Chapter 1

General introduction
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Molecular oxygen (O₂) appeared on earth approximately 3500 million years ago when the first phototrophic oxygenic microorganisms evolved. Before this event the earth atmosphere and oceans were essentially devoid of free oxygen. The precise history and control of atmospheric O₂ is still a matter of dispute. However, it is generally accepted that for a long period (from 1700 to 2900 million years) the O₂ concentration remained below 2% because the rate of supply of reduced substances (H₂, CO and H₂S from volcanoes and Fe²⁺ from seawater/basalt interaction) exceeded the photolytic production of O₂ (Kasting et al., 1992). Today the air contains about 20.9% of O₂ and the major part (estimated > 90%) of the biological cycling of elements is performed by O₂-producing or O₂-consuming organisms during the phototrophic formation of organic matter counteracted by its decomposition through aerobic respiration (Stumm & Morgan, 1981; Zehnder, 1982). In spite of the fully oxic modern atmosphere, the heterogeneity of most environments still supports the establishment of low-oxygen and anoxic habitats.

Analysis of microbial ecosystems in situ in combination with studies on microbial cultures in the laboratory have provided detailed information on the functioning of microorganisms under both fully oxic and anoxic conditions. However, knowledge of the ecology and physiology of microorganisms in low-oxygen environments is still scarce.

Molecular oxygen in microbial metabolism.

Metabolic functions of oxygen

The solubility of oxygen in water is relatively low. For example, the oxygen concentration in pure water at 20° C equilibrated with air at a pressure of 1 atm. (dissolved oxygen tension 159 mm Hg or, 21.2 kPa) is about 284 μM (9.1 mg/liter⁻¹). This concentration is lowered by increase in temperature and pressure and the presence of solutes (e.g. medium salts or glucose).

Bacteria utilize O₂ for two purposes (Morris, 1984). During aerobic respiration O₂ acts as a terminal acceptor for the electrons temporarily stored in co-enzymes (e.g. NAD(P), FAD, FMN) during the oxidation of reduced energy-substrates. This process is performed by membrane associated cytochrome oxidases catalysing the transfer of electrons to O₂, reducing the dioxygen molecule to O₂⁻, H₂O₂ or 2H₂O.

Cytochrome oxidases are responsible for more than 90% of biological oxygen consumption (Poole et al., 1985). Nitrate, some oxidized metal ions or organic compounds, sulphate, protons or carbon-dioxide may be used as terminal electron acceptors in the absence of O₂. However, O₂ allows the generation of more ATP and hence more biomass and is thus the preferred electron acceptor.

Another purpose for using O₂ is its role as a reactant for the primary attack of various compounds during which oxygen molecules are inserted into substrate molecules. Monoxygenases catalyse the transfer of one and dioxygenases of both atoms of the O₂ molecule into a substrate. Thus O₂ is a co-substrate for the activation and catabolism of aliphatic and aromatic hydrocarbons such as methane, octane or benzene. Although synthesis of microbial cell-components usually does not require O₂, some microorganisms (including yeasts and fungi) need molecular oxygen in oxygenase reactions for a restricted number of biosynthetic purposes.

Oxygen inhibition

Besides these useful metabolic functions also negative aspects are associated with the chemical character of oxygen. The biological reduction of O₂ to water has to be well controlled because partial reduction may lead to the generation of superoxide (O₂⁻), hydrogen peroxide (H₂O₂), hydroxyl radical (OH•) and singlet oxygen (¹O₂), all of which have been implicated in the destruction of vital cell-components (Morris, 1976). In addition, inhibition of microbial growth by O₂ may be due to high redox values or drainage of reducing power needed for biosynthesis. Some strategies employed by bacteria to avoid the lethal potential of oxygen are: (1) Induction of super- and per-oxide decomposing enzymes (superoxide dismutases, catalases and peroxidases); (2) Synthesis of compounds absorbing radicals (carotenoids, melanoids); (3) Avoidance of the reduction of O₂ (no synthesis of O₂-reducing enzymes or transition into a metabolically dormant state upon exposure to O₂); (4) Maintenance of a low internal O₂-concentration (increased respiration by futile cycles, excretion of slimes or formation of cell-aggregates retarding O₂ diffusion, negative aerotaxis).

Bacteria are classified in different groups depending on their requirements for and level of tolerance to oxygen. Different definitions have
been used throughout the literature. The generally accepted growth responses of various metabolic types of microorganisms are summarized in Figure 1.

![Figure 1: Typical growth responses of different metabolic types of bacteria to oxygen concentration in the range from 0 to 10 μM. At concentrations above air saturation the growth of strict aerobes, facultative (an)anaerobes and tolerant anaerobes may also become repressed. Obviously, the position of the curves relative to the value of 10 μM is dependent on the actual species involved.](image)

### Responses of heterotrophic microorganisms to low oxygen-concentrations

Aerobic bacteria utilize O₂ for their respiration. For strictly (or obligately) aerobic bacteria O₂ is an essential terminal electron acceptor for membrane energization and for ATP-synthesis through electron transport dependent phosphorylation (Brock & Madigan, 1988). Aerobes adapt to low O₂-tensions by boosting their O₂-affinity. This can be accomplished by raising the concentration of components of the respiratory chain such as cytochrome oxidases (increasing V_{max}) and/or by synthesizing alternative cytochrome oxidases with lower half-saturation constants for O₂ (decreasing K_{m}) (Harrison, 1976; Rice & Hemphling, 1978). When the O₂-concentration and/or the rate of O₂-supply drops to such low values that insufficient O₂ can be captured to match the supply of carbon-substrates (O₂-limitation) the internal concentration of reduced co-enzymes such as NADH increases dramatically. Under these conditions the metabolism of strict aerobes becomes choked and carbon substrates can no longer be converted entirely into structural biomass, carbon-dioxide and water. Physiological responses to dispose of the excess reducing power include the excretion of catabolic (e.g. TCA intermediates) and anabolic (e.g. amino acids) metabolites, the synthesis of intra-cellular "storage" molecules such as polyhydroxalkanoates (e.g. pHB) or polysugars (e.g. trehalose) and induction of fermentation pathways enabling the formation of reduced molecules such as H₂, alcohols or organic acids (Dawes, 1986; Vollbrecht, 1987; Gunsalus, 1992). The type and amount of electron-sink products formed depends on the degree of oxygen limitation and the specific microorganisms involved.

In contrast to strict aerobes, facultative aerobes (or facultative anaerobes) can also grow in the absence of O₂ by using alternative electron acceptors or fermentative pathways. The switch-over from oxic to anoxic conditions is accompanied by the induction of a complete set of enzymes needed for anoxic growth, regulated genetically at the level of DNA-transcription (Guest, 1992; Gunsalis, 1992). The capability to completely switch over between aerobic and anaerobic types of metabolism appears to be an important adaptation to environments with fluctuating oxygen tensions (Gottschal & Szewzyk, 1985).

Anaerobic bacteria are unable to grow at the expense of the reduction of O₂ alone. They may be arranged in groups on the basis of their degree of O₂-tolerance. Growth of strict (or obligate) anaerobes is severely inhibited even by very low levels of O₂. For most strictly anaerobic bacteria exposure to atmospheric oxygen concentrations is lethal. Moderate anaerobes are less sensitive and are able to grow (sub-optimally) at low O₂ concentrations, whereas tolerant anaerobes endure atmospheric oxygen without significant suppression of growth or loss of viability. Anaerobic bacteria have been divided in groups, based on (1) their capacity to grow on agar plates incubated under atmospheres with various oxygen-concentrations, (2) their ability to survive periods of exposure to air, or (3) the distance of growth from the surface of agar stab-cultures incubated under oxygen-containing atmospheres (Morris, 1976). Mainly due to deficiency of quantitative information on the effect of oxygen on the growth of most anaerobic bacteria, the classification of these organisms is
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not well defined. In spite of the apparent adverse effects of $O_2$ on most anaerobes, the influence of $O_2$ on the physiology of these microorganisms appears to be a field of exciting new discoveries. Recently it was found that the capacity of $O_2$-respiration, coupled to ATP formation, is widespread among sulphate reducing bacteria, though these classical anaerobes are generally not able to grow at the expense of aerobic respiration alone (Danneberg et al., 1992). Many obligately fermenting microorganisms, such as lactate- and propionate-acid bacteria, also actively reduce significant quantities of $O_2$ commonly through the activity of flavoprotein enzymes including NADH-NADPH- or pyruvate-oxidases (Bentzen & Larsen, 1989; Condon, 1987; Cove, et al., 1987; De Vries, et al., 1978; Morris, 1984; Thomas & Pera, 1983; Tseng et al., 1991). Besides a function as a detoxification mechanism, diversion of reducing equivalents to $O_2$ enables a more complete oxidation of the carbon substrate often resulting in a significant increase of the ATP- and biomass-yield. It is now recognized that many anaerobes grow optimally in the presence of low concentrations of $O_2$. Occasionally, such microorganisms are therefore referred to as microaerophiles (microaerophilic anaerobes) (Danneberg et al., 1992; Stouthamer, et al, 1979).

Table 1: Comparison of half-saturation constants of some $O_2$-utilizing enzymes. $K_{m}^{O_2}$ values of enzymes were determined at physiological temperatures with their natural substrates as reductants.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>$K_{m}^{O_2}$ ($\mu M$)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome o</td>
<td>various bacteria</td>
<td>0.2-4</td>
<td>Poole, 1983</td>
</tr>
<tr>
<td>Cytochrome d</td>
<td>various bacteria</td>
<td>0.018-0.38</td>
<td>Poole,1983</td>
</tr>
<tr>
<td>Cytochrome aa$_3$-oxidase</td>
<td>prokaryotes</td>
<td>4-7</td>
<td>Poole, 1983</td>
</tr>
<tr>
<td>Pyruvate oxidase</td>
<td>mitochondrial</td>
<td>0.3-3.0</td>
<td>Bentzen &amp; Larsen, 1989</td>
</tr>
<tr>
<td>NADH oxidase</td>
<td>Bacteroidaceae sp.</td>
<td>160</td>
<td>Bentzen &amp; Larsen, 1989</td>
</tr>
<tr>
<td>NADPH oxidase</td>
<td>Bacteroidaceae sp.</td>
<td>300</td>
<td>Bentzen &amp; Larsen, 1989</td>
</tr>
<tr>
<td>Catechol-1,2-dioxygenase</td>
<td>Pseudomonas sp.</td>
<td>1340</td>
<td>Dorn &amp; Knackmuss, 1978</td>
</tr>
<tr>
<td>Catechol-1,2-dioxygenase</td>
<td>Pseudomonas arvilla</td>
<td>19</td>
<td>Sahler &amp; Klecka, 1985</td>
</tr>
<tr>
<td>Catechol-2,3-dioxygenase</td>
<td>Pseudomonas sp.</td>
<td>9</td>
<td>Sahler &amp; Klecka, 1985</td>
</tr>
<tr>
<td>Catechol-2,3-dioxygenase</td>
<td>Pseudomonas putida</td>
<td>25</td>
<td>Sahler &amp; Klecka, 1985</td>
</tr>
<tr>
<td>Melilotate-hydroxylase</td>
<td>Pseudomonas sp.</td>
<td>50</td>
<td>Sahler &amp; Klecka, 1985</td>
</tr>
<tr>
<td>Ornicol-hydroxylase</td>
<td>Pseudomonas putida</td>
<td>69</td>
<td>Sahler &amp; Klecka, 1985</td>
</tr>
<tr>
<td>Phenol-hydroxylase</td>
<td>Trichosporum cutaneum</td>
<td>53</td>
<td>Sahler &amp; Klecka, 1985</td>
</tr>
<tr>
<td>Protocatechuate-3,4-dioxygenase</td>
<td>Pseudomonas aeruginosa</td>
<td>44</td>
<td>Sahler &amp; Klecka, 1985</td>
</tr>
<tr>
<td>Protocatechuate-4,5-dioxygenase</td>
<td>Comamonas testosteroni</td>
<td>303</td>
<td>Dorn &amp; Knackmuss, 1978</td>
</tr>
<tr>
<td>Tryptophan-2,3-dioxygenase</td>
<td>Pseudomonas sp.</td>
<td>53</td>
<td>Sahler &amp; Klecka, 1985</td>
</tr>
<tr>
<td>Luciferase</td>
<td>Photobacterium fischeri</td>
<td>19</td>
<td>Lloyd et al., 1981</td>
</tr>
</tbody>
</table>
their specific oxygenases and oxidases. Microbial growth will become oxygen-limited at oxygen-concentrations below those required for saturation of these oxygen reducing enzymes. There is considerable variation in the kinetic properties of different oxygenases and oxidases (Table 1). For example, values of the (Michaelis-Menten) half-saturation constant (K<sub>v</sub>) for O₂-binding by cytochrome oxidases are considerably lower than the K<sub>v</sub>-values reported for aromatic dioxygenases. Indeed, for most aerobic bacteria utilizing for example sugars or aliphatic organic acids the rate of growth is essentially independent of the O₂ concentration above 10 μM, whereas significantly higher oxygen tensions are necessary to obtain maximum growth of organisms catabolizing aromatic compounds.

Minimum concentrations for consumption of substrates by bacteria (thresholds) have been described for various substrates, such as acetate and hydrogen (Widdel, 1988). It is not clear if such threshold concentrations can also be found for O₂-utilization by aerobic bacteria. Thermodynamic calculations indicate that the oxidation of organic matter according to equation (1) is exergonic to below an O₂ tension of 1.3 x 10⁻²² atm., corresponding to an O₂ concentration of approx. 1.5 x 10⁻¹⁶ M. However, below an O₂-concentration of 0.1 nM the reaction rate of the cytochrome oxidases with the highest O₂-affinity reported is less than 1% of the maximum rate (Table 1). Hence, O₂-concentrations below 0.1 nM probably permit only very slow growth of aerobic bacteria.

\[ \text{(1) } \text{CH}_3\text{O} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} \]
\[ \Delta G^\circ = -125.1 \text{ kJ/ equiv}; \text{ Zehnder & Stumm (1988)} \]

Low-oxygen habitats

The quantitatively most important sites for the mineralization of organic matter are soils, sediments and water columns of rivers, lakes and seas. In these environments the potential rate of O₂-consumption is often higher than the rate of O₂-supply. Consequently, (oxic and) anoxic areas are established, bridged by a gradient of decreasing oxygen concentration. Oxygen profiles have been documented for a wide variety of environments. The size of these gradients can extend from the micrometer-range, at eutrophic locations rich in readily metabolizable organic matter, up to above 100 meters in stratified seas or oceans (Figure 2). Oxygen-limiting conditions prevail at the interfaces of oxic and anoxic areas.

Field studies have indicated that these low-oxygen habitats are often sites of enhanced microbiological activity. The major reason probably is that strong coupling between metabolism of aerobic and anaerobic bacteria occurs at the
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Concentration

Fig. 3: Idealised example of two-dimensional cross-diffusion, resulting in microbial growth at oxic/anoxic interfaces in for example soils, sediments or stratified lakes. At the contact sheets of oxic and anoxic zones, oxygen meets products of anaerobic metabolism.

contact sheets of oxic and anoxic zones. Particularly when $O_2$ gradients are steep, aerobic and anaerobic bacteria live with very little spatial separation. During the anoxic decomposition of organic material, reduced compounds such as fermentation products, ammonium, sulphide and methane are formed. At the location where these substrates diffuse out of the anoxic zone and meet $O_2$, they are utilized by aerobic bacteria (Figure 3). Mathematical simulation of this principle of two-dimensional cross-diffusion demonstrated that it results in the formation of narrow concentrated regions of intense biological activity in which growth is limited by the rate of diffusion of the substrates. Under such circumstances substrates decrease in a linear way from their sources, become very low in the region of intense growth and are absent passed the zone of microbial growth (Koch, 1992).

A classical illustration of cross-diffusion leading to sequential depletion of electron donors and acceptors, followed by the generation of spatially separated horizons of microorganisms, is the Winogradsky column (Figure 4). With Winogradsky columns ecosystems containing various layers of phototrophic, lithotrophic and heterotrophic microbes can be simulated on a laboratory scale (Brock & Madigan, 1988). More defined laboratory models developed to study microbial growth in gradients are bidirectional linked continuous cultures, such as the gradostat and various gel-stabilized plate-systems (Herbert, 1988; Wimpenny, 1988; Wimpenny et al., 1988). Some examples of natural ecosystems containing oxic/anoxic interfaces are discussed below.

Stratified lakes

The spatial distribution of microbial communities as related to concentrations of resources has probably been studied most comprehensively in the water column of stratified lakes (Jones, 1987; McDonough et al., 1986; Wynn-Williams, 1992). In many lakes, in temperate climates particularly during summer, the circulation of water is insufficient to replenish the $O_2$ consumed during the oxidation of organic matter. The water column of a typical stratified lake can be divided in three major compartments: (1) the epilimnion, the oxic top layer (usually 5 to 10 meters) of (wind) circulated water; (2) the metalimnion, the zone of relatively sharp physical (temperature) and chemical (e.g. $O_2$, $NH_4^+$, $Mn^{2+}$, $Fe^{2+}$, $H_2S$ and $CH_4$) gradients; and (3) the hypolimnion, the
anoxic stagnant layer also stratified in response to (usually) light and the presence of electron acceptors other than O$_2$. The habitats present at these physico-chemical gradients are occupied by narrow bands of a variety of chemolithotrophic microorganisms oxidizing ammonia, iron, sulphide or methane (Figure 5). The abundance and production of anoxicogenic phototrophic and heterotrophic bacteria is also often maximal at the interface of metalimnion and hypolimnion where oxygen approaches undetectable low levels (McDonough et al., 1986; Lovell & Konopka, 1985).

**Marine waters**

A remarkable feature of the vertical distribution of O$_2$ in oceans is the dissolved oxygen minimum layer usually present between 300 and 1000 m depth (Figure 6). The O$_2$-minimum zone is probably the result of the (restricted) circulation of ocean water and the biological O$_2$-utilization during the decomposition of particulate organic matter accumulating at a depth of 800-1000 m (Karl, 1982; Millero & Sohn, 1992). Particularly in regions of high primary productivity the elevated heterotrophic microbial activity can result in a nearly complete depletion of oxygen within the O$_2$-minimum zone.

Marine waters with oxic/anoxic interfaces are present in some basins and fjords with restricted vertical circulation and in coastal environments during periods of high organic load. Examinations of the vertical distribution of microbial biomass in the Black Sea, the Cariaco Trench and the Farmvaren Fjord demonstrated the presence of thin, dense layers of viable microorganisms at the of O$_2$/H$_2$S or O$_2$/CH$_4$ interfaces. On the basis of such observations Karl (1982) suggested that the microbiological processes occurring at the oxic/anoxic interfaces are of global significance with regard to organic carbon transformations.
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Sediments

In marine and freshwater sediments O₂ penetrates from less than 1 mm in systems with high microbiological productivity such as microbial mats, to over 10 cm in sediments where the vertical stratification is disturbed by animal activity or to at least several decimeters in the bottom of the deep-sea (Andersen & Helder, 1987; Jørgensen, 1992; Lloyd et al., 1986; Revsbech & Jørgensen, 1986; Revsbech et al., 1980; Sweerts, 1990; Vischer, 1992).

Marine microbial mats are highly productive ecosystems composed of layers of oxygenic cyanobacteria, colourless sulphur bacteria, anoxygenic phototrophic bacteria and sulphate reducing bacteria, tightly packed within the top millimetres of the sediment. The distribution of oxygen consumption in the upper zone is typically characterized by a maximum in the layer of cyanobacteria, which exude oxygen and electron donors, and a second peak in a narrow zone just at the oxic/anoxic interface, due to substrates (mainly sulphide) diffusing up from the anoxic mat layers below and the supply of O₂ through diffusion from above or in situ production by oxygenic phototrophs (Figure 7) (Jørgensen et al., 1992).

Soils

Soils are composed of conglomerates of minerals (e.g. sand, clay) and organic matter in a matrix of water and gas distributed in pores. The O₂ content of soils is governed by the complex interplay between these structural and physical features (e.g. temperature) which determine the diffusive fluxes of O₂ and other substrates in the sites where microbes reside. Oxygen diffuses much (about 10⁴ times) faster through soil pores than through the interstitial pore water. The opposite is true for diffusion of most carbon-substrates. The overall microbial activity in soils (empirically measured as the rate of CO₂ formation) is at a maximum where the limiting effects of substrate diffusion and O₂-supply are equal which usually occurs at a soil volumic water content of about 0.5-0.6 m³/m³ (Myrold & Tiedje, 1985; Focht, 1992; Skopp, 1990) (Figure 8). Interestingly, this maximum of soil microbial activity coincides with the transition from oxic (e.g. nitrification) to anoxic (e.g. denitrification) microbial metabolism. Greenwood (1961) measured respiration (O₂-consumption and CO₂-production) by microbial populations in water-saturated soil crumbs supplied with different organic substrates. He observed that oxygen limitation of microbial metabolism (> 50% reduction of respiration rate) and switch over from oxic to anoxic metabolism occurred when the mean dissolved O₂-concentration became less than about 1-3 µM.

Soils can be totally oxic, e.g. many drained sands, to virtually anoxic such as in water-logged swamps and marshes where the presence of O₂ is restricted to the top layer and the proximity of roots and animal burrows (Figure 9). Aggregated soils contain a multitude of both oxic and anoxic sites. The dissolved oxygen concentration in soils drops with increasing depth and from the outside to the centre of aggregates. Anoxic centres are often present in soil aggregates of various textures with a radius larger than 1 cm (Greenwood, 1961; Sextone et al., 1985; Smith, 1977; Smith & Arah, 1986). The numerous vertical and radial O₂ gradients present in most soils permit oxic and anoxic processes to proceed simultaneously in
VOlUMETRIc WATER CONTENT, \( \beta \), (m^3/m^3)

Fig. 8: Conceptual plot of microbial activity as a function of soil water content. Also indicated by straight lines are the theoretical limits of activity posed by either the flux or total substrate (from Skopp et al., 1990).

close proximity (Skopp et al., 1990; Reddy et al., 1989). In soils containing a large quantity of aggregates the sum of the areas containing such low oxygen concentrations may constitute a relatively large fraction of the total soil volume.

The rhizosphere is often a localized area of low \( \text{O}_2 \) concentration within an either predominantly anoxic or oxic environment (Patruquin et al., 1983; Reddy, 1989; Smith, 1986). Many plants growing in water-logged soils (e.g., rice) transport \( \text{O}_2 \) from the leaves via the aerenchym through the roots into the adjacent anoxic soil. In addition, transpiration of plants, effecting a dehydration of the soil, results in an increased rate of \( \text{O}_2 \) diffusion in the soil. In contrast, microbial respiration enhanced by root exudates and also respiration by the roots themselves reduces the rhizosphere \( \text{O}_2 \) concentration. Plants profit from buffering the soil \( \text{O}_2 \) concentration at a low value because it optimizes the symbiotic (oxygen-sensitive) nitrogen fixation by bacteria such as \textit{Azospirillum} spp. living in the rhizosphere (Hill, 1988).

The rhizobia-containing root nodule systems of leguminous plants are extreme examples of microbial ecosystems with plant-regulated low \( \text{O}_2 \) tensions of less than 0.0002 atm. (approx. 0.2 \( \mu \text{M} \text{O}_2 \)) (Revsbech and Jørgensen, 1985). The tissue of these nodules contains leghaemoglobin, a high-affinity \( \text{O}_2 \)-binding protein, which facilitates the efficient transport of \( \text{O}_2 \) at low free dissolved concentration to the microaerophilic \( \text{N}_2 \)-fixing endosymbiotic bacteria (Brock & Madigan, 1988).

**Microniches in oxic environments**

Oxygen depleted microniches are present in aggregates of particulate organic matter in essentially oxic surface layers of marine- and fresh-waters, sediments and soils. These aggregates, such as marine snow or faecal pellets, are highly nutrient enriched and heavily colonized by vigorously respiring microbial communities. In a way they can be considered as oases in a desert. Oxygen gradients can be very steep resulting in anoxic regions in the centres of aggregates larger than a few millimetres. These are sites of activity of microbes requiring low concentrations of oxygen or none at all. Such activities include nitrogen fixation, denitrification, sulphate reduction and methanogenesis (Aldredge & Cohen, 1987; Jørgensen, 1977, Lloyd, 1989; Pearl, 1990; Sieburth, 1987).

**Impact of oxygen fluctuations**

In all ecosystems discussed above the \( \text{O}_2 \)-tension is not only variable in space but also in time. Shifts in \( \text{O}_2 \)-gradients are induced by physical changes such as day/night, summer/winter, wet/dry and hot/cold rhythms or biological factors such as activities of soil or sediment inhabiting animals, altering the rates of \( \text{O}_2 \)-supply and \( \text{O}_2 \)-consumption. In the zones of \( \text{O}_2 \)-limited growth these fluctuations are to some extent damped by the activity of the local microbial communities which readily adapt their metabolism to in- or decreased \( \text{O}_2 \) or carbon-substrate availability. Larger variations, however, cause those microbes located close to oxic/anoxic interfaces to be
Biodegradation of halogenated carbon compounds

The decomposition of organic compounds is not only ruled by the prevailing environmental conditions and the nature of the microbial communities, but of course also by the chemical structure of the individual molecules. For example, the rate of degradation of components from oak leaf litter decreased in the order, sugars > hemicellulose > cellulose > lignin > waxes > phenols (Minderman, 1968). Recalcitrance of natural compounds usually does not result in malfunctioning of ecosystems. In contrast, many man-made compounds not present naturally in the environment (xenobiotics) are toxic for living organisms. Particularly halogenated carbon compounds, including pesticides (e.g. DDT), insulators (e.g. polychlorinated biphenyls), chemical solvents and intermediates (e.g. chlorinated benzenes and chlorinated ethenes) or by-products (e.g. chlorinated dibenzodioxins) are known to persist in the environment, may accumulate in animals and thus are serious pollutants (Stumm & Morgan 1981). Knowledge of the microbial degradation of these chemicals is important for predicting their fate in nature and for the development of technologies for the treatment of waste streams and polluted areas.

Microorganisms transform xenobiotics either haphazardly while growing on other substrates (co-metabolism) or by utilizing them as the carbon and/or energy sources for growth (Commandeur & Parsons, 1990; Reineke W & Knackmuss, 1988; Rockkind-Dubinsky et al, 1987). There are large differences in the rates and mechanisms of biodegradation of halogenated aliphatic or aromatic compounds under oxic and anoxic conditions. When O₂ is available, particularly aromatic compounds are more readily degraded than under anoxic conditions because mono- and dioxygenases efficiently catalyse cleavage of aromatic rings. However, oxygenases are generally inhibited by halogen substituents and therefore for the oxidative catabolism of halogenated aromatics the removal of halogen substituents is critical. Consequently, aerobic bacteria usually fail to metabolize polyhalogenated compounds and in that sense the most heavily halogenated xenobiotics are particularly persistent under oxic conditions.

Under anoxic conditions polyhalogenated compounds can be reductively dehalogenated (Holliger, 1992; Kuhn & Sufita, 1989; Mohn & Tiedje, 1992). Although reductive dehalogenations usually appear to be co-metabolic reactions, some anaerobic bacteria may use chlorinated compounds as terminal electron acceptors in energy-yielding reactions. In general, increasing halogen substitution (i.e. increasing oxidation-state) of carbon compounds makes them thermodynamically more favourable for reductive dehalogenation. Indeed, in many cases heavily halogenated compounds are relatively rapidly

### Table 2:

**Some chlorinated xenobiotic compounds displaying enhanced microbial mineralization when exposed to sequential anoxic and oxic conditions.**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Synonym</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tetrachloroethylene</td>
<td>Perchloroethylene</td>
<td>Fathepure &amp; Vogel, 1991</td>
</tr>
<tr>
<td>Chloroform</td>
<td>Trichloromethane</td>
<td>Fathepure &amp; Vogel, 1991</td>
</tr>
<tr>
<td>Hexachlorobenzene</td>
<td>HCB</td>
<td>Fathepure &amp; Vogel, 1991</td>
</tr>
<tr>
<td>1,1-Bis(p-methoxyphenyl)-2,2,2-trichloroethane</td>
<td>Methoxychlor</td>
<td>Beunink &amp; Rehm, 1988</td>
</tr>
<tr>
<td>1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane</td>
<td>DDT</td>
<td>Beunink &amp; Rehm, 1990</td>
</tr>
<tr>
<td>4-Chloro-2-nitrophenol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polychlorinated biphenyls</td>
<td>PCB's</td>
<td>Abramowitz, 1990</td>
</tr>
</tbody>
</table>
dehalogenated by reduction, but unfortunately partially dehalogenated products often accumulate. These products, however, are available for further oxidative biodegradation. Consequently, sequential anoxic and oxic conditions, enabling cooperation of anaerobic and aerobic bacteria with supplementary degradative capabilities, are stimulating or even essential for complete mineralization of many polyhalogenated compounds (Table 2). Such conditions can be brought about by oxygen fluctuations or spatial separation of anaerobic and aerobic bacteria. More efficient coupling of activity of anaerobic and aerobic bacteria, performing reductive and oxidative reactions simultaneously in close cooperation, may be possible at low oxygen concentrations. This would be an important step forward in the development of treatment systems for complete biological mineralization of polychlorinated carbon compounds.

Aims and outline of this thesis

The aim of the research reported in this thesis was to obtain fundamental information on the ecology and physiology of (aerobic, facultatively anaerobic, anaerobic and microaerophilic) heterotrophic bacteria under conditions with limiting concentrations of oxygen. Furthermore, it was investigated whether the combined activity of anaerobes and aerobes can in principle be applied to the mineralization of polychlorinated carbon compounds.

The experimental approach was to analyze the behaviour of bacteria in laboratory model systems, including pure cultures, defined mixed cultures and enrichment cultures, in controlled oxygen-limiting environments simulated in chemostats.

Research was focused on (1) selection, isolation and identification of various physiological types of heterotrophic bacteria under oxygen-limitation (Chapters 2 and 5), (2) analysis of metabolic interactions between anaerobic and aerobic bacteria (Chapters 2, 3, 4 and 7), (3) physiological adaptations to various levels of oxygen-availability (Chapters 3 and 5), and (4) potential application of combined activity of anaerobes and aerobes at low oxygen-concentrations to degrade chlorinated carbon compounds (Chapters 6 and 7).

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