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Gas metabolism of microorganisms

R. Bachofen

Institute of Plant Biology, University of Zürich, Zollikerstr. 107, CH-8008 Zürich (Switzerland)

Summary. Microorganisms produce or consume various gases, all found in the atmosphere. All are members of the biogeochemical cycles of carbon, hydrogen, nitrogen, oxygen, and sulfur. Environmental conditions favoring gas production and consumption are discussed in general and also with respect to repository conditions.
Key words. Gas; oxygen; hydrogen; carbon dioxide; methane; nitrogen oxides; sources; sinks.

As early as 100 years ago studies on bacterial metabolism provided evidence of a great metabolic diversity of the various microorganisms investigated. Among the substrates and metabolic products a few are of gaseous nature or readily volatile at ambient temperature. All of these compounds are found in the atmosphere at least in trace amounts. Some of these gases play an important role in the global climatic changes occurring at the present time. The concentration of the various gases in the atmosphere is determined by the activity of the individual sources and sinks of the compounds, which may be of physical, chemical or biological nature. Several general reviews or symposia on microbial gas generation have been published[11,33,35].

Little information is available on the microbial activities of mixed populations in natural environments including repositories for specific wastes and over long time periods[16]. Microorganisms are known to be present in all habitats, they have been found even in rocks, oil wells or in deep subsoil. In some of these environments microorganisms must have been separated from the surface for millions of years and have survived until their isolation in present times. Their metabolism may differ from microorganisms living in recent habitats. In soil, microbial activities were recently found far below the surface[8,20]. Therefore, it must be concluded that no sterile natural habitats exist on earth except probably for very hot active volcanic environments. To estimate the metabolic activities in a repository, soil systems may be taken as good models[30].

The gases found in the soil atmosphere are in principle the same as the ones in the atmosphere, mainly N_2, O_2 and CO_2. Besides these, trace gases from various microbial activities are present. A list of the gases produced or consumed by microorganisms and the respective metabolic pathways are given in table 1. All these gases are members of the quantitative dominant biogeochemical cycles of the elements C, N and S. Landfill gas has been analyzed. Besides the atmospheric gases listed in table 1, a wide range of volatile trace compounds has been found including various hydrocarbons, alcohols, esters, terpenes and chlorinated organic substances. Not all of these originate from microbial metabolism. The main difference between these semiaerobic systems and the soil atmosphere is the high proportion of CO_2 (30–40%) and methane (40–50%) while oxygen may be as low as 1%[37].

Table 1. Gases produced or consumed by microorganisms. Rates of global production and consumption are given in 10^{14} g/year[4,36].

Gas	Production	Consumption
H_2	0.01–0.07	1.2
O_2	10^3*	10^3
Carbon compounds		
CO_2	$3 \cdot 10^3$	$3 \cdot 10^3$*
CH_4	5.3–8.3	?
CO	0.2–1.2	5.0
Hydrocarbons	?	?
Ethylene	?	0.07 (for USA)
Volatile fatty acids	?	?
Nitrogen compounds		
N_2	?	1.75
N_2O	1.6–2.6	?
NO_x	0.77	6.0
NH_3	11.9	?
Sulfur compounds		
H_2S	0.98	?
SO_2	?	?
$(CH_3)_2S$	?	?
$(C_2H_5)_2S$	?	?

* by all phototrophic organisms.

In aerated soil oxygen concentration hardly drops below 18% and CO_2 may increase up to 2%. When diffusion is limited by the texture of the material and high moisture combined with high microbial activities, oxygen will, however, rapidly become limiting and CO_2 will rise to 10% or more. The solubility of gases in water is an essential property determining the concentration and distribution of these compounds in the system. Substances that are ionized in water, such as CO_2, NH_3, or H_2S, are more soluble than gases like O_2 or N_2 and may therefore accumulate in the liquid phase (table 2). The metabolism of a microbial population may change from aerobic to anaerobic at O_2 concentrations under 1%. However, in an inhomogeneous system like soil or repository material, even well-aerated aggregates larger than 3 mm will be inhomogeneous and probably anoxic in the center. Therefore, anaerobic processes like denitrification and sulfate reduction are often observed in obviously aerobic systems suggesting the presence of anaerobic microenvironments[44]. Interestingly enough, obligate anaerobic bacteria can often be isolated in high numbers even in the uppermost layers of soil.

Table 2. Diffusion constants in air and water and solubility of atmospheric gases in water at 20 °C[13].

	Diffusion constant ($cm^2 \cdot s^{-1}$)		Solubility in water
	Air	Water	($ml \cdot ml^{-1}$)
CO_2	0.161	$0.177 \cdot 10^{-4}$	0.878
CO	0.153		0.023
O_2	0.205	$0.180 \cdot 10^{-4}$	0.031
N_2	0.205	$0.164 \cdot 10^{-4}$	0.015
N_2O			1.300
NO			0.073
NH_3			0.899 ($g \cdot ml^{-1}$)
H_2	0.349	$0.634 \cdot 10^{-4}$	0.019
H_2S		$0.177 \cdot 10^{-4}$	0.044

Data on microbial reaction rates usually originate from pure culture studies in the laboratory. Such experiments may provide excellent information on pathways and enzymatic reactions. However, they are hardly useful for the quantitative estimation of the reactions occurring in a natural habitat with constantly changing physical and chemical environmental conditions. This is especially true for the gas metabolism of microbial populations. Field data under natural conditions are available only for a few of the gases where sensitive instruments and an adequate sampling technique have been developed.

The carbon cycle (fig. 1) contains the gases CO_2, CO, and CH_4 and is strongly coupled with the cycles of oxygen and hydrogen. CO_2 and carbon in living or dead biomass are the most actively cycled carbon reservoirs, while carbonaceous rocks and fossil fuel have only a negligible turnover in nature.

In a closed system in the absence of light, the carbon cycle is reduced to predominantly heterotrophic systems (fig. 2).

Microbial populations and their metabolic activities differ greatly between aerobic and anaerobic habitats. Some carbon compounds such as certain aromatics are thought to be degraded only in oxic environments while other reactions, e.g. methanogenesis, are restricted to strictly anoxic conditions. In nature, in structured solid phase systems such as soil or sediments, transport of gases is limited to diffusion processes. In the presence of excess organics oxygen as electron acceptor is often consumed faster than it is supplied. In such systems we regularly observe a spatial zonation from oxic to anoxic systems. In the case of a closed system such as a waste repository a temporal succession must be assumed, starting from oxic conditions and slowly changing to anoxic ones[44]. Such cases have been modelled by Arter et al.[3]. In natural chemically stratified environments compounds produced in an anoxic environment may diffuse into oxic zones and become transformed there again to CO_2. If a repository with its barriers is believed to be a closed system, products of anaerobic metabolism due to incomplete decomposition will accumulate as long as organic substrates are available.

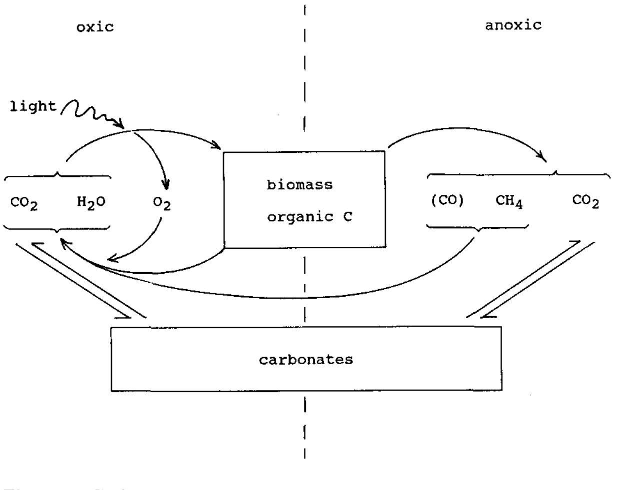

Figure 1. Carbon cycle with gases related to microbial metabolism.

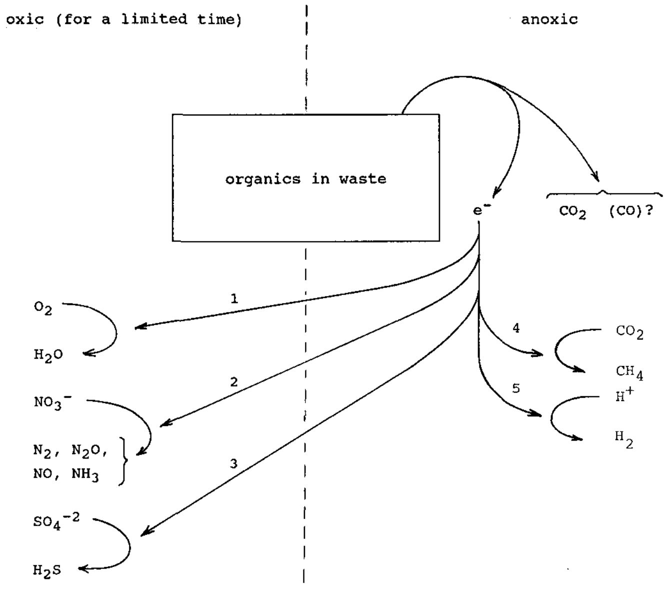

Figure 2. Carbon cycle in a closed system such as a repository with limited amounts of electron acceptors O_2, NO_3^- and SO_4^{-2}. Numbers denote the sequence of electron acceptors used.

The cycles of hydrogen and oxygen which are closely coupled to the carbon cycle are discussed together with the carbon cycle. Important gases belonging to these cycles are:

Carbon dioxide is the most important source of carbon for autotrophic carbon assimilation and thus for all life on earth in general. The phototrophic organisms, higher plants, algae, and phototrophic bacteria, act as the major sink for CO_2. CO_2 is also the substrate for biomass formation of chemolithotrophs and is of some importance in heterotrophs growing on certain carbon sources. These two types of organisms will be present in repositories. Furthermore, CO_2 is the electron acceptor for methanogens and acetogens. These activities act therefore also as sinks for CO_2.

On the other hand, respiration and fermentation reactions are massive sources of CO_2. CO_2 is the exclusive

Table 3. Reactions of denitrification and dissimilatory nitrate reduction[24].

$NO_3^- \rightarrow NO_2^-$	*Thiobacillus thioparus, Lysobacter antibioticum*
$NO_3^- \rightarrow N_2O$	*Achromobacter nephrii, Aquaspirillum itersonii, Pseudomonas* sp.
$NO_3^- \rightarrow N_2$	*Paracoccus denitrificans, T. denitrificans, Rhodopseudomonas sphaeroides, Alcaligenes eutropha, Hyphomicrobium* sp., *Pseudomonas* sp., *Halobacterium* sp.
$NO_2^- \rightarrow N_2$	*Neisseria* sp., *Flavobacterium* sp.
$N_2O \rightarrow N_2$	*Vibrio succinogenes*
$NO_3^- \rightarrow NH_3$	*Escherichia coli, Klebsiella pneumoniae, K. aerogenes, Campylobacter* sp.

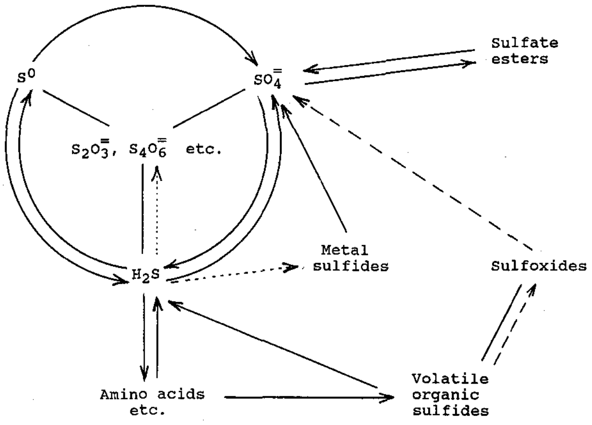

Figure 3. The microbial sulfur cycle[45]. Solid lines denote known biological reactions, dashed lines possible ones and dotted ones abiological reactions.

carbon compound produced in complete degradation either under aerobic conditions or in the absence of oxygen but in the presence of nitrate or sulfate as electron acceptor. It is also released in large amounts during fermentative anaerobic metabolism. The yearly global turnover of carbon through CO_2 is in the order of 10^{11} t.

Oxygen is produced by green organisms in the light through the process of photosynthesis allowing the present life of man and higher animals on the surface of the earth. For all these organisms oxygen is the electron acceptor in energy conversion, i.e. in respiration. A wide range of microorganisms also exhibit respiratory activities to sustain their metabolism. In a closed system in which chemoorganotrophic organisms dominate and an excess of oxidizable carbon compounds are present oxygen will be rapidly depleted resulting in a change from oxic to anoxic conditions. Some respiring organisms switch to alternative electron acceptors, e.g. nitrate or sulfate, both leading to new gaseous compounds, N_2, N_2O, NO, and H_2S, respectively. These compounds of the nitrogen and the sulfur cycles will be discussed later. While the concentration of oxygen in the atmosphere of the earth hardly changes, it may show greater variations in active sinks and locations in which diffusion is limited compared to air, e.g. water, sediments and soil. This regulates the activities of alternative respiratory pathways, e.g. the reduction of nitrate or sulfate. However, the presence or absence of oxygen not only determines the metabolism of the microorganisms but is also extremely important for the oxidation state of minerals in the environment such as iron, manganese or sulfur. Respiration not only produces CO_2 and consumes O_2, but also forms water to close the hydrogen cycle to photosynthesis. Since the amount of water in a system will often govern growth and metabolic activities of the microorganisms present, formation of water may accelerate rates of microbial processes. Due to the different pool sizes the global turnover of CO_2 is around $3 \cdot 10^{-1}\,y^{-1}$, for O_2 $2 \cdot 10^{-3}\,y^{-1}$ and $2 \cdot 10^{-6}\,y^{-1}$ for water[4].

Carbon monoxide in the atmosphere originates from burning biomass, from photochemical oxidation of methane, or from a photobiological side reaction. It is also produced in anoxic environments during methanogenesis. A few microorganisms are known to use CO as their carbon and energy source under oxic conditions, while methanogens and acetogens convert CO to methane or bacterial biomass under anoxic ones[12,36].

Molecular hydrogen is formed by the action of the enzyme hydrogenase which catalyzes the reaction

$$2\,H^+ + 2\,e^- \rightleftarrows H_2$$

Such enzymes are characterized by the presence of iron and acid-labile sulfur, in some cases also of nickel and selenium. The function of these enzymes is the disposal of excess electrons from the cells in anaerobic systems by the formation of hydrogen gas, e.g. in fermentations in the absence of a suitable electron acceptor. Gaseous hydrogen is also liberated in the absence of molecular nitrogen in fermentations or in photosynthesis through the enzyme nitrogenase.

Fermentation of many substrates such as carbohydrates and other carbon containing compounds including polymers like cellulose, starch, proteins or hydrocarbons may result in the formation of variable amounts of molecular hydrogen as well as of CO_2[10]. Some twenty strictly or facultative chemotrophic bacteria have been investigated with respect to hydrogen production[23]. For thermodynamic reasons, accumulation of hydrogen in a closed system will inhibit the rate of degradation of the organics by the hydrogen producing organisms. In natural habitats hydrogen is normally removed by a hydrogen-consuming microflora. Most of the hydrogen cycling globally is present in its bound form either as water or as biomass.

Methane is found in the atmosphere at concentrations of about 1.4 ppm and originates mainly from the anaerobic decomposition of organic material by microorganisms in anoxic environments such as waterlogged soils, swamps and marshes, sediments, and the intestine of animals[15,29]. Methane is formed from acetate or from hydrogen and CO_2, both present in anoxic ecosystems. Methanogenic organisms thus act as sinks for acetate and CO_2, as well as for hydrogen. Labelled methane has been observed to be produced from the leachate of a waste repository demonstrating microbial activity in the disposal site[16].

Dissolved CO and H_2 have been measured in ocean water and found to be present on the average as $6 \cdot 10^{-5}$ ml/l water and $3 \cdot 10^{-5}$ ml/l water, respectively, while the maximum values for both gases reach 10^{-3} ml/l water[36]. The concentration of CO varies with time, both gases exhibiting great variation in concentration with the depth of the water. Bacterial cell numbers correlate strongly with the distribution of the dissolved CO and H_2 in the water column. Compared to the concentrations found in the atmosphere, water from the ocean surface and also from freshwater systems is supersaturated with both gases suggesting a high microbial production rate in these ecosystems. On the other hand both gases are taken up through the soil surface by microorganisms[26].

In the biogeochemical nitrogen cycle nitrogen is present in various oxidation states ranging from −III in NH_3 to +V in NO_3^-. Molecular nitrogen in the atmosphere and nitrogen in rocks where almost all of the soil nitrogen is localized form a large but slowly cycling reservoir. Inorganic nitrogen salts are readily water soluble and thus easily distributed in the environment by water. The N-cycle is dependent on the activity of specific microorganisms (fig. 4), more so than the cycles of carbon, hydrogen and oxygen.

The assimilatory pathways of NO_3^- and NH_3 are the entry of nitrogen into organic material and found in green plants, fungi and many bacteria. In contrast, three processes in the nitrogen cycle are restricted exclusively to microorganisms: nitrification, denitrification and nitrogen fixation.

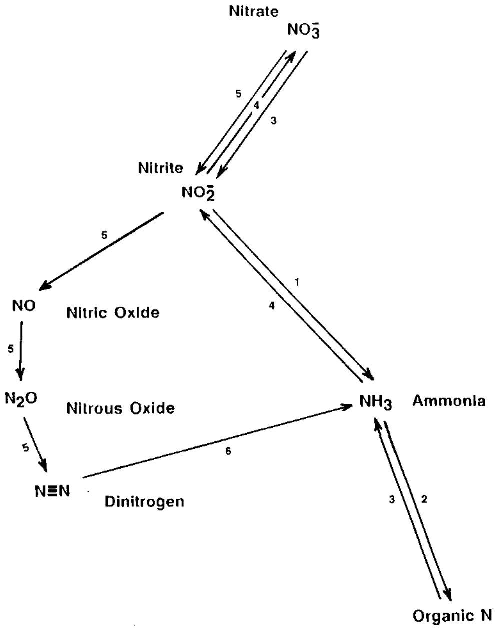

Figure 4. Nitrogen cycle[31]. 1 = assimilatory nitrate reduction, 2 = nitrogen immobilization into organic nitrogen, 3 = ammonification, 4 = nitrification, 5 = denitrification, 6 = nitrogen fixation.

Nitrate is formed in oxic ecosystems from ammonia through nitrification by nitrifiers, a small group of chemolithotrophs. Since cations (NH_4^+) are strongly adsorbed by clay particles or humic substances and therefore better retained in soil than anions (NO_3^-), nitrification acts in a soil system as a nitrogen mobilization process.

Besides assimilatory nitrate reduction which leads finally to the production of proteins, nitrate can be reduced by a dissimilatory process either to ammonia (nitrate-ammonification) or by denitrification to N_2. Denitrification and nitrate-ammonification occur mainly in systems low in or lacking the electron acceptor oxygen. NH_3 is volatile at alkaline pH and denitrification yields various gaseous intermediates such as NO and N_2O. The product of denitrification is a mixture of N_2 with these nitrogen oxides. The composition of the gas is dependent on the organisms present as well as on environmental factors[2,21]. Nitrogen oxides are formed as the main products by organisms lacking the last enzymatic steps of denitrification[31,38,39]. The sequence of denitrification is as follows:

$$NO_3 \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Of the various organisms known for denitrification, some reduce NO_3^- only under anoxic conditions while in others denitrification and respiration using oxygen as electron acceptor occur simultaneously. NO and N_2O are also produced during nitrification[24,27].

Finally, NH_3 formed from molecular N_2 and H_2 through the process of nitrogen fixation balances the losses in bound nitrogen due to denitrification. A variety of procaryotic organisms is known to be capable of catalyzing this process.

Since environmental conditions, especially the presence of oxygen, regulate reactions in the nitrogen cycle, natural environments are usually spatially zonated concerning the products and reactions of the N-cycle, nitrification predominately taking place under oxic conditions, denitrification predominately under anoxic ones[6]. In a closed system such as a repository, a succession of various reactions with time is expected to occur as in the C-cycle.

In the *sulfur cycle*, sulfur is present in the oxidation states from −II in H_2S to +VI in SO_4^-. Of the various sulfur compounds found in nature only H_2S and some organic sulfur compounds, e.g. methylsulfides and mercaptanes, are volatile. H_2S stems from the degradation of biomass and other sulfur-containing organic material and from the microbial reduction of sulfate and other oxidized S-compounds in anaerobic habitats[25,41,43,45]. Reductive sulfur transformations are catalyzed by various sulfate reducing bacteria using organic compounds or molecular hydrogen as electron donors. The formation of H_2S may alter the microbial population drastically

Table 4. Volatile sulfur compounds produced or degraded by microbial processes[22].

Compound	Formula	
Hydrogen sulfide	H_2S	Metabolic intermediate in assimilation, product of sulfate reduction and anaerobic degradation of organic compounds
Dimethylsulfide	$(CH_3)_2S$	Major volatile organic sulfur compounds
Dimethyldisulfide	$(CH_3)_2S_2$	
Methylsulfide	CH_3HS	
Carbonylsulfide	COS	
Carbondisulfide	CS_2	
Thiophene	C_4H_4S	Found in numerous compounds, also in oil and coal

due to its toxic effect. Furthermore, heavy metals necessary as trace elements for growth are precipitated as metal sulfides and are thus no longer available for the cells. The yearly production of H_2S is estimated to be around 10^{10} t.

Furthermore, anaerobic decomposition of S containing amino acids or proteins yields methylsulfide and dimethylsulfide (DMS), which are reduced to H_2S and CH_4 in anaerobic, to dimethylsulfoxide (DMSO) and finally to sulfate in aerobic conditions[22]. Other organic sulfur containing volatile compounds formed are CS_2 and COS (table 4). A variety of microorganisms are known to produce such organic sulfur compounds in pure culture from sulfur containing substrates[19].

When gas production was studied in batch cultures with sulfate-reducing bacteria (a system which can be taken as a temporary analog of an anaerobic waste repository), a variety of gases were detected. CO_2 and H_2S dominated at the beginning of growth, followed by H_2 which appeared after a few days and reached a concentration of 15 – 35% depending on the species present. Traces of CH_4, C_2H_6 and possibly C_3H_8, of CH_3SH and C_2H_5SH, of SO_2, CS_2 and COS were also found[18]. In a lactate-sulfate medium a ratio of 2.3 – 2.5 to 1 for CO_2 to H_2S was observed instead of theoretically 2 to 1. Whether the various trace compounds found are of special significance in a repository remains to be examined, however, they indicate active sulfate reduction. The simultaneous production of hydrogen points to fermentation parallel to sulfate reduction, this is also the case when sulfate is present in non-limiting concentrations. Increasing amounts of H_2S may inhibit the cytochrome system of the sulfate reducers and precipitate the iron of the medium thereby limiting cytochrome biosynthesis and thus growth.

Rates of H_2S production in lake and sea sediments are found to be in the wide range of 0.0002 to 19 mg H_2S $l^{-1} \cdot d^{-1}$ depending on the chemical and physical environment[45].

There are striking similarities between the biogeochemical cycles of nitrogen and sulfur:

- plants take up both elements in the form of the most oxidized species
- both elements are components of the organic matter in soil
- under oxic conditions chemolithotrophic microorganisms form the most oxidized species in both cases
- in the oxidized form both elements are easily lost from soil by leaching
- under anoxic conditions both elements are reduced by microorganisms and are released into the atmosphere

Soil has been found to act as a sink as well as a source for various atmospheric gases due to the presence of highly active microbial populations in its upper layers. Some of their metabolic activities have been determined in laboratory as well as in field experiments. They are dependent on temperature, content of organic material and water, and of the concentration of oxygen and other gases.

Several aspects have to be kept in mind when estimating or predicting rates of microbial gas formation and consumption in natural systems and in waste repositories: composition and availability of the carbon source and other nutrients, the presence of oxygen, or physical parameters such as temperature or water potential. In repositories microorganisms cannot be excluded from the waste material and will also be present in the backfill material and the host rock. Possible microbial activities must therefore be included in safety considerations over the long time span in which the repository is expected to retain the radioactive material[37].

Most radioactive waste contains various organic materials such as cellulose or ion-exchange resins[7] which are degraded by microorganisms. Furthermore, as seen in other papers in this volume, bitumen used for stabilization is degraded significantly under aerobic and also under anaerobic conditions[1,34,42]. CO_2 and CH_4 formation has been reported in laboratory degradation studies[9]. In landfills with domestic waste large amounts of these gases are released. For safety reasons they have to be removed from the site by pumps.

Only a few studies have tried so far to estimate gas production in actual repositories of radioactive waste[5,17,28,40]. Gas may be produced by radiolysis, by thermal or chemical corrosion, and by microbial degradation. Various organic materials have been tested as artificial waste in the same type of canisters used in repositories. CO_2 production of up to 0.025 mg $\cdot$ d^{-1} per g waste under aerobic and about 3 times more under anaerobic conditions was observed[5,28]. Astonishingly, no other gases than CO_2 were detected. CO_2 may also stem from the solubilization of concrete used as barrier through the action of acids formed by microorganisms[14,32].

In this context the message on the news of the Swiss radio corporation on March 24th, 1990, that in a US repository for radioactive waste in Hanford (Washington), hydrogen gas had escaped from canisters about 20 years old, demonstrates that many questions are still open. No information is available on the mechanism by which the gas was formed.

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