referring to the volume, the most important component of the plants. Wood has a lignin portion between 18 and 30 % of dry matter. Lignin forms with the cellulose fibres a complex, lignocellulose. Referring to its structure wood can be compared with reinforced concrete. Steel and cellulose fibres take up tensile stress, concrete and lignin take up compressive strain. The decomposition of lignin is relatively slow. In regard to its chemical attitude lignin is built-up in quite a complex way. The structure is three-dimensional. The basic module of the giant molecule consists of alcohols with ring-shaped residuals.

Decomposition is exclusively realised by aerobic processes. Fungi are active in wood and fungi and bacteria in the soil. One of the most active wood decomposing fungi is the white-rot fungus. The decomposition products of lignin are playing a decisive role at humus production in the soil. Wood has a especial high nitrogen consumption. Thus, during biowaste treatment attention has to be paid to the fact that in the original material are not too many wooden parts. On the other hand can a substance, containing a relatively high volume of nitrogen, be 'slimmed' with wooden material.

1.5.4.5 Proteins

All organisms consist to a large part of proteins. Proteins are big molecules which are built from amino acids. The nitrogen content is relatively high.

Proteins are decomposed by fungi and bacteria

- aerobically and
- anaerobically.

Exoenzymes split proteins out of the cells in smaller components, which can be taken up by the cells (compare *Chapter 1.4.2 Enzymes*). The protein fragments are split to amino acids in the cell. These can be built either in cell-inherent proteins or decomposed under generation of energy. One of the first decomposition steps is the separation of the nitrogen from the amino acid (desamination). Hereby ammonium is created (ammonification). The carbon skeleton of the amino acids is further decomposed over the citrate cycle (see *Figure 1.17*).

1.6 Summary

Microorganisms is a collective term for small, mostly unicellar pro- and eukaryotes. Prokaryotes (bacteria) have no nucleus. Eukaryotes have a nucleus and other cell organelles (chloroplasts and mitochondria) (see *Chapter 1.3 Structure and compound of cells and bacteria*).

The high metabolic efficiency is established by the great surface-volume-ratio. The microbial growth corresponds to an exponential function and depends on the con-

centration of the substrate (see *Chapter 1.4 Growth and metabolism of microorganisms*).

Microorganisms are playing the central role in the global carbon and nitrogen loop on account of their ability for decomposition of organic material (see *Chapter 1.2.2 Position of the microorganisms in the animated nature*).

Microorganisms, especially bacteria, provide an extremely large range of energy and carbon generation. They can exist under aerob or anaerob conditions or under both (see *Chapter 1.5 Metabolism and energy recovery*).

The substrate decomposition is realised by enzymes which reduce the activation energy of a reaction. Enzymes have certain transformation velocities and are substrate specific. Microorganisms can decompose all compounds from biological origin. The degradation of big molecules is at first realised out of the cell by exoenzymes (see *Chapter 1.4.2 Enzymes*).

Figure 1.20 summarised the most important aerobic decomposition ways of the microorganisms.

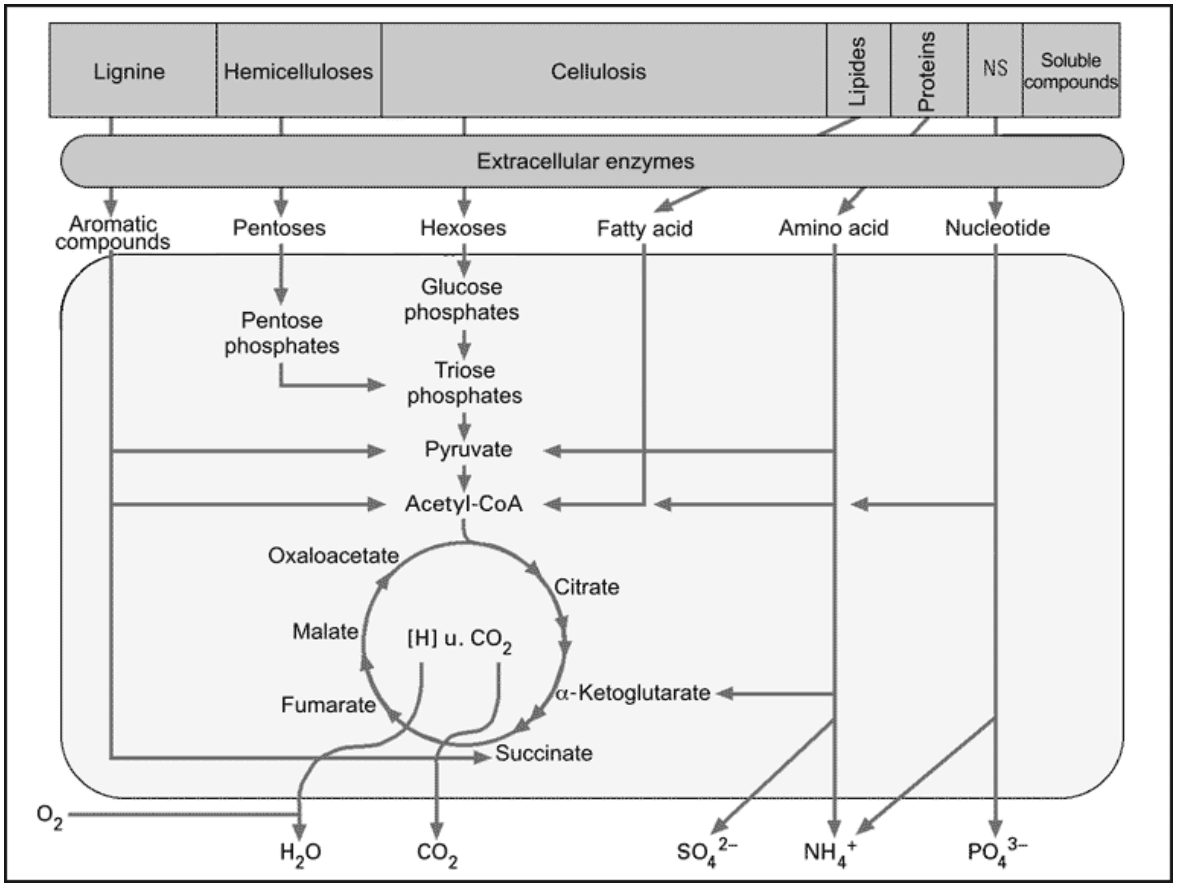

Fig. 0.20: Aerobic decomposition of biomass

For further literature we recommend [3]:

1.7 Literature

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