Microbial activity in the terrestrial subsurface

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Summary. Little is known about the layers under the earth's crust. Only in recent years have techniques for sampling the deeper subsurface been developed to permit investigation of the subsurface environment. Prevailing conditions in the subsurface habitat such as nutrient availability, soil composition, redox potential, permeability and a variety of other factors can influence the microflora that flourish in a given environment. Microbial diversity varies between geological formations, but in general sandy soils support growth better than soils rich in clay. Bacteria predominate in subsurface sediments, while eukaryotes constitute only 1–2% of the microorganisms. Recent investigations revealed that most uncontaminated subsurface soils support the growth of aerobic heteroorganotrophic bacteria, but obviously anaerobic microorganisms also exist in the deeper subsurface habitat. The microorganisms residing below the surface of the earth are capable of degrading both natural and xenobiotic contaminants and can thereby adapt to growth under polluted conditions.

Key words. Aquifers; groundwater; deep subsurface microorganisms; eukaryotes; prokaryotes; microbial metabolism; oligotrophic conditions; xenobiotic compounds.

Introduction

The layers under the earth's crust (1–100 m) have remained unexplored both chemically and microbiologically, and as a result, little is known about the fate of pollutants from the earth's surface in the substrata. Few studies are available about shallow aquifers, sediments, and well waters [18, 25, 26, 29, 71, 74]. The continuous deterioration of the quality of groundwater necessitates the acquisition of more knowledge about fundamental processes in the deeper subsurfaces. The use of synthetic compounds (various xenobiotics including pesticides, fertilizers etc.) has increased tremendously in the past few decades and the resulting pollution has been involved in the contamination of groundwater and subsurface environments with organic compounds [33]. Our knowledge of the transformations catalyzed by microorganisms in many natural ecosystems, especially in the deeper subsurface and groundwater, are insufficient.

Only recently investigations have begun to probe the problems caused by toxic waste dumps and landfills. The leachate from such landfills can percolate into subsurface zones and contaminate groundwater. Although groundwater is more difficult to contaminate than surface water, reclamation of contaminated aquifers will also be much more difficult. It is known that surface microorganisms can transform and mineralize many organic compounds, but in the deeper subsurface, where limited sources exist for nutrients like phosphorus and nitrogen, electron acceptors like oxygen, nitrate, and sulfate, and other minerals that serve as trace elements for microbial growth, it is likely that many compounds will be only partially degraded. Since microbial transformations often lead to compounds with different water solubilities, the local mobility and leaching properties might change. Several metals can also be changed by microorganisms either by varying the oxidation state or by producing metal complexes. Microbial processes can either dissolve or precipitate metals and thereby change their mobility in the soil.

Further investigations on the subsurface environment are required in the near future. Analysis should include a number of subsurface environmental factors that can influence microbial activity and therefore also the transformation of xenobiotic chemicals: temperature, pH, redox potential, availability of electron acceptors, salinity and hydrostatic pressure, porosity of the soil, chemical recalcitrance and solubility, chemical and physical ad- and desorption on soil particles. The types of microorganisms involved vary according to the nature of different electron acceptors and, therefore, the pathways may differ under various redox conditions.

The study and characterization of subsurface microbial populations is necessary. It is important to know how they survive under natural oligotrophic conditions and how they react when organic contaminants enter in their environments. Kinetic studies of the metabolism of xenobiotic compounds at different substrate concentrations are important and will allow predictions about persistence in groundwater to be made.

The subsurface habitat

The shallow and the deep subsurface

Soil profiles can differ in their thickness, chemical constitution, aeration, color, texture and water content; therefore, different soil sources support microbial communities differing in size and activity [2]. The subsurface provides a habitat for prokaryotic and eukaryotic microorganisms alike, but bacteria are the most abundant. Because the habitat may differ from one geographic region to another and from one subsurface depth to another, a large variety of different microorganisms have been detected and characterized.

Physiological factors, temperature, pH, salinity, redox conditions, porosity, texture of soil material, etc., all dictate the conditions in a given habitat. Organic carbon supplies are the most common growth-limiting factors.

With the exception of clay and rock formations, most subsurface environments provide enough permeability for subsurface microorganisms to survive[46].

In uncontaminated subsurface environments oligotrophic conditions prevail and therefore sufficient oxygen should be present. However, it is always possible to isolate anaerobic microorganisms in such environments, indicating that special microhabitats exist that allow anaerobic microorganisms to survive in these areas. In recent years, several reports have indicated that subsurface microorganisms are able to degrade xenobiotic compounds under anaerobic conditions. In some cases, degradation of certain compounds is possible exclusively under anaerobic conditions[10,11].

Nutrients

Substrate concentrations are critical for growth of microorganisms in deep subsurface regions. Unpolluted subsurface soil usually contains low concentrations of organic carbon[68], and the microorganisms are exposed to oligotrophic conditions. Experiments with subsurface soil have shown that when a diluted nutrient medium that simulates oligotrophic conditions is used, more microorganisms grow than in a nutrient medium with usual substrate concentrations. However, other studies have shown that many subsurface microorganisms can readily adapt to normal substrate concentrations. On the other hand, in some cases the substrate concentration has to reach a certain concentration before transformation occurs. It has also been shown that degradation is slower when the substrates are present in low concentrations[8]. At the other extreme, when substrate concentrations reach high levels, microbial metabolism might also be inhibited.

Inorganic nutrients especially nitrogen and phosphorus, might be limited when too many organic compounds leach from the surface into the subsurface environment. The low solubility of phosphate salts might be one reason that phosphorus is limited in these regions[44]. Other elements like magnesium, potassium, calcium and trace elements necessary for microbial growth are usually available in concentrations high enough to support microbial growth under oligotrophic conditions.

Pore size

In surface soils, solid materials comprise half of the volume[2]. The other half is occupied by water and gases such as carbon dioxide, nitrogen and oxygen. Porosity is required for movement of water. Higher water mobility correlates with an increased supply of organic compounds and therefore a higher number of microorganisms in these soils. Samples from sandy subsurface areas generally contain more living microorganisms than soil samples which contain a higher amount of clay and silty sand.

Aeration and moisture are directly related[2]. Air moves into the pores which are not occupied by water. Because both carbon dioxide and oxygen influence microbial metabolism, the pore size has a big influence on the redox potential and thereby the physiological conditions of a certain habitat.

Electron acceptors

The presence or absence of a certain electron acceptor will dictate the redox potential in the environment. The redox potential in a certain environment will control the range and distribution of microorganisms.

Furthermore, the redox potential also influences various inorganic compounds in the subsurface area. Under anaerobic conditions the solubilities of iron and manganese can change when oxidized by soil microorganisms. Most unpolluted aquifers contain enough oxygen and therefore remain aerobic. Polluted groundwater often contains bacteria which are enriched and able to metabolize xenobiotic chemicals. However, the supply of oxygen and mineral nutrients can be limited and therefore rapid degradation of these chemicals is often inhibited.

Anaerobic metabolism usually requires electron acceptors like nitrate, sulfate, or carbon dioxide. Nitrate and sulfate concentrations are usually low in the deep subsurface, but denitrifying and sulfate-reducing bacteria are usually abundant in these soils. Many of these microorganisms are able to metabolize organic compounds completely to carbon dioxide when sufficient nitrate or sulfate is available. In the absence of oxygen, nitrate or sulfate, methanogenic consortia can degrade organic matter to methane and carbon dioxide. Microbes may also participate in inorganic reactions such as the oxidation or reduction of iron or manganese. Some chemolithotrophic bacteria can obtain energy from the oxidation of H_2, Fe^{2+}, Mn^{2+}, H_2S and other elemental compounds. Under anaerobic conditions and in the presence of fermentable substrates, manganese and iron can be reduced by soil microorganisms.

Temperature, pH, hydrostatic pressure and salinity

Metabolism and growth of microorganisms is dependent on temperature and pH. Psychrophiles are able to grow even at temperatures below 0 °C whereas thermophiles are able to survive at 99 °C or higher. The temperature in the top few meters of the surface can vary seasonally. Degradation in the earth's upper crust is therefore limited during the cooler seasons of the year. At depths below 10–20 m the temperature of the subsurface soil has a constant air temperature[44]. In deeper layers the temperature increases by 3 °C per 100 m depth. Because most bacteria are mesophiles and can grow at a range between 10 and 45 °C, temperature will have little effect on microbial metabolism in the deeper subsurface. Microbial growth is also dependent on a favorable pH. Most bacteria are able to grow in a pH range between 5 and 9. Extremes in pH might limit the variety of microbes. However, most aquifers are buffered with carbon dioxide, so that extremes of pH are rare. It is therefore unlike-

ly that pH will have a big effect on microbial activities in the deeper subsurface.

Like temperature and pH hydrostatic pressure has only minor effects on the metabolism of organic compounds and on microbial growth in deeper subsurface soils. Hydrostatic pressure increases by approximately 1 atmosphere per 10 m. Experiments with surface microorganisms show no decrease in metabolic activity at higher hydrostatic pressure. Groundwater salt concentration increases with depth since more salts are dissolved during penetration of the water through the subsurface soil. Experiments with subsurface soils from a depth as low as 500 m, however, show that even in the deeper subsurface area microbial life is still present, and these soils are as metabolically active as soils from 10 m depth. These results indicate that in most cases the salt concentration found at lower depths will have little effect on microbial activity.

Sampling and characterization of subsurface microorganisms

Sampling

Only in recent years have the techniques for aseptically sampling soil from the deeper subsurface been developed. Knowledge about microorganisms indigenous to subsurface soil is scarce because the sampling technology necessary for aseptic sampling has been either too costly or lacking. Dunlap and coworkers [16] have described a sampling method that consists of drilling a borehole to the desired depth and then taking a soil sample with a core barrel. Only the inner part of the remaining soil sample, which has not come in contact with any unsterile materials, is investigated. These uncontaminated soil samples should contain the microbial population indigenous to that particular soil layer.

In 1984 the U.S. Department of Energy initiated a program on microbial ecology and activity in the deeper subsurface. The sediment samples were taken from boreholes drilled at the Savannah River Plant in Aiken, South Carolina. To obtain aseptic soil material from as far as 250 m under the earth a new sampling technique was used [55]. The samples were collected with steam-cleaned or autoclaved core liner. The subsurface samples were isolated ahead of the recirculating drilling fluids. The outer sediment portions were removed with sterile tools and only the remaining inner part of the undisturbed sediment core was investigated. This advanced sampling procedure should have guaranteed the collection of an uncontaminated soil sample from the deep subsurface to permit study of the abundance of microbes and microbial activity in these areas.

Methods for characterizing subsurface microorganisms

In general, the same methods for characterizing microbes in surface soil are used to study microorganisms in the subsurface environment. Light and electron microscopy allow direct counting of microorganisms in subsurface samples. The total bacterial density in the soil is determined primarily by epifluorescence microscopy using the acridine orange direct count method [16]. Microscopy also gives an indication of the morphological structure (size, form, gram-type, cell structure, etc.). However, the test does not distinguish between dead and live cells and gives no indication about the activity of the microbes in the environment. In general, the estimated number of microorganisms in a soil sample is much higher by using the acridine orange method than by plate counts or most probable number techniques. Microscopic analysis provides information about distribution and morphology, but provides no information about the physiology of microorganisms in the environment.

Plate counts and most probable number counts are also often used to determine the abundance of viable microorganisms in deeper subsurface samples. Quantities determined by plate counts are between 20 and 80 % of the totals from acridine orange counts for equivalent samples, varying based on medium and substrates [4].

Plate counts and most probable number techniques can determine whether certain special microorganisms exist in a habitat by using defined media, substrates, electron acceptors and culture conditions. Growing microorganisms on agar plates gives information not only about the number of colonies present under these special conditions, but also about the color, relative size and morphological characteristics of the distinct colony types; this provides useful information about the diversity in a certain environment. On the other hand, it should be noted that agar plates cause selection of organisms with characteristic growth parameters or special substrate requirement [53]. Microorganisms grown on agar plates do not necessarily represent the majority of microorganisms active in a certain habitat [69]. The most probable number assay usually provides results similar to those obtained from plate counts. Fungi are enumerated by growth in a dextrose agar containing antibiotics like penicillin and streptomycin, or by growth on a potato-dextrose medium at lower pH, which inhibits growth of bacteria. Algae are grown on mineral salts agar medium and exposed to light [62].

Additional methods that are used to measure the abundance of microbial life in the deeper subsurface include incorporation of [1-^{14}C]acetate into lipids, methyl-[^{3}H]thymidine into DNA and reduction of 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl-tetrazolium chloride (INT). These methods are used to indicate the metabolic activity in subsurface material [6, 54, 56, 63, 67, 77]. Microorganisms with active respiratory enzymes can be estimated microscopically by counting the red-purple INT-formazan granules [77].

Smith et al. [63] estimated the metabolic activity of bentic marine sediment by measuring the incorporation of radiolabeled methyl-[^{3}H]thymidine into bacterial DNA and the incorporation of sodium [1-^{14}C]acetate into

lipids. The deep aquifer sediment microbiota are able to assimilate [1-^{14}C]acetate into lipid fatty acids and methyl-[^{3}H]thymidine into DNA within minutes or hours of sample retrieval, indicating that incorporation of radiolabeled compounds is a useful method to estimate the indigenous activity of microbes in the deeper subsurface[56].

Biochemical indicators

Several molecular markers (ATP, GTP, muramic acid, phospholipids, etc.) can serve as biochemical indicators. Because all of these biomarkers are dependent on different physiological conditions, they serve as qualitative indicators for the presence of microbial life in a particular environment. Karl[32] suggested that adenosine-5′-triphosphate (ATP) can be used as an indicator for metabolic activity. Because ATP can interact with several compounds present in the soil, a special extraction procedure has been developed that could extract all ATP from the sample material[72].

Muramic acid levels reflect the gram-type which is dominant in a certain habitat. Furthermore, methanogens, which belong to the Archaebacteria, have a completely different cell wall structure that lacks muramic acid.

Parker and coworkers[52] use the hydroxyfatty acid content, released from lipid A of lipopolysaccharides to estimate the level of gram-negative bacteria in sediments. Because there is a rapid turnover of lipopolysaccharides in nature only the active gram-negative population would be enumerated.

Phospholipids can serve as biomarkers for special types of bacteria. Methoxy-, cyclopropyl-, unsaturated- or hydroxycyclopropyl-fatty acid composition is diagnostic of specific bacteria. However, the composition of lipids is also dependent on environmental conditions such as temperature, pH, growth substrate, etc. Growth substrates can act as precursors in fatty acid biosynthesis; organisms grown on acetate contain higher amounts of even straight chain acids, whereas organisms grown on propionate contain more odd straight chain acids. Fatty acids in the C_{12} to C_{19} range are common in bacteria[61].

Isolated bacterial communities of different respiratory types from sediments display different fatty acid composition characteristics. Cyclopropyl fatty acids are present in significant amounts in cultures grown under aerobic conditions. Facultative aerobes contain higher amounts of C_{18}:1w7 than other cultures. Facultative anaerobes on the other hand contain high amounts of C_{12}:0 and about double the amount of C_{16}:1w7 as saturated C_{16}:0 fatty acids. Branched chain iso and anteiso C_{15}:0 are usually found in prokaryotic but rarely in eukaryotic organisms[61]. Fatty acid composition can serve as a species-specific biomarker[66].

Diversity, abundance and distribution of microorganisms in the subsurface

Transport

Sargent and Fliermans[59] propose two alternative origins for subsurface microbial populations. 1) Microorganisms might have been transported from the surface by surface water percolating downward to the groundwater table, and 2) microorganisms might have occupied the deep subsurface habitat during sediment deposition. This latter interesting hypothesis would suggest that subsurface microorganisms have been isolated from surface bacteria for thousands of years, and this might explain why a large variety of different microorganisms have been detected in subsurface soil.

Horizontal movement of subsurface organisms could occur by groundwater movement into aquifers. For instance, Harvey and coworkers[27] have demonstrated that in sandy subsurface soils microorganisms can be transported over long distances by water movement. In polluted groundwater more microorganisms are found in the water phase. Lateral migration of microorganisms by water movement through aquifer sediments is another possible explanation for colonization of the subsurface area.

Diversity in subsurface soil

Microorganismal diversity and morphology varies from geological formation to geological formation[4], and in general diversity correlates with population density[62]. Fliermans[20], using the API test method, determined that over 85 % of the 1100 bacteria isolated from subsurface soil are presently only at one depth, indicating that the subsurface habitat supports a large variety of physiologically different microorganisms. With respect to temperature, exclusively mesophilic bacteria inhabit the subsurface[4], and only in samples where the pH of the pore-water falls below 4.7 are cell counts adversely affected[4, 62]. Lowest cell counts are obtained from samples with high clay content, whereas sediments with a low clay content and a larger proportion of sand show relatively high cell counts[4]. Sinclair and Ghiorse[62] estimate a total population density of 10^7 cells/g d.wt in sandy subsurface soils at the Savannah River Plant. Although subsurface microorganisms are clearly adapted to oligotrophic conditions, 10–50 % of deep subsurface organisms can easily tolerate high substrate concentrations.

Although Fliermans and Balkwill[21] suggest that the deeper subsurface microflora at Savannah River Plant may be more diverse and may contain a larger proportion of forms that grow readily in the laboratory, subsurface soil samples from other sources[9, 67] display microbial activities up to 1000-fold lower than those at the surface. Of all subsurface microorganisms, less than 2 % are eukaryotic[4, 62], but eukaryotes can be detected in sediment samples of almost all geological formations at the Savannah River Plant[4]. Surprisingly, a variety of

algae can also be found in deeper subsurface sediments [62].

In general, gram-positive bacteria show lower metabolic activity than gram-negative bacteria. At the Savannah River Plant, most of the bacteria isolated from subsurface sediment samples are rod-shaped (81 %), the rest being either pleomorphic or cocci. Gram-negative bacteria are dominant (86 %) in transmissive aquifer sediments, while the proportion of gram-positive bacteria increases from 43 % to 63 % in samples from nontransmissive zones [4]. Sinclair and Ghiorse [62] find that gram-negative bacteria prefer sandy soil, such that in samples consisting of greater than 50 % clay only gram-positive bacteria can be detected. Coliforms are also detected in soil layers 30–150 m below the surface of the Savannah River Plant site, but in most samples fecal streptococci are absent indicating that these organisms might not belong to the indigenous flora of the subsurface habitat [30]. In contrast to the observations from the Savannah River Plant site where gram-negative bacteria predominate, Balkwill and Ghiorse [3] find that in aquifers in Oklahoma, 85–90 % of all microorganisms in subsurface samples are prokaryotic gram-positive coccoid rods. Water communities from a Pleistocene sandy aquifer in the Lower Rhine Region, FRG, contain relatively low proportions of gram-positive bacteria (< 11 %), whereas in sediment samples the gram-positive isolates constitute 35–43 % [34]. Water communities are less active than populations obtained from sediment samples. Not surprisingly communities obtained from water samples have a completely different composition than communities obtained from sediment samples. Sediment samples have a more diverse microbial constitution than water samples, because the chemical composition of groundwater samples is more homogeneous than that of sediment samples [34, 73].

Aerobes predominate in subsurface regions, but there are often anaerobes present, since aerobic soil samples always contain anaerobic microenvironments where anaerobes survive [31]. For instance, nitrifying bacteria can be found at the Savannah River Plant, and the ammonium oxidizers represent the major class of nitrifying bacteria [20]. Microaerophilic microorganisms are found throughout the subsurface profile and many of these microbes are able to fix nitrogen [24]. Denitrification occurs in all samples obtained from the Savannah River Plant site as well as in subsurface soils of Jutland and Zealand [42]. Denitrification appears to be limited by nitrate supply [23] and by availability of organic carbon sources [42].

Sulfate-reducing bacteria are the most abundant class of chemolithotrophic bacteria at the Savannah River Plant site, and generally coexist with sulfate-reducing bacteria, which can be detected in many subsurface layers [30]. As with heterotrophs, in samples with a high clay content, the levels of sulfate-reducing bacteria decrease. On the other hand, the presence of these anaerobes does not correlate with the sulfate concentration in the groundwater. Even though the natural water does not maintain a redox potential favorable for growth, sulfate-reducing bacteria can also be found at relatively high abundance in most of the wells at a Montana groundwater aquifer [15]. It has been postulated that these bacteria are most likely active in the adsorbed state, a microenvironment where the redox conditions are conducive to sulfate reduction. Sulfate-reducing bacteria could also be observed in sediments in the deep coastal plain of Maryland [13].

Although methanogens are universally low in abundance, they can be found in most subsurface environments [7, 13, 30]. Subsurface environments from Lula (Oklahoma), Traverse City (Michigan), and Summit Lake (Wisconsin) are inhabited by diverse communities of bacteria [67]. Ghiorse and Balkwill [25] suggest that only a small part of the total population is metabolically active and that the organisms constituting this part are adapted for survival under oligotrophic conditions. That the subsurface environment can be quite diverse is underscored by Rengpipat et al. [57], who have isolated a novel halobacterium from a deep subsurface sandstone core (490 m).

In conclusion, the subsurface contains a large variety of microorganisms that are well adapted to oligotrophic conditions. Sandy soil appears to support microbial life best. Cell viability does not appear to decrease with increasing depth. Diversity is greatest where microbial communities flourish. Greater than 98 % of the microorganisms are prokaryotes. In transmissive aquifer sediments, gram-negative bacteria are dominant, whereas in layers with a higher proportion of clay the numbers of gram-positive bacteria increase. Most uncontaminated subsurface soils are aerobic and the bacteria found in these soils belong to the group of heteroorganotrophs, but denitrifying, sulfate-reducing and methanogenic communities can also be found in deeper subsurface soils.

Microbial activity in subsurface soils

Metabolism of naturally occurring compounds under aerobic conditions

Subsurface microorganisms are able to metabolize a large variety of natural and xenobiotic compounds under aerobic as well as under anaerobic conditions. Hicks and Fredrickson [28] studied the growth potential of the indigenous microflora in deeper subsurface samples by quantifying the amount of $^{14}CO_2$ produced from radiolabeled compounds. Different samples from similar geological formations showed quite similar microbial activities. In most subsurface samples aerobic degradation of acetate was faster than the degradation of phenol and 4-hydroxybenzoate. Sandy sediment samples with high cell counts had the highest potential for acetate and phenol degradation, whereas biodegradation from samples with a larger

Table 1. Natural substances degraded aerobically by samples from subsurface material or groundwater

Compound	Source	Reference
Acetate, phenol, 4-methoxybenzoate	Subsurface soil from Savannah River Plant, South Carolina	Hicks and Fredrickson[28]
Benzene	Aquifer in Texas	Wilson et al.[76]
Glucose	Subsurface soil from Savannah River Plant	Madsen and Bollag[43]
Glucose, glutamic acid, acetate, stearate, benzoate	Subsurface soil at Williamsburg, Ontario	Ward[72]
Glutamic acid, glycolic acid, phenylalanine, cinnamic acid, lignocellulose	Groundwater in Marmot Basin, Alberta	Ladd et al.[39]
4-Hydroxybenzoate	Subsurface sediment from Oklahoma	Beloin et al.[6]
Phenol	Aquifer material from Oklahoma	Suflita and Miller[64]
Toluene	Shallow aquifer in Oklahoma	Wilson et al.[73]

clay content was much slower or absent. Consisting predominantly of gram-negative bacteria, the distribution of microorganisms isolated from groundwater in a subalpine forest region was similar to that commonly found in other soils. Mineralization of lignocellulose and cinnamic acid by microorganisms obtained from a mountain stream was slower than from groundwater. More bacteria were found in the groundwater, which also showed greater metabolic activity per bacterium. Isolated bacteria could grow at 10 °C, indicating that the organisms are well adapted to their environment. Natural substrates like glucose, glutamic acid, acetate, and benzoate as well as xenobiotic compounds like nitrilotriacetate (NTA) were rapidly degraded in the subsurface soils from Williamsburg, Ontario[71]. Degradation of the natural compounds, with exception of benzoate, was even faster under nitrate-reducing conditions. The pesticides parathion and 2,4-D were more recalcitrant and only small amounts were converted under aerobic conditions. Further examples of naturally occurring compounds that were degraded under aerobic conditions are listed in table 1.

Metabolism of naturally occurring compounds under anaerobic conditions

Jones et al.[30] used lactate, formate, and acetate as substrates to evaluate microbial activity under anaerobic conditions in the deep subsurface soil of the Savannah River Plant site. Whereas lactate and formate were degraded under anaerobic conditions in most samples, the time required for degradation of the substrates varied greatly. Methanogenesis occurred in general 1–3 months after lactate or formate disappeared. The water-saturated transmissive zones harbored the highest number of sulfate-reducing bacteria, while layers rich in clay showed little anaerobic activity. In some samples acetate and methane accumulated during incubation, indicating that subsurface soil from the Savannah River Plant site contains reserves of fermentable carbon sources.

Phenol and benzoate were also tested as substrates because they were naturally occurring compounds. Anaerobic benzoate degradation was associated with soil layers capable of methane production[30], but no degradation of phenol occurred in subsurface soil samples from the Savannah River Plant under anaerobic conditions. Degradation of hydroxybenzoate under sulfate-reducing conditions required a shorter lag period than in methanogenic incubations[38]. When samples from the sulfate-reducing and methanogenic sites were amended with nitrate, degradation of these 3 hydroxybenzoates required an even shorter lag period, indicating that the availability of an electron acceptor can influence the initial anaerobic biotransformation mechanism.

Biotransformation in groundwater of St. Louis Park, Minnesota, also occurred under anaerobic conditions[17]. Methane and methanogenic bacteria were found only in water samples from the contaminated zones, indicating that anaerobic biotransformation, and not dilution effects, was responsible for the disappearance of phenolic compounds.

Further examples of naturally occurring compounds which are degraded under anaerobic conditions are listed in table 2.

Metabolism of xenobiotic compounds under aerobic conditions

The large variety of microorganisms found in the deep subsurface are able to transform not only natural compounds under aerobic and anaerobic conditions, but also a large number of xenobiotic contaminants. In general, contaminated habitats contain acclimated microbial populations able to transform these xenobiotics under the existing redox conditions. Usually pollution has stimulatory effects on the growth of subsurface microorganisms at concentrations below toxic levels. Ogunseitan et al.[50] found that bacteria from contaminated aquifers contained more plasmids and thereby adapt episomally to their contaminated environment. Microorganisms obtained from contaminated subsurface soil and groundwater samples were able to metabolize anthracene, dibenzofuran, fluorene and naphthalene under aerobic conditions[41]. That degradation was faster in highly contaminated wells suggests that microbial populations continually adapt to these chemicals. This increased trans-

Table 2. Natural substances degraded anaerobically by samples from subsurface material or groundwater

Compound	Condition(s)	Source	Reference
Benzene, toluene ethylbenzene, *o*-xylene	Methanogenic	Aquifer material	Wilson et al.[73]
Benzoate	Sulfate-reducing, methanogenic	Subsurface soil from Savannah River Plant	Jones et al.[30]
Glucose	Methanogenic	Subsurface soil from Savannah River Plant	Madsen and Bollag[43]
Glucose	Nitrate-reducing	Subsurface soil from Zealand	Lind and Eiland[42]
Glucose, acetate glutamate, stearate, benzoate	Nitrate-reducing	Subsurface soil from Williamsburg, Ontario	Ward[71]
o-, *m*-, *p*-Hydroxybenzoate	Nitrate-reducing, sulfate-reducing, methanogenic	Anoxic aquifer slurries	Kuhn et al.[38]
Phenol	Methanogenic	Methanogenic aquifer material from Oklahoma	Suflita and Miller[64]
Succinate	Nitrate-reducing	Subsurface soil from Savannah River Plant	Francis et al.[23]

formation potential in more contaminated groundwater also correlated with the abundance of organisms capable of degrading the contaminants.

Soil microorganisms and enrichment cultures from subsurface sediments and groundwaters contaminated with trichloroethylene (TCE) from the Savannah River Plant site have been examined[54]. Although in heavily contaminated areas (greater than 500 mg/l) no activity could be detected, microorganisms could be isolated from less contaminated waters. As with unpolluted subsurface soils, sandy aquifers contained large and active communities of microorganisms. The activity in such layers can be as much as 100–1000 times higher than that in the near surface soil. Phelps and coworkers[54] found that in highly contaminated sediment samples more than 90 % of the colony forming units were fungal; this was consistent with the detection of phospholipids with 23 carbons in length, as typical for eukaryotes. Although it is well known that TCE is transformed by methanotrophs, other organisms can also metabolize this chemical. For instance, Nelson and coworkers[48] isolated a trichloroethylene-degrading, nonmethanotrophic, gram-negative, rod-shaped bacterium from a polluted water sample.

Rogers et al.[58] studied microbial degradation of alkylpyridines under aerobic and anaerobic conditions in groundwater. Biotransformation under aerobic conditions was dependent on the molecular weight of the alkylpyridines: lighter alkylpyridines were generally transformed faster than the heavier analogs. Degradation of alkylpyridines also took place under anaerobic conditions, but the molecular effects and the specific substitution of the ring did not affect rates as much as degradation under aerobic conditions.

Degradation of aldicarb occurs in soil samples under aerobic as well as under anaerobic conditions[51]. Aldicarb disappeared rapidly under aerobic conditions whereas it persisted longer under anaerobic conditions. On the other hand, the metabolites produced disappeared faster under anaerobic conditions. Aromatic substances (3,4,5-trichloroguaiacol, 3,4-dichlorophenol, 2,4,5-trichlorophenol and pentachlorophenol) were transformed faster when inoculated with a mixed culture from humic lake water than with one from a clear lake[40].

In general, a habitat that has been exposed to certain xenobiotic compounds over a longer time period contains a well-acclimated indigenous microflora, able to convert xenobiotic substances under the prevalent redox-conditions.

Further examples of xenobiotic compounds which are degraded under aerobic conditions are listed in table 3.

Metabolism of xenobiotic compounds under anaerobic conditions

Certain xenobiotic compounds may persist under aerobic conditions, and are sometimes only transformed in the absence of oxygen. The following examples show that the subsurface habitat contains a number of microorganisms able to transform a large number of different chemicals. Because anaerobic degradation is slow and microorganisms capable of degrading these man-made compounds might be present only in low numbers, long lag periods are observed before transformation occurs. This long latency is true especially when the subsurface soil is exposed to a certain chemical for the first time.

It has been shown that methanogenic mixed cultures obtained from subsurface habitats are capable of dehalogenating chlorinated contaminants. Microorganisms from an actively methanogenic aquifer bordering the landfill were able to metabolize chlorophenols like 2-, 3- and 4-chlorophenol, 2,4-, and 2,5-dichlorophenol by replacing the halogen substituents with a hydrogen atom[64]. No degradation of these compounds took place either with soil samples from a nonmethanogenic site or under aerobic conditions, suggesting either that dehalogenating bacteria were absent in nonmethanogenic sedi-

Table 3. Xenobiotic substances degraded aerobically by samples from subsurface soil or groundwater

Compound	Source	Reference
Aldicarb	Subsurface soil from Albany	Ou et al.[51]
Acenaphthylene, acenaphthene, 2-methylnaphthalene, 2-methylindene, 3-methylindene, indene	Contaminated groundwater from Iowa	Ogawa et al.[49]
Anthracene, dibenzofuran, fluorene, naphthalene	Contaminated subsurface soil and groundwater samples from Conroe, Texas	Lee et al.[41]
Alkylpyridines	Groundwater	Rogers et al.[58]
Bromodichloromethane	Shallow water-table aquifer	Wilson et al.[73]
Chlorobenzene, 1,2-dibromoethane	Subsurface soil from Oklahoma	Wilson et al.[73]
o-, *m*-, *p*-Dichlorobenzene, *o*-, *m*-, *p*-xylene	Aquifer material	Kuhn et al.[35]
1,2-Dichloroethylene, trichloroethylene, tetrachloroethylene, vinylidene chloride, vinyl chloride	Lake sediment	Fogel et al.[22]
Hexachloroethane	Aquifer in Ontario	Criddle et al.[14]
Indole	Subsurface soil from Savannah River Plant	Madsen and Bollag[43]
Naphthol, naphthalene, acenaphthene	Soil-water system	Mihelic and Luthy[47]
Nitrilotriacetate	Subsurface soil from Williamsburg, Ontario	Ward[71]
p-Nitrophenol	Surface soil from Dryden	Scow et al.[60]
Quinoline	Subsurface soil	Brockman et al.[12]
1,2,4-Trichlorobenzene, p-chlorophenol, p-nitrophenol	Aquifer in Oklahoma	Aelion et al.[1]
Trichloroethylene	Subsurface soil from Savannah River Plant	Phelps et al.[54]
3,4-Dichlorophenol, 2,4,5-trichlorophenol, pentachlorophenol, 3,4,5-trichloroguaiacol	Humic water	Larsson et al.[40]
Trichloroethylene	Shallow aquifer material	Mayer et al.[45]
Trichloroethylene	Subsurface soil from Savannah River Plant	Fliermans et al.[19]
Trichloroethylene	Unsaturated sandy soil	Wilson and Wilson[75]
Trichloroethylene	Polluted water sample from Pensacola	Nelson et al.[48]

Table 4. Xenobiotic substances degraded anaerobically by samples from subsurface soil or groundwater

Compound	Condition(s)	Source	Reference
Aldicarb	Fermentative	Subsurface soil from Albany	Ou et al.[51]
Alkylpyridines	Fermentative	Groundwater	Rogers et al.[58]
o-, *m*-, *p*-Aminobenzoate, benzamide, *N*-methylbenzamide, *p*-toluamide, *p*-benzosulfonic acid	Sulfate-reducing, methanogenic	Aquifer slurry from sulfate-reduced and methanogenic site, respectively	Kuhn and Suflita[36]
Aniline, m-toluidine	Sulfate-reducing	Aquifer slurry from sulfate-reduced site	Kuhn and Suflita[36]
2-, 3-, 4-Chlorophenol, 2,4- and 2,5-dichlorophenol	Methanogenic	Methanogenic aquifer material from Oklahoma	Suflita and Miller[64]
1,1- and 1,2-Dichloroethylene, trichloroethylene, 1,2-dibromoethane	Methanogenic	Aquifer material	Wilson et al.[76]
o-, *m*-, *p*-Cresol	Sulfate-reducing	Shallow toxic aquifer	Suflita et al.[65]
1,1-Dichloroethene, 1,2-dichloroethene	Fermentative	Organic sediment from the Everglades	Barrio-Lage et al.[5]
Indole, pyridine	Methanogenic	Subsurface soil from Savannah River Plant	Madsen and Bollag[43]
Naphthol, naphthalene, acenaphthene	Nitrate-reducing	Soil-water system	Mihelcic and Luthy[47]
Nitrilotriacetate	Nitrate-reducing	Subsurface soil from Williamsburg, Ontario	Ward[71]
Phenolic compounds	Methanogenic	Groundwater from Minnesota	Ehrlich et al.[17]
Quinoline	Fermentative	Subsurface soil	Brockman et al.[12]
2,3,4,5-Tetrachloraniline	Methanogenic	Polluted aquifer	Kuhn and Suflita[37]
Tetrachloroethylene	Methanogenic		Vogel and McCarty[70]
o-, *m*-, *p*-Xylene	Nitrate-reducing	Aquifer material	Kuhn et al.[35]

ments or that environmental factors precluded their metabolic activity on chlorophenolic substrates. Phenol was metabolized in all of the tested groundwater habitats, aerobic as well as anaerobic.

Each habitat contains different populations of microorganisms with special characteristics. Chloroanilines are transformed under methanogenic conditions, whereas *N*-substituted benzenes are preferentially degraded in a sulfate-reducing environment. For instance, 2,3,4,5-tetrachloroaniline is biologically dehalogenated in polluted aquifers under methanogenic but not under sulfate-reducing conditions[37]. The transformation of a variety of *N*- and *S*-substituted aromatic compounds is also dependent on the physiological condition of the habitat[36]. Nitrogen-substituted benzenes are biotransformed while sulfonated ones are more recalcitrant under anaerobic conditions. Carboxylated anilines are more readily biodegraded under anaerobic conditions than aniline or methylated anilines, and a second *N*-alkyl group makes the compound even more resistant to anaerobic degradation. Vogel and McCarty[70] observed that tetrachloroethylene is transformed by reductive dehalogenation to trichloroethylene, dichloroehtylene, vinyl chloride and carbon dioxide under methanogenic conditions. Biotransformation does not always lead to products less toxic than the original contaminant. For instance, vinyl chloride, produced during transformation of tetrachloroethylene, is very persistent under anaerobic conditions and is furthermore very toxic.

Additional examples of xenobiotic compounds which are degraded under anaerobic conditions are listed in table 4.

In conclusion, the subsurface habitat contains an active microflora which is able to degrade a large variety of natural as well as xenobiotic compounds. Some of these compounds are more easily degraded under aerobic conditions, others under anaerobic conditions. The availability of certain electron acceptors is important for the degradation of organic substances. McNabb and Dunlap[46] have postulated that essentially all naturally occurring organic compounds are utilized by microorganisms under suitable growth conditions. Microorganisms in the deep subsurface are relatively active in situ, and therefore play a significant role in influencing groundwater chemistry and quality[14].

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