Environmental Biotechnology

Concepts and Applications

Edited by
Hans-Joachim Jördening and Josef Winter

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Preface

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The growing awareness of environmental problems, caused especially by the predominate use of fossil resources in connection with pure chemical pathways of production, has led the focus on those alternatives, which sounds environmentally more friendly. Here, biotechnology has the chance to influence and improve the quality of the environment and production standards by:

- introduction of renewable instead of fossil raw materials
- controlled production of very specific biocatalysts for the
- development of new and environmentally improved production technologies with less purified substrates and generation of fewer by-products
- bioproducts as non-toxic matters, mostly recyclable.

Some impressive studies on industrial applications of biotechnology are published in two OECD reports, which summarized, that biotechnology has the potential of a reduction of operational and/or capital cost for the realization of more sustainable processes (OECD1, OECD2). However, until today the sustainability of technical processes is more the exception than the rule and therefore so-called "End-of-Pipe"-technologies are absolutely necessary for the treatment of production residues.

In 1972 the Club of Rome published its study "Limits of Growth" and prognosted an upcoming shortage of energy and primary resources as a consequence of exponential growth of population and industry (Meadows et al. 1972). Although the quantitative prognoses of Dennis Meadows and his research team have not been fulfilled, the qualitative statements are today well accepted. Aside of a shortage of resources for production of commodities the limits for an ecologically and economically compatible disposal of production residues and stabilized wastes have to be more and more taken into consideration. The limits for disposal of solid and liquid pollutants in soil and water or of waste gases in the atmosphere are a major issue, since soil, water and air are no longer able to absorb/adsorb these emissions without negative consequences for ecology and life in general. The ultimate oxidation product of organic residues by incineration or – more smooth – by biological respiration in aquatic or terrestric environment led to a significant increase of the carbon dioxide content of the atmosphere in the last centuries and thus influences the overall climate. This increase is abundantly attributed to combustion of fossil fuels by traffic and of fossil fuels and coal for industrial production processes and house heating. Increasing concentrations of carbon dioxide in the atmosphere from incineration of fossil energy sources and from decomposition of organic matter are the main reason for the greenhouse effect.

Whereas the pollution of soil with waste compounds and subsequently with their (bio)conversion products generally remains a locally restricted, national problem, as long as evaporation of volatile compounds into the air or solubilization of solids in rain or groundwater can be prevented, emissions into water or the atmosphere are spreading rapidly and soon reach an international dimension. A disturbance of the equilibrium of the natural cycles of carbon, nitrogen, phosphate, sulfur or halogen compounds causes an ecological imbalance and endangers nature. In the Brundtland-report "Our common future" (Hauff 1987) a discussion was started about "sustainable development". The practical realization of this concept was suggested at the "Conference on Environment and Development" of the United Nations in Rio de Janeiro in 1992 and enforced as an action programme in the so-called Agenda 21. A sustainable development to maintain the basis for future generations is contra-indicated by exploitation of non-regenerative energy and material resources and a shortening of life cycles (e.g. in information technologies).

A life cycle assessment is required to reduce or at least to bring to everybodies attention the flood of waste material. By the obligate demand for recycling of waste components, which is fixed in European Council Directive 91/156/EEC and e.g. translated to the German waste law (KrW/AbfG 1996), production and the use of commodities should minimize the amount of wastes. The practicability of this approach must be demonstrated in industrialized countries and then should be adopted by less developed or developing countries.

Environmental biotechnology initially started with wastewater treatment in urban areas at the turn of the 19/20th century (Hartmann 1999) and has been extended among others to soil remediation, off gas purification, surface and groundwater cleaning, industrial wastewater purification, deposition techniques of wastes in sanitary landfills and composting of bioorganic residues, mainly in the second half of the 20 century.

The available processes for the protection of the terrestric and aquatic environment were summarized in the first edition of "Biotechnology" still in one volume. Some ten years later in the second edition of "Biotechnology" the development in the above mentioned environmental compartments was updated and decribed by experts in the field from Europe and the United States of America. Although the description was kept very stringent, the above mentioned areas of environmental processes finally were issued in 3 volumes. Volume 11a of "Biotechnology" was subtitled "Environmental Processes I – Wastewater Treatment" (edited in 1999) and was devoted to general aspects and the process development for carbon, nitrogen and phosphate removal during wastewater treatment and anaerobic sludge stabilization. Volume 11b of "Biotechnology" was subtitled "Environmental Processes II – Soil Decontamination" (edited in 2000) and summarized microbial aspects and the processes that were applied for soil (bio-)remediation and Volume 11 c, subtitled “Environmental Processes III – Solid Waste and Waste Gas Treatment, Preparation of Drinking water” (edited in 2000) covered general aspects, microbiology and processes for solid waste treatment, waste gas purification and potable water preparation.

The new book “Environmental Biotechnology” covers what we think the most relevant topics of the previous volumes 11 a, b and c of “Biotechnology” in a comprehensive form. The invited authors were given the opportunity to update their contributions when a significant progress was achieved in their field in recent years. For instance, although many alternatives were existing in the past for domestic sewage treatment to remove nitrogenous compounds, the development of new biological processes for nitrogen removal in the laboratory and in pilot scale-dimension was reported recently. These processes work with a minimized requirement for an additional carbon source. Although these processes are not yet widely applicated in praxi, they are investigated in detail in pilot- or demonstration-scale in single wastewater treatment plants. The results seem to be promissing and might get importance in the future.

The authors and the editors of the new book hope that the presented comprehensive overview on processes of environmental biotechnology for liquid, solid and gaseous waste treatment will help students and professional experts to obtain a fast fundamental information and an overview over the biological background and general process alternatives. This might then be a useful basis or starting point to tackle a specific process in more detail.

Josef Winter, Claudia Gallert, Hans-Joachim Jördening
Karlsruhe and Braunschweig, September 2004

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1
Bacterial Metabolism in Wastewater Treatment Systems

Claudia Gallert and Josef Winter

1.1
Introduction

Water that has been used by people and is disposed into a receiving water body with altered physical and/or chemical parameters is defined as wastewater. If only the physical parameters of the water were changed, e.g., resulting in an elevated temperature after use as a coolant, treatment before final disposal into a surface water may require only cooling close to its initial temperature. If the water, however, has been contaminated with soluble or insoluble organic or inorganic material, a combination of mechanical, chemical, and/or biological purification procedures may be required to protect the environment from periodic or permanent pollution or damage. For this reason, legislation in industrialized and in many developing countries has reinforced environmental laws that regulate the maximum allowed residual concentrations of carbon, nitrogen, and phosphorous compounds in purified wastewater, before it is disposed into a river or into any other receiving water body However, enforcement of these laws is not always very strict. Enforcement seems to be related to the economy of the country and thus differs significantly between wealthy industrialized and poor developing countries. In this chapter basic processes for biological treatment of waste or wastewater to eliminate organic and inorganic pollutants are summarized.

1.2
Decomposition of Organic Carbon Compounds in Natural and Manmade Ecosystems

Catabolic processes of microorganisms, algae, yeasts, and lower fungi are the main pathways for total or at least partial mineralization/decomposition of bioorganic and organic compounds in natural or manmade environments. Most of this material is derived directly or indirectly from recent plant or animal biomass. It originates from carbon dioxide fixation via photosynthesis (→ plant biomass), from plants that served as animal feed (→ detritus, feces, urine, etc.), or from fossil fuels or biologically or geochemically transformed biomass (→ peat, coal, oil, natural gas). Even the carbon portion of some xenobiotics can be tracked back to a biological origin, i.e., if these substances were produced from oil, natural gas, or coal. Only because the mineralization of carbonaceous material from decaying plant and animal biomass in nature under anaerobic conditions with a shortage of water was incomplete, did the formation of fossil oil, natural gas, and coal deposits from biomass occur through biological and/or geochemical transformations. The fossil carbon of natural gas, coal, and oil enters the atmospheric CO_2 cycle again as soon as these compounds are incinerated as fuels or used for energy generation in industry or private households.

Biological degradation of recent biomass and of organic chemicals during solid waste or wastewater treatment proceeds either in the presence of molecular oxygen by respiration, under anoxic conditions by denitrification, or under anaerobic conditions by methanogenesis or sulfidogenesis. Respiration of soluble organic compounds or of extracellularly solubilized biopolymers such as carbohydrates, proteins, fats, or lipids in activated sludge systems leads to the formation of carbon dioxide, water, and a significant amount of surplus sludge. Some ammonia and H_2S may be formed during degradation of sulfur-containing amino acids or heterocyclic compounds. Oxygen must either be supplied by aeration or by injection of pure oxygen. The two process variant for oxygen supply differ mainly in their capacity for oxygen transfer and the stripping efficiency for carbon dioxide from respiration. Stripping of carbon dioxide is necessary to prevent a drop in pH and to remove heat energy. Respiration in the denitrification process with chemically bound oxygen supplied in the form of nitrate or nitrite abundantly yields dinitrogen. However, some nitrate escapes the reduction to dinitrogen in wastewater treatment plants and contributes about 2% of the total N_2O emissions in Germany (Schön et al., 1994). Denitrifiers are aerobic organisms that switch their respiratory metabolism to the utilization of nitrate or nitrite as terminal electron acceptors, if grown under anoxic conditions. Only if the nitrate in the bulk mass has been used completely does the redox potential become low enough for growth of strictly anaerobic organisms, such as methanogens or sulfate reducers. If anaerobic zones are allowed to form in sludge flocs of an activated sludge system, e.g., by limitation of the oxygen supply, methanogens and sulfate reducers may develop in the center of sludge flocs and form traces of methane and hydrogen sulfide, found in the off-gas.

Under strictly anaerobic conditions, soluble carbon compounds of wastes and wastewater are degraded stepwise to methane, CO_2, NH_3, and H_2S via a syntrophic interaction of fermentative and acetogenic bacteria with methanogens or sulfate reducers. The complete methanogenic degradation of biopolymers or monomers via hydrolysis/fermentation, acetogenesis, and methanogenesis can proceed only at a low H_2 partial pressure, which is maintained mainly by interspecies hydrogen transfer. Interspecies hydrogen transfer is facilitated when acetogens and hydrogenolytic methanogenic bacteria are arranged in proximity in flocs or in a biofilm within short diffusion distances. The reducing equivalents for carbon dioxide reduction to methane or sulfate reduction to sulfide are derived from the fermentative metabolism, e.g., of clostridia or *Eubacterium* sp., from β oxidation of fatty acids, or the oxidation of alcohols. Methane and CO_2 are the main products in anaerobic environments where sulfate is absent, but sulfide and CO_2 are the main products if sulfate is present.

1.2.1
Basic Biology, Mass, and Energy Balance of Aerobic Biopolymer Degradation

To make soluble and insoluble biopolymers – mainly carbohydrates, proteins, and lipids – accessible for respiration by bacteria, the macromolecules must be hydrolyzed by exoenzymes, which often are produced and excreted only after contact with respective inductors. The exoenzymes adsorb to the biopolymers and hydrolyze them to monomers or at least to oligomers. Only soluble, low molecular weight compounds (e.g., sugars, disaccharides, amino acids, oligopeptides, glycerol, fatty acids) can be taken up by microorganisms and be metabolized for energy production and cell multiplication.

Once taken up, degradation via glycolysis (sugars, disaccharides, glycerol), hydrolysis and deamination (amino acids, oligopeptides), or hydrolysis and β oxidation (phospholipids, long-chain fatty acids) proceeds in the cells. Metabolism of almost all organic compounds leads to the formation of acetyl-CoA as the central intermediate, which is used for biosyntheses, excreted as acetate, or oxidized to CO_2 and reducing equivalents in the tricarboxylic acid (TCA) cycle. The reducing equivalents are respired with molecular oxygen in the respiration chain. The energy of a maximum of only 2 mol of anhydridic phosphate bonds of ATP is conserved during glycolysis of 1 mol of glucose through substrate chain phosphorylation. An additional 2 mol of ATP are formed during oxidation of 2 mol of acetate in the TCA cycle, whereas 34 mol ATP are formed by electron transport chain phosphorylation with oxygen as the terminal electron acceptor. During oxygen respiration, reducing equivalents react with molecular oxygen in a controlled combustion reaction.

When carbohydrates are respired by aerobic bacteria, about one third of the initial energy content is lost as heat, and two thirds are conserved biochemically in 38 phosphoanhydride bonds of ATP. In activated sludge reactors or in wastewater treatment ponds that are not loaded with highly concentrated wastewater, wall irradiation and heat losses with the off-gas stream of aeration into the atmosphere prevent self-heating. In activated sludge reactors for treatment of highly concentrated wastewater, however, self-heating up to thermophilic temperatures may occur if the wastewater is warm in the beginning, the hydraulic retention time for biological treatment is short (short aeration time), and the air or oxygen stream for aeration is restricted so as to supply just sufficient oxygen for complete oxidation of the pollutants (small aeration volume).

The conserved energy in the terminal phosphoanhydride bond of ATP, formed during substrate chain and oxidative phosphorylation by proliferating bacteria is partially used for maintenance metabolism and partially for cell multiplication. Partitioning between both is not constant, but depends on the nutritional state. In highly loaded activated sludge reactors with a surplus or at least a non-growth–limiting substrate supply, approximately 50% of the substrate is respired in the energy metabolism of the cells and 50% serves as a carbon source for cell growth (Table 1.1). The biochemically conserved energy must be dissipated to be used for the maintenance metabolism of existing cells and cell growth.

If the substrate supply is growth-limiting, e.g., in a low-loaded aerobic treatment system, a higher proportion of ATP is consumed for maintenance, representing the energy proportion that bacteria must spend for non-growth–associated cell survival metabolism, and less energy is available for growth. Overall, more of the substrate carbon is respired, and the ratio of respiration products to surplus sludge formed is higher, e.g., around 70% : 30% (Table 1.1). In a trickling filter system, an even higher proportion of the substrate seems to be respired. This might be due to protozoa grazing off part of the biofilm.

For comparison, Table 1.1 also summarizes carbon dissipation in anaerobic methanogenic degradation. Only about 5% of the fermentable substrate is used for cell growth (surplus sludge formation) in anaerobic reactors, whereas 95% is converted to methane and CO_2, and most of the energy of the substrates is conserved in the fermentation products.

1.2.1.1 Mass and Energy Balance for Aerobic Glucose Respiration and Sewage Sludge Stabilization

In most textbooks of microbiology, respiration of organic matter is explained by Eq. 1, with glucose used as a model substance. Except for an exact reaction stoichiometry of the oxidative metabolism, mass and energy dissipation, if mentioned at all, are not quantified. Both parameters are, however, very important for activated sludge treatment plants. The surplus sludge formed during wastewater stabilization requires further treatment, causes disposal costs, and – in the long run – may be an environmental risk, and heat evolution during unevenly high-loaded aerobic treatment may shift the population toward more thermotolerant or thermophilic species and thus, at least for some time, may decrease the process efficiency.

$$1\ \text{mol}\ C_6H_{12}O_6 + 6\ \text{mol}\ O_2 \rightarrow 6\ \text{mol}\ CO_2 + 6\ \text{mol}\ H_2O + \text{heat energy} \qquad (1)$$

Table 1.1 Carbon flow during (A) aerobic degradation in an activated sludge system under (a) saturating and (b) limiting substrate supply[a] and during (B) anaerobic degradation.

(A)	Aerobic degradation:
	(a) Saturating substrate supply = high-load conditions
	1 unit substrate carbon → 0.5 units CO_2 carbon + 0.5 units cell carbon
	(b) Limiting substrate supply = low-load conditions
	1 unit substrate carbon → 0.7 units CO_2 carbon + 0.3 units cell carbon
(B)	Anaerobic degradation:
	1 unit substrate carbon → 0.95 units (CO_2 + CH_4) carbon + 0.05 units cell carbon

[a] Estimated from surplus sludge formation in different wastewater treatment plants.