- 122 Tiedge, H., and Schäfer, G., Symmetry in F<sub>1</sub>-type ATPases. Biochim. biophys. Acta 977 (1989) 1-9.
- 123 Tiedge, H., Schäfer, G., and Mayer, F., An electron microscopic approach to the quaternary structure of mitochondrial F<sub>1</sub>-ATPase. Eur. J. Biochem. 132 (1983) 37-45.
- 124 Tsuprun, V. L., Mesyanzhinova, I. V., Kozlov, I. A., and Orlova, E. V., Electron microscopy of beef heart mitochondrial F<sub>1</sub>-ATPase. FEBS Lett. 167 (1984) 285-290.
- 125 Velours, J., Esparza, M., Hoppe, J., Sebald, W., and Guerin, B., Amino acid sequence of a new mitochondrially synthesized proteolipid of the ATP synthase of Saccharomyces cerevisiae. EMBO J. 3 (1984) 207-212.
- 126 Verburg, J. G., and Allison, W. S., Tyrosine α244 is derivatized when the bovine heart mitochondrial F<sub>1</sub>-ATPase is inactivated with 5'-pfluorosulfonylbenzoylethenoadenosine. J. biol. Chem. 265 (1990) 8065-8074.
- 127 Vignais, P. V., and Lunardi, J., Chemical probes of the mitochondrial ATP synthesis and translocation. A. Rev. Biochem. 54 (1985) 977– 1014.
- 128 Vogel, P. D., and Cross, R. L., Adenine nucleotide-binding sites on mitochondrial F<sub>1</sub>-ATPase. Evidence for an adenylate kinase-like orientation of catalytic and noncatalytic sites. J. biol. Chem. 266 (1991) 6101-6105.
- 129 Von Meyenburg, K., Jorgensen, B. B., Nierlsen, J., Hausen, F. G., and Michelson, O., The membrane-bound ATP synthase of *Escherichia coli*: a review of structural and functional analysis of the *atp* operon. Tokai exp. clin. Med. 7 (1982) 23-31.
- 130 Wagner, R., Ponse, G., and Strotmann, H., Binding of 2'(3')-O-(2,4,6-trinitrophenyl)-adenosine-5'-diphosphate opens the pathway for protons through the chloroplast ATPase complex. Eur. J. Biochem. 161 (1986) 205-209.
- 131 Walker, J. E., Saraste, M., and Gay, N. J., The unc operon. Nucleotide sequence, regulation and structure of ATP-synthase. Biochim. biophys. Acta 768 (1984) 164-200.
- 132 Walker, J. E., Lutter, R., Dupuis, A., and Runswick, M. J., Identification of the subunits of F<sub>1</sub>-F<sub>0</sub>-ATPase from bovine heart mitochondria. Biochemistry 30 (1991) 5369-5378.
- 133 Wang, J. H., Functionally distinct β subunits in F<sub>1</sub>-adenosine triphosphatase. J. biol. Chem. 260 (1985) 1374-1377.
  134 Webb, M. R., Grubmeyer, C., Penefsky, H. S., and Trentham, D. R.,
- 134 Webb, M. R., Grubmeyer, C., Penefsky, H. S., and Trentham, D. R., The stereochemical course of phosphoric residue transfer catalyzed by beef heart mitochondrial ATPase. J. biol. Chem. 255 (1980) 11637-11639.

- 135 Weber, J., Lücken, U., and Schäfer, G., Total number and differentiation of nucleotide binding sites on mitochondrial F<sub>1</sub>-ATPase. An approach by photolabeling and equilibrium binding studies. Eur. J. Biochem. 148 (1985) 41-47.
- 136 Weber, J., Schmitt, S., Grell, E., and Schäfer, G., Differentiation of the nucleotide-binding sites on nucleotide-depleted mitochondrial F<sub>1</sub>-ATPase by means of a fluorescent ADP analogue. J. biol. Chem. 265 (1990) 10884-10892.
- 137 Williams, N., Hullihen, J.M., and Pedersen, P.L., The proton adenosine triphosphatase complex of rat liver mitochondria. Temperature-dependent dissociation-reassociation of the F<sub>1</sub>-ATPase subunits. Biochemistry 23 (1984) 780-785.
- 138 Wise, J. G., Site-directed mutagenesis of the conserved β subunit tyrosine 331 of *Escherichia coli* ATP synthase yields catalytically active enzymes. J. biol. Chem. 265 (1990) 10403-10409.
- 139 Wise, J. G., and Senior, A. E., Catalytic properties of the *Escherichia coli* proton adenosine triphosphatase: evidence that nucleotide bound at noncatalytic sites is not involved in regulation of oxidative phosphorylation. Biochemistry 24 (1985) 6949-6954.
- 140 Wong, S.-Y., Matsuno-Yagi, A., and Hatefi, Y., Kinetics of ATP hydrolysis by F<sub>1</sub>-ATPase and the effects of anion activation, removal of tightly bound nucleotides, and partial inhibition of the ATPase by covalent modification. Biochemistry 23 (1984) 5004-5009.
- 141 Xue, Z., and Boyer, P. D., Modulation of the GTPase activity of the chloroplast F<sub>1</sub>-ATPase by ATP binding at non-catalytic sites. Eur. J. Biochem. 179 (1989) 677-681.
- 142 Yoshida, M., The synthesis of enzyme-bound ATP by the F<sub>1</sub>-ATPase from the thermophilic bacterium PS3 in 50% dimethyl sulfoxide. Biochem. biophys. Res. Commun. 114 (1983) 907-912.
- 143 Yoshida, M., and Allison, W.S., Characterization of the catalytic and noncatalytic ADP binding sites of the F<sub>1</sub>-ATPase from the thermophilic bacterium PS3. J. biol. Chem. 261 (1986) 5714– 5721

0014-4754/92/040351-12\$1.50 + 0.20/0 © Birkhäuser Verlag Basel, 1992

## **Mini-Review**

### The importance of microbiology in waste management

M. Gandolla a and M. Aragno b

<sup>a</sup> Ente Smaltimento Rifiuti del Sottoceneri (ESR), CH-6934 Bioggio, and <sup>b</sup>Laboratoire de Microbiologie de l'Université, P.O. Box 2, CH-2007 Neuchâtel (Switzerland)

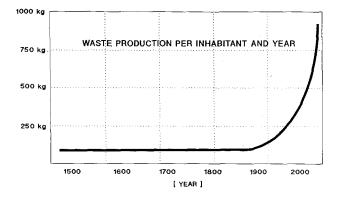
Abstract. Until a hundred years ago, the waste products from human activities were returned into the environment and underwent the biosphere's natural elimination processes without there being any long-term charge on the environment. During the last century, the increase in the amount of refuse has been accompanied by a decrease in its quality, mainly due to the production and dispersal of heavy metals and xenobiotic compounds. Both useful and noxious microbial processes have been underestimated in applied research in the field of waste management which, until now, has dealt mainly with artificial technologies. This paper presents some examples of microbiological processes occurring in waste treatment, particularly dumping, waste incineration, composting and biomethanization. Key words. Microbiology; aerobic processes; anaerobic processes; waste disposal; landfills; landfill topsoil; biogas; biomethanization; percolating waters; tetrachloroethylene, anaerobic biodegradation; vinyl chloride, anaerobic production; incineration; biofilter; composting; Aspergillus fumigatus.

#### Introduction

In the past, mankind was much better integrated into the biosphere's natural cycles. His wastes were generally recycled by the environment without major problems. Then, mainly in the last hundred years, this equilibrium was upset by three causes with synergetic effects:

- The dramatic increase in population;
- the widespread utilization, followed by diffusion into the environment, of toxic metals previously kept out of the biosphere because they were concentrated in ores;
- the tremendous increase in the production and dispersion of xenobiotic compounds. These organic chemical compounds differ so much from those of biological origin that they are biodegradable at best with difficulty, often not at all.

Thus, while the amount of waste grew, the possibility of its being returned to the environment without causing damage decreased considerably (fig. 1). The present situation is critical <sup>1</sup>, and urgent interventions are necessary. Until recently, man wrongly assumed that he was able to manage and control his environment, substituting technological means for nature. The failure of this concept is now becoming more and more evident. Our knowledge of natural processes, which could help us to integrate our activities in them, is still insufficient. A typical case is the subject of this paper, microbiological aspects of waste management.



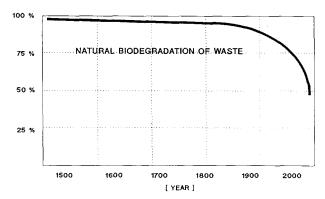


Figure 1. Tendencies in the evolution of the amount and biodegradability of waste produced by mankind <sup>1</sup>.

#### The present situation

Whatever kind of waste treatment is used, a mass equal to the initial waste mass will be returned to the environment. Waste disposal into the environment occurs in two ways: either by dispersal of the derivatives into the biosphere (sediments, soil, water, air), or by concentration (e.g. in landfills), in order to exclude them from the biosphere. Dispersal should only be allowed if it is environmentally acceptable in the long term. Therefore, waste treatment ideally has a double aim: to produce derivatives whose dispersal is acceptable (e.g. composts, certain gases), and to concentrate the dangerous compounds (e.g. heavy metals) and isolate them more or less indefinitely from the biosphere.

More than 90% of the mass of urban waste in the world is deposited in more or less controlled landfills; less than 10% is incinerated. The situation is the opposite in Switzerland, where about 85% of the total urban waste is incinerated, whereas less than 15% is put in landfills. However, incineration involves the dumping of the residue, which amounts to about 25% of the initial waste volume.

The procedures applied in waste management technology are of three types: physical (sorting, compacting); chemical (combustion, chemical treatment of gaseous and liquid emissions); and biological (composting, anaerobic digestion, biofiltration). Biological processes can either be undesirable, and have to be controlled and minimized, or they are necessary, and should be used and optimized.

### Microbiological processes in landfills

A landfill containing organic material of biological origin (paper, cardboard, domestic, agricultural and some types of industrial wastes) can be compared to a huge bioreactor, in which biological degradations will occur, either aerobically or anaerobically depending on the way the dumping is conducted <sup>2</sup>.

Modern, compacted landfills mainly behave as anaerobic bioreactors, in which a highly structured microbial association, the methanogenic syntrophy, converts the anaerobically degradable materials into a mixture of methane and carbon dioxide. It is necessary to drain this gas to prevent biogas migration and the formation, outside the waste mass, of mixtures of air and biogas, which present combustion or even explosion hazards and the risks of asphyxia either through lack of oxygen or through excess of carbon dioxide. This drainage gas also constitutes an interesting renewable energy source.

Optimization of the functioning of the methanogenic microflora is necessary in order to accelerate the stabilization of the waste mass, and to avoid accumulation or emission into the percolating water and into the atmosphere of the low molecular weight organic intermediates characteristic of incomplete degradations. In normally managed landfills, however, the biological activity is far from optimal, owing to the coarse heterogeneity of the

material deposited, the scarcity of water, and the lack of available nitrogen and phosphorus compounds.

The bioreactor properties of the landfill can be optimized or at least improved in various ways <sup>3</sup>. Sorting out the organic fraction of the waste, grinding it, adding material rich in nitrogen and phosphorus (such as sewage sludge) and inoculating the whole mass with active biomass (either by adding freshly digested sewage sludge or by recirculating the percolating water) will result in a higher production of better quality biogas, a decrease of the liquid and volatile emissions, and an overall shortening of the time necessary to stabilize the landfill biologically, or at least to reduce its activity to a level where it can be controlled by spontaneous processes.

The optimization also results in the purification of the percolating water by the landfill itself. On leaving it, the water is fairly stabilized biologically, and will mainly need chemical treatment. Moreover, the existence of a balanced methanogenic community strongly reduces the concentration of volatile fatty acids which are intermediates in the methanization process. This helps to maintain a neutral pH, thus avoiding the solubilization of most heavy metals and fixing them in the landfill.

The surface of the landfill in contact with the atmosphere, generally a soil cover, should act as an aerobic biofilter, able to oxidize methane and volatile compounds diffusing from the inside of the landfill. This biofilter effect must be optimized so that the soil cover can take over from the active draining processes as soon as possible after landfill completion, once the drainage of the gas is no longer economic (fig. 2).

Thermogenic, aerobic processes may occur spontaneously at the periphery of the landfill, either due to composting of organic material following contact with air, or to biological oxidation of methane/air mixtures. Such events can be dangerous, because the high temperatures reached  $(60-80\,^{\circ}\text{C})$  could damage the material used for isolating the landfill, and even lead to autocombustion processes. A better understanding of these hazards could help in avoiding them (fig. 3).

The responses of the microbial community in the landfill to the introduction of xenobiotic compounds is generally not known. Such a compound might be toxic to some members of the community, and inhibit the part they normally play in the degradation process. The compound might be degraded, and the normal activity restored more or less rapidly. On the other hand, it might remain undegraded, or degradation might be partial, sometimes leading to the production of still more toxic substances. This was observed, for example, with tetrachloroethylene, a widely used compound of low toxicity. In the presence of an active, anaerobic methanogenic flora, it is sequentially converted into tri- and dichlorinated compounds, and finally to the highly toxic vinyl chloride<sup>4</sup>. Biotests for the detection of such phenomena, in order to decide whether a compound can be deposited in a landfill or not, are essential.

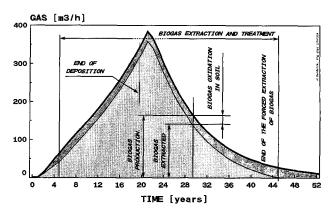


Figure 2. Example of the evolution of biogas production and extraction during and after the period when a landfill was in use.



Figure 3. Hot vent caused by a spontaneous aerobic composting process in the neighbourhood of an old landfill.

Other compounds that must definitely not be deposited in a landfill are antibiotics. Although they are generally biodegradable, their presence can lead to the selection and diffusion of resistent bacterial strains which could eventually transfer their resistance to potential pathogens.

Acute or opportunistic pathogens – fungi, bacteria and viruses – could survive or even develop in a landfill environment, from which they could be dispersed by the wind or by animals (birds or rodents), or inhaled by workers on the site. This is particularly important in the case of epidemic infectious diseases, such as pig plague or bovine spongiform encephalopathy, where large amounts of infectious waste have to be disposed of. It is a matter for concern that only in a few regions in the world is it possible to eliminate such waste correctly.

The material utilized for waterproofing the bottom of the landfill, in order to avoid contamination of the groundwater, can also in the long term be subject to biodegradation, either aerobic or anaerobic <sup>5, 6</sup>.

Microbiological problems linked to incineration

Problems similar to those of a landfill could also arise from the garbage tank of an incineration plant, where potential pathogens present in the waste (e.g. from hospitals or laboratories) might find favorable conditions.

During the short-term storage of the waste mass in a container prior to incineration, significant amounts of molecular hydrogen (more than 20% v/v) may be produced. This appears to be a biological phenomenon, occurring during the transition phase between aerobic and anaerobic degradation <sup>7</sup>. In this way, explosive hydrogen/air mixtures might be formed. Similar hydrogen production has also been observed in slag heaps from incinerators, although the origin of the hydrogen (chemical or biological) has not yet been ascertained. Molecular hydrogen could be removed from the container gases by means of a biofilter, in which aerobic, hydrogen-oxidizing (knallgas) bacteria would be responsible for its oxidation to water <sup>7,8</sup>.

The incineration process applied to organic waste rarely allows a complete mineralization of the materials. In general the ashes and the slag still contain several percent of organic materials, which will undergo further biological degradation after deposition or even after the utilization of these materials, e.g. for road construction. Not only an incomplete mineralization, but a net synthesis of organic compounds of unknown biodegradability can also take place during the incineration process. For example, this seems to be the case with organochlorines <sup>9</sup>.

### Composting

Composting is an aerobic, often thermogenic degradation process, producing a stabilized fraction of humigenic material that can be recycled in agricultural and other soils as fertilizer. In order to obtain a good quality compost, respecting the tolerance limits for heavy metals and other environmental pollutants, it is necessary to use uncontaminated substrates, like home-sorted kitchen wastes or garden and forest wastes.

The quality of the compost depends on the substrate(s) and on the process itself. The classical composting system in heaps is simple and economical; it can be used on a small scale within the community. However, it can lead to environmental problems, such as groundwater pollution or, if not well managed, to the emission of noxious odors.

Another aspect of heap-composting must be seriously taken into account. At temperatures between 40 and 55 °C, there is a massive development of thermophilic fungi, including the cellulolytic *Aspergillus fumigatus* <sup>10</sup>. *A. fumigatus* is known to be a powerful allergen, and even an opportunistic pathogen that can provoke lung aspergillosis or aspergilloma. Concentrations of *A. fumigatus* colony forming units (cfu) up to 10<sup>7</sup> g<sup>-1</sup> dry weight were measured in 1 to 2-month-old compost heaps (table). During the turning over of these heaps, cfu concentra-

Mean concentration of Aspergillus fumigatus in 1-2-month-old organic waste compost heaps at different depths. Data from Beffa et al. 10

| Depth[cm] | Temperature [°C] | A. fumigatus<br>[cfu/g d. wt] |
|-----------|------------------|-------------------------------|
| 0-10      | 33               | 15.0*10 <sup>6</sup>          |
| 25-35     | 50               | 4.8*104                       |
| 55-65     | 70               | < 20                          |
| 95-105    | 53               | < 20                          |

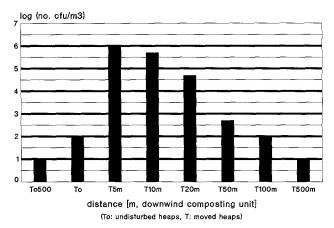


Figure 4. Concentration (colony forming units (cfu)/m³) of Aspergillus fumigatus in the air, downwind from displaced compost heaps. From Beffa et al. 10.

tions in the air as high as  $10^6/\text{m}^3$  were recorded (fig. 4). The normal concentration is between 0 and  $100 \text{ cfu/m}^3$ . Although it requires a more sophisticated technology and cannot be applied to small-scale plants, composting in a bioreactor eliminates several of the problems caused by heap-composting. The introduction of air under pressure allows a better control of the temperature which, as a rule, is higher and more uniform in the whole mass. The time necessary to reach stabilization is shortened, and the volume and ground area needed are greatly reduced. The emission of odors is easier to control, e.g. by biofilters. A procedure in two phases is, however, necessary, as the high temperature reached in the first phase excludes some essential microbial activities, such as nitrification. These will only take place during a post-composting maturation phase, at a lower temperature.

In a high-temperature bioreactor, A. fumigatus is not only inhibited, but is also killed by the heat, and will not be found during the first part of the process. It could develop during the lower temperature maturation phase, but such high spore numbers will not be reached, probably because of the exhaustion in this phase of most of the substrates it could metabolize.

# Biomethanization

Organic wastes with a relatively low content of ligneous material, such as plant kitchen wastes, fruit and vegetables wastes from agriculture and the canning industry, as well as manure from industrial farming, and in some cases sewage sludge, could be advantageously treated by a biomethanization process <sup>11</sup>. In principle, this procedure should take place in a liquid suspension, either at medium (35 °C) or high (60 °C) temperatures. The advantages of this method are the production of a high quality biogas as well as hygienization, deodorization and a relative stabilization of the waste mass. The residue can be combined with ground ligneous material before composting. The time required for compost stabilization is then much shortened.

To function correctly, biomethanization involves the use of thermally regulated biodigesters. The substrate composition (e.g. dry weight, nitrogen, carbon and phosphorus contents), should be optimized and the biologically important parameters (pH, volatile fatty acids, gas composition) continuously or regularly monitored. This requires qualified personnel. Such a system is therefore only economical if used on a large scale.

#### Conclusion

Environmentally acceptable waste management is essential for the long-term maintenance of our civilization. Every human activity results in the production of refuse. During this century, mainly during the last decades, there has been a dramatic growth of waste production induced by an uncontrolled 'progress'. However, the main problem is not the quantitative increase in waste, but the modification of its nature and composition. The production, widespread distribution and dispersal into the environment of new compounds, often xenobiotics, has unavoidably led to a drastic change in the composition of wastes, making their return to the environment difficult. A major complication is that this development does not only concern special industrial refuse, but also both solid and liquid wastes from urban communities, so that the treatment of such wastes by conventional systems becomes problematic.

During the last decades, it has generally been thought that technology could bring solutions to any type of problem, and particularly to environmental ones. Because of such ideas, the study of natural processes was largely neglected. This was particularly evident in the treatment of solid urban wastes. The importance of microbiological research in this field was considerably underestimated.

A better knowledge of the microbiological processes which actually occur or might potentially intervene in waste management systems would lead to their optimization. In consequence, the environmental load could be reduced, and at the same time the efficiency and security of the treatment procedures could be improved.

However, the necessity for an interdisciplinary approach by microbiologists, agronomists, medical scientists, engineers and chemists, is not yet sufficiently acknowledged by most of the national and international associations and commissions, such as the International Solid Waste Association (ISWA) and the Swiss Federal Wastes Commission. A widespread consciousness of the importance of microbiology for waste management and environmental protection in general is urgently needed.

Acknowledgments. The authors would like to acknowledge the participation in the *A. fumigatus* research project of Trello Beffa, Pierre Goumovski, Bibiane Schlunegger, Paolo Selldorf, and Roland Stettler, members together with the authors of the GCME (Groupe Compost-Médecine-Environnement), and also the correction of the manuscript by Catherine Fischer. This research was in part sustained by funds from the ESR, Bioggio, and from the Swiss National Science Foundation. The subject matter of this paper was presented during the 50th Annual Congress of the Swiss Society of Microbiology, Basel 1991.

- 1 Swiss National UNESCO Commission. The urban waste: a global problem. Berne 1990.
- 2 Aragno, M., in: The Landfill, Reactor and Final Storage, pp. 15-38. Lecture Notes in Earth Sciences, vol. 20. Ed. P. Baccini. Springer Verlag, Heidelberg 1989.
- 3 Gandolla, M., and Grabner, E., Wasser, Energie, Luft 75 (1983) 241.
- 4 Maspoli, G., Test di degradazione anaerobica di rifuiti speciali previsti per il deposito in discariche controllate di rifuiti solidi urbani. Diploma Work, University of Neuchâtel (CH) 1991.
- 5 Brunner, C., Wolf, M., and Bachofen, R., FEMS Microbiol. Lett. 43 (1987) 337.
- 6 Wolf, M., Mikrobieller Abbau von Bitumen. Ph.D. thesis, Zürich University, Zürich 1988.
- 7 Dugnani, L., Wyrsch, I., Gandolla, M., and Aragno, M., FEMS Microbiol. Ecol. 38 (1986) 347-351.
- 8 Dugnani, L., Wyrsch, I., Gandolla, M., and Aragno, M., Rifiuti Solidi 1 (1987) 163–168.
- 9 Lange, M., Proc. of the congress Dioxin- und Nox Minimierungstechniken, München, 20–21 Sept. 1990.
- 10 Beffa, T., Selldorf, P., Gumovski, P., Dunoyer-Geindre, J., Stettler, R., Schlunegger, B., Gandolla, M., and Aragno, M., Proc. 50th Annual Congress, Swiss Society of Microbiology, Basel 1991.
- 11 Glauser, M., Gandolla, M., and Aragno, M., in: Bioenvironmental Systems, vol. 3, pp. 143-225. Ed. D. Wise. CRC Press, Boca Raton (USA) 1987.

0014-4754/92/040362-05\$1.50 + 0.20/0 © Birkhäuser Verlag Basel, 1992