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DISTRIBUTION OF CONTAMINANTS AND THE SELF-PURIFYING POTENTIAL FOR AROMATIC COMPOUNDS IN A CARBONIZATION WASTEWATER DEPOSIT

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Abstract

The Schwelvollert, a highly colored carbonization wastewater deposit located in Saxony-Anhalt (eastern Germany) was the subject of extensive analytical studies. When sampled in autumn the pyrolysis effluent lake was found to be stratified in three distinct layers, each exhibiting different physicochemical and microbiological characteristics. Although no oxygen was detectable in the whole deposit, aerobic bacteria were shown to predominate among pollutant degraders: in the upper layers about 40-60% degraders of phenolic compounds in heterotrophic microbial communities were found. Neither nitrate-reducers nor sulfate-reducing or methanogenic consortia that degraded benzoate were detectable. However, nitrite-reducing benzoate degraders were found with MPNs ranging from 0.03 to 20/mL. The attempt to identify aerobic isolates from the top layer with the commercially available BIOLOG and API 20NE identification systems revealed their unsuitability for the determination of specific pyrolysis effluent bacteria. Although the aerobic bacteria with specific degradation abilities are not likely to use their abilities under *in-situ* conditions, they represent a degradation potential which might be useful for aerobic remediation strategies.

1. Introduction

Wastewater from the low-temperature carbonization of lignite is a substantial component of eastern Germany's spectrum of hazardous wastes inherited from the past. Lignite processing was the main industry in

East Germany for decades. During the production of briquettes, coke and tar, pyrolysis effluents arose which then were mostly discharged into rivers, open mining holes, or pressed into deep Zechstone layers. The products of pyrolytic processing served as domestic and industrial energy sources or as raw material for the chemical industry.

From 1950 to 1968 pyrolysis effluents from the nearby coking plant at Deuben were discharged into a disused opencast mining pit in the administrative district of Weissenfels (Federal State of Saxony-Anhalt, Germany). Known as the Schwelvollert, the thus created disposal site nowadays extends over approximately 9 ha. The lake has a maximum depth of 27 m and a volume of approximately 2 million m^3. Its low temperature, small surface and deep position compared to the surroundings have prevented it from undergoing thorough seasonal mixing. Polluted with large amounts of phenols and their polymeric autoxidation products, the water is dark brown in color so that only the very surface warms up in summer. In addition to phenol and polyphenols, their more toxic alkylated derivatives have also been detected [1, 2]. These compounds react with oxygen to form the colored polymers which are described by the term synthetic or anthropogenic humic matter (AHM). The AHM has similar features to natural humic matter [1, 3] and is just as resistant to biodegradation [cf. 4 - 6]. The autoxidation of phenolic compounds has not yet ceased, as the abiotic oxygen consumption of deposit water shows [2]. However, other anthropogenic compounds such as nitro or halogenated aromatics have not been found among the pollutants.

The Schwelvollert deposit is the subject of basic studies within the research project "Remediation of dangerous wastes from lignite pyrolysis". Various remediation methods are to be evaluated with respect to their efficiencies for working out guidelines for the remediation of such contaminated sites. Both ecological needs and economical restrictions are to be taken into account for an appropriate solution. Hence, the goal of our work is to restore a detoxified ecosystem using a minimum of technological means. As technology is intended to be merely employed to trigger or optimize processes of intrinsic bioattenuation, the exploitation of microbiological cleansing capacities will play an important part in this soft remediation strategy.

In order to find out about the biodegradation potential of the indigenous microbiota for aromatic compounds, aerobic isolates gathered in arbitrary samples (the size of which was 48 colony-forming units each, cf. [7, 8]) were characterized physiologically and anaerobic most-probable-number enrichments were performed from different depths of the deposit. Moreover, we were interested in the determination of bacterial species from this severe environment. Consequently, the efficacy of commercially available identification systems for isolates was tested. Two test kits were compared: the BIOLOG system, the usefulness of which for the determination of environmental taxa is stressed by the supplier (Biolog Inc., Hayward, Calif., USA), and the API 20NE system (Biomerieux, Marcy-l'Etoile, France). Apart from the results of the microbiological studies, analytical data are presented in this paper to present insight into the *in-situ* microbial cleaning-potential.

2. Materials and Methods

2.1. Sampling

Water samples were taken from depths of 0.5 m, 2 m, 5 m, 8 m, 15 m and 27 m from the Schwelvollert deposit (located in Trebnitz, district of Weissenfels, Saxony-Anhalt, Germany). Water from each depth was pumped through a silicone hose (diameter 10 mm) with a hose pump (model 504 S/RL, Watson-Marlow Inc., Falmouth, UK) from the center of the lake. About 15 min after water started running, sterile 500mL flasks were filled right up to the top. Afterwards the samples were transported chilled on ice and processed in the laboratory within a few hours. Sampling for the determination of aerobic organisms was performed on 25 October 1993, with that for anaerobic bacteria taking place on 25 March 1994 and 23 May 1995.

2.2. Physicochemical Analyses

The oxygen content in the deposit was measured by means of an Oxi 196 oxygen meter from WTW (Wissenschaftliche-Technische Werkstätten, Weilheim, Germany). The detection limit was about 0.1 mg/L.
The Dissolved Organic Carbon (DOC) and Inorganic Carbon (IC) were determined using a TOC 5000-analyzer (Shimadzu Corp., Kyoto, Japan). To measure the DOC, samples were pre-filtered (polyethersulfone filter, 0.45 µm pore size; Filtron Technology Corp., Northborough, Massachusetts) and then analyzed according to the firm's instructions.
To determine the Biological Oxygen Demand (BOD), nutrients were added [9] to each sample, together with 2 mg/L allylthiourea in order to prevent nitrification [10]. The BOD was then measured manometrically with a WTW BOD-analyzer 1002 (Wissenschaftlich-Technische Werkstätten, Weilheim, Germany) using the Warburg principle.
The determination of the Chemical Oxygen Demand (COD) was carried out as a DIN-compatible micro method with chromo-sulfuric acid [11].
Steam-Distillable Phenolic Compounds were measured pursuant to DIN 38409 [12].
Nitrate, nitrite and sulfate were determined ion-chromatographically (DX 100, Dionex Corp., Sunnyvale, Calif., USA) using the AS4A-Sc ion-exchange column with AG4A-Sc pre-column. 1.8 mmol Na_2CO_3/ 1.7 mmol $NaHCO_3$ with a flow rate of 2 mL/min was employed as mobile phase. The conductivity or conductivity and UV-absorption (in the case of the determination of nitrate) were measured for detection. The detection limit was about 0.1 mg/L each. Prior to injection, organic compounds (such as aromatic compounds and AHM etc.) were removed by running 1 mL over an Onguard-P-cartridge (Dionex Corp.).
Sulfide was measured using the Ag 500 ion-selective silver/sulfide electrode and the R 502 reference electrode from WTW. Ammonium was determined electrochemically using a NH_3-electrode (Ingold, Steinbach, Germany) according to WTW's instructions. The measurements were taken using samples from 2 September 1993.

2.3. Microbiological Analyses

The determination of total cell counts was performed with acridine orange (AO; Serva, Heidelberg, Germany) as prescribed by HOBBIE et al. [13] and the slightly modified Nuclepore instructions (Costar, Cambridge, Massachusetts, USA): to improve the distribution of the cells on the 0.2µm Nuclepore filters, 10 µL Tween 20 (Serva) were added to each 10 mL diluted sample. Counting was performed using a Jenalumal microscope (Zeiss Jena, Jena, Germany; excitation filter: KP 490, beam splitter filter: LP 510, barrier filter: LP 490). About 30 - 50 of the 0.01mm^2 microscopic fields were evaluated each at 1,000x magnification.

To determine viable cell counts (following BRUNIUS and LUNDGREN [14, 15]) at least 1 mL of the sample was filtered through a 0.2µm Nuclepore filter (cf. total cell counts' determination). 2 mg fluorescein diacetate (FDA, Serva) was dissolved in 1 mL acetone and 20 µL of the solution diluted with 1 mL physiological (0.9%) sodium chloride solution. This mixture was placed on the membrane for 5 minutes. Counting was then performed with the Jenalumal microscope as described above.

In order to determine colony-forming units (CFUs), 0.1mL aliquots of tenfold diluted samples (in physiological sodium chloride solution) were plated onto R2A agar [16] (Difco Laboratories, Detroit, Michigan, USA). For the selection of fungi, 100 mg/L gentamycin sulfate (Serva) was added. The numbers of fungi were also determined on malt extract agar (Merck). Counting was performed after 10 days' incubation at 20°C.

Two different test systems were employed for bacterial identification: a total of 70 bacterial strains from the top layer of the deposit were tested with the BIOLOG identification system (Micro Log 1 version 3.5, Biolog Inc., Hayward, Calif., USA) and the API 20NE system (Biomerieux, Marcy-l'Etoile, France) in order to find out whether these test kits were useful for the determination of isolates from the deposit. 20 of the isolates were degraders of phenolic compounds; the others were selected due to their different morphologies and pigmentation in order to test a wide variety of bacteria from the Schwelvollert. Because of their slow growth the test strains had to be incubated for up to 4 days at 30°C on tryptic soy agar (TSA, Difco) or R2A agar (Difco). In order to look for fluorescent pseudomonads, ten-fold serially diluted samples from the surface were plated on King B agar (Difco).

The degradation potentials of communities of aerobic heterotrophic bacteria were determined by the physiological characterization of each of the 48 pure cultures forming an arbitrary sample according to BECKER [7, 8]. However, instead of microtitration plates, glass bottles were used due to the high volatility of alkylated phenols: the ability to degrade different hydroxylated aromatic compounds was tested in 500µL batch assays in 4mL culture tubes with aluminium screwcaps (Schütt Labortechnik, Göttingen, Germany). Test substrates of analytical grades were added up to double the final concentration (50 mg/L) in basic mineral solution [8]. The media were inoculated with 250 µL of cell suspensions in physiological sodium chloride solution (turbidity according to McFarland standard tube No. 5). After 14 days at 20°C colorimetric evaluations with Folin-Ciocalteu's phenol reagent (Merck, Darmstadt, Germany) were performed (50 µL of

each 1:5 diluted phenol reagent and 10% aqueous sodium carbonate solution added produces a blue color reaction in the presence of phenol) [8]. The most-probable-numbers (MPN) of nitrate or nitrite reducers, sulfate-reducing and methanogenic consortia were determined using benzoate as carbon source. All MPN tests were carried out in triple assays. The composition of the basal medium was as follows: 0.2 g KH_2PO_4, 0.3 g NH_4Cl, 0.4 g $MgCl_2$ x 6 H_2O, 0.15 g $CaCl_2$ x 2 H_2O, 0.25 g Na-benzoate (or 0.5 g in the case of 10mL inocula), 2.5 g $NaHCO_3$ for methanogens, 0.5 g $NaHCO_3$ for the other ecophysiological groups, and 0.4 mg resazurin per liter of distilled water. Vitamins were added in accordance with EICKELBOOM [17], trace elements as described by PFENNIG and LIPPERT [18], amended with 1.2 mg Na_2SeO_3 x 5 H_2O and 1.6 mg Na_2WO_4 x 2 H_2O per liter. Nitrate and nitrite reduction tests were dosed with 1.0 g KNO_3 and 0.5 or 1.0 g KNO_2 per liter. 0.3 g $FeSO_4$ x 7 H_2O and 1 g $NaSO_4$ per liter was added to sulfate reduction assays. (Double the concentrations of group-specific chemicals were added in the case of 10mL inocula.) The basal medium was used without further additions for tests on methanogenic consortia.

Anaerobic conditions were ensured by (i) working in the anaerobic glove box and (ii) by adding precultured cells of *Serratia marcescens* strain UFZ B229 (from the central culture collection of the UFZ Centre for Environmental Research Leipzig-Halle) prior to inoculation. For all assays to be handled in the anaerobic glove box (i) anaerobic culture tubes (18 x 150 mm, Bellco Glass Inc., Vineland, N.J., USA) with gas impermeable butyl rubber septum-type stoppers fixed with aluminum crimp seals were employed and filled with 10 mL (for the 10 mL inocula) or 9 mL (for all other dilutions) of the respective media, which had been reduced with Na-dithionite. After sealing the tubes were autoclaved. They were inoculated with syringes: 10mL and 1mL volumes were added from the sample bottle. The 1mL assays were then serially diluted by removing 1 mL each and adding it to fresh tubes filled with 9 mL medium. The tests with the biological reductant (ii) were performed as follows: studies on nitrite and sulfate reduction were carried out in screw cap culture tubes. Tubes used for 10mL inocula were 150 x 16 mm in size and for smaller volumes 98 x 16 mm (Schütt Labortechnik, Göttingen, Germany). Tests on methanogenic organisms were performed in the pressure-safe anaerobic culture tubes (18 x 150 mm, Bellco Glass Inc.). The media were filled into the respective tubes leaving room for inoculum and then sterilized by autoclaving. Prior to sample inoculation, *Serratia marcescens* strain UFZ B229 was added [cf. 19, 20]. This strain was precultured overnight on R2A agar at 30°C. The cell lawn from 1 plate was suspended in 5 mL sterile physiological sodium chloride solution and 50 µL was added to the respective assays. Then samples were added in 10mL, 1mL, 0.1mL and 0.01mL aliquots. Afterwards the tubes were filled with medium right up to the top and caps screwed on tightly or stoppers fixed with seals.

The MPN test tubes were incubated for 10 to 12 weeks (or 4 months in the case of methanogenic tests) at 20°C. After incubation, the nitrate and nitrite assays were checked using Merckoquant nitrate and nitrite test stripes (Merck). Sulfate reduction became visible as blackening. The formation of methane was measured gas-chromatographically (GACH 21.3 gas chromatograph, Betriebskontrollgerätewerk KG, Berlin-Oberschöneweide, Germany; 2m PPN column, diameter: 4 mm, pore size: 100 - 120 µm; carrier gas: H_2; thermal conductivity detector). Benzoate was determined by its UV absorption spectrum in a range from 220

to 300 nm after appropriate dilution of the samples. All evaluations were performed in comparison with benzoate-free controls, and the most-probable-numbers were read from tables published by ALEF [21].

3. Results

3.1. Physicochemical State of the Schwelvollert Deposit

Studies on the extent of the pollution showed that Steam-Distillable Phenolic Compounds, the DOC, BOD, COD and NH_4^+-nitrogen far exceed innocuous concentrations in the Schwelvollert deposit (Figs. 1, 2, 3). For comparison, according to Germany's administrative waste disposal regulation, the upper limits for the discharge of refuse dump seepage into the receiving watercourse are 20 mg/L for the BOD, 200 mg/L for the COD and 50 mg/L for ammonium [22]. (Although water fleas were observed at the very surface in summer time [rain water layer], the lake water proved to be harmful to *Paramecium caudatum*, *Poecilia reticulata* and *Ankistrodesmus falcatus* [23]. Furthermore, the water turned out to be highly toxic to *Photobacterium phosphoreum* [especially the bottom water with EC 50 = 13.5 mL/L]. While in the upper layers toxic effects are mainly caused by low-molecular compounds, at the bottom high-molecular fractions of the AHM proved most toxic [2].)

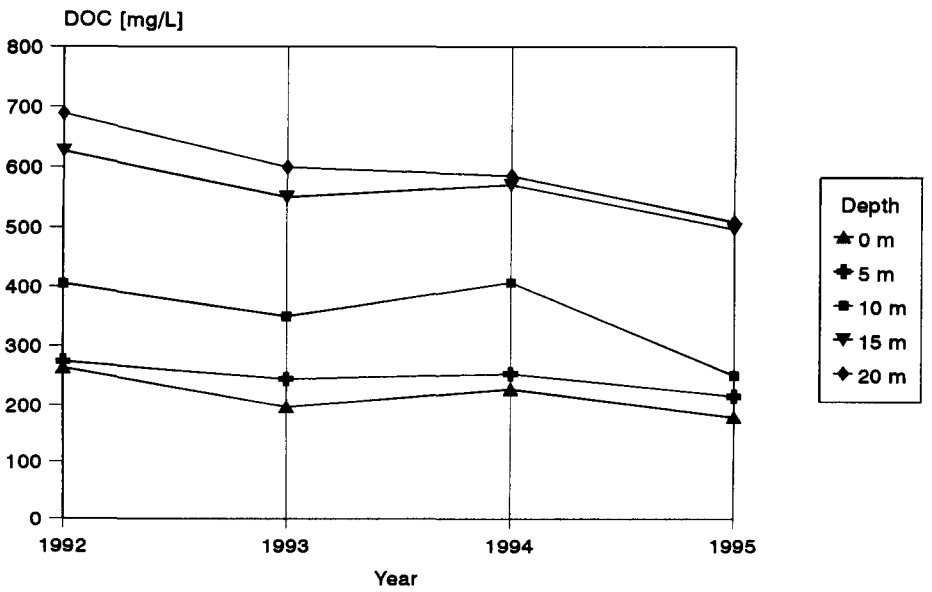

Figure 1: DOC load in the Schwelvollert deposit over recent years

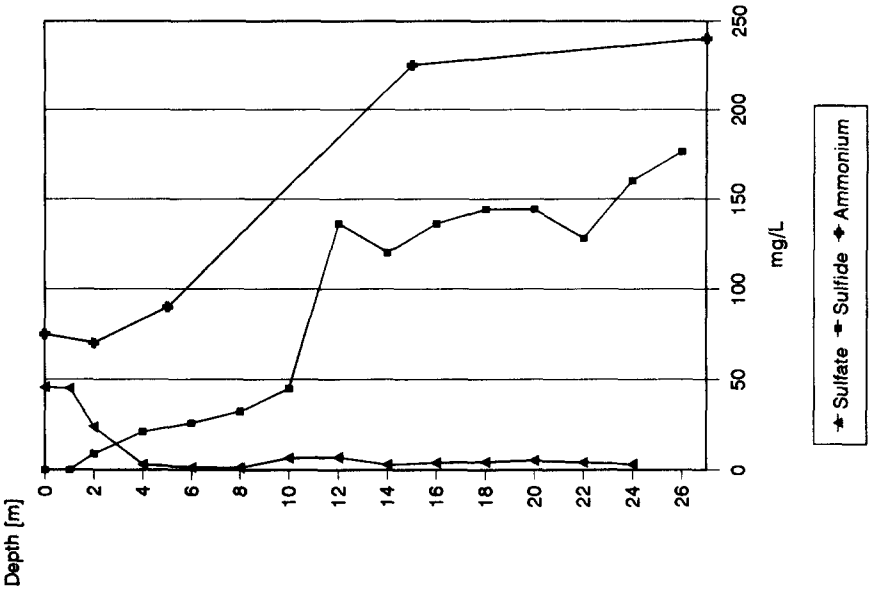

Figure 3: Depth profiles of the contents of selected ions

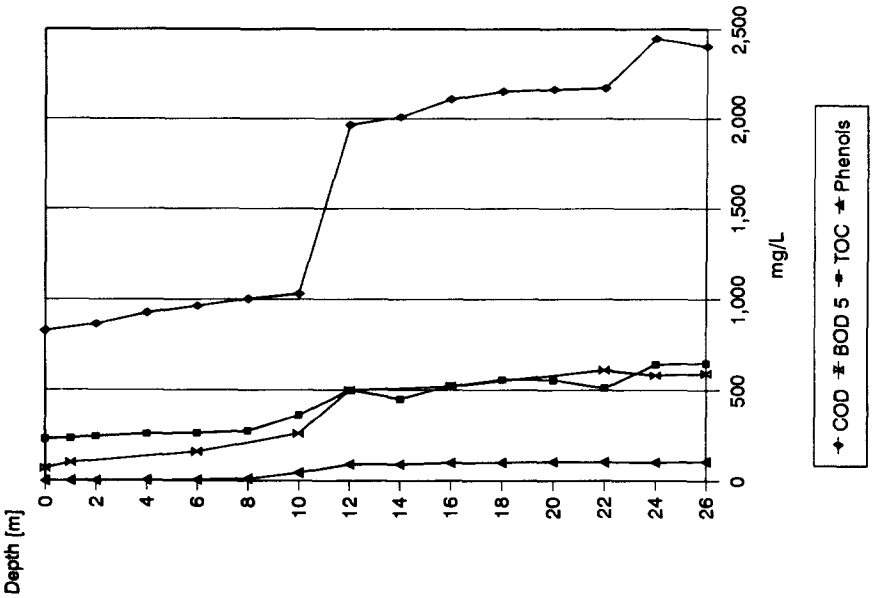

Figure 2: Depth profiles of pollutants determined as sum measurands

Depth profiles of different physicochemical parameters and contaminant concentrations showed two strikingly corresponding transitions, indicating the stratification of the lake in three distinct layers: one transition was located at a depth of about 4 m and another at about 12 m (Figs. 2, 3, 4). When sampled in autumn the temperature decreased from 7.6 to 6.0°C towards the bottom (Fig. 4). In the course of a year the temperature fluctuated in the upper zone from about 6 to 22°C, whereas below 6 to 7 m steady temperatures of 5 to 6°C were measured. The pH proved to be neutral (7.3 - 7.8) and almost constant from top to bottom due to carbonate concentrations ranging between 100 to 170 mg/L. However, even in the surface layer no oxygen was measurable - the whole lake is anaerobic. Among oxidized compounds sulfate was found in significant amounts in the surface water, but approached zero at 4 - 5 m with the simultaneous increase in the sulfide content, thus indicating a chemocline at this level. Neither nitrate nor nitrite was detected. Below 12 m the contents of carbon compounds, sulfide and ammonium increased sharply towards the bottom (Figs. 2, 3), characterizing the distinct bottom layer with high contaminant concentrations.

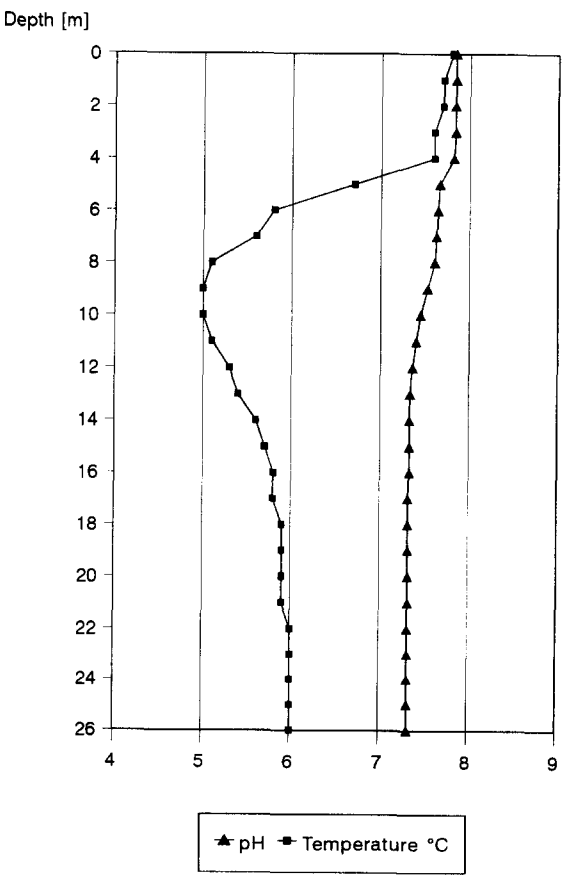

Figure 4: Depth profiles of temperature and pH

3.2. Anaerobic Microbiota

It was shown that the determination of viable cell counts with fluorescein diacetate works with some bacteria, but apparently has its limitations with anaerobes. Studies with enrichment cultures of anaerobes (phenol degraders in consortium with methanogens) and with actively metabolizing pure cultures (*Methanosaeta concilii* DSM 2139, *Desulfovibrio salexigens* DSM 2638) revealed that these organisms are unable to sufficiently hydrolyze fluorescein diacetate to be recognized as active cells when tested following the methods described by G. BRUNIUS and B. LUNDGREN [14, 15].

Since there is no common measure for anaerobic bacteria, different physiological groups were quantified separately. Because benzoate (as activated benzoyl-coenzyme A) is known to be the final aromatic intermediate in the anaerobic transformation of several classes of aromatic compounds [24, 25], it was chosen as carbon source in most-probable-number (MPN) assays to determine the cell densities of anaerobic degraders of aromatic compounds (nitrate/nitrite reducers, sulfate-reducing and methanogenic consortia). Tests were performed with liquid media, because solidified media are considered to be rather unselective [26]. Some denitrifying (and fermentative) bacteria are only able to reduce nitrite [27 - 29]: in order to detect these bacteria and because the ones that denitrify nitrate can reduce nitrite as well [21], nitrite and nitrate were added as electron acceptor in parallel tests. According to ALEF [21], one advantage of employing nitrite instead of nitrate in denitrification assays is that, in contrast to nitrate, nitrite (at a concentration of 0.1%) inhibits many non-denitrifying bacteria. Among the latter, the nitrite-reducing fermentative bacteria are also able to tolerate comparatively high concentrations of nitrite under anoxic conditions [29].

Two different techniques to ensure anaerobic conditions were compared: (i) working under the anaerobic glove box and (ii) adding precultured cells of *Serratia marcescens* strain UFZ B229 prior to inoculation with lake samples [cf. 19, 20]. The strain *S. marcescens* UFZ B229 was shown to accumulate nitrite on reduction of nitrate in Nutrient Broth (Merck). However, the nitrite concentration used in the denitrifiers' assays was tolerated and had no obvious growth limiting effect. Moreover and just as important, benzoate was not used as carbon source by this strain.

Only nitrite-reducing organisms that degraded benzoate were found with the MPN tests (Fig. 5). Bacteria reducing nitrate (methods i), sulfate or carbonate (methods i and ii) and simultaneously degrading benzoate were not even detected in 10 mL sample volumes. However, methanogenic (0.4 - 2.0 MPN/mL) and sulfate-reducing consortia were enriched on ascorbate, which we tried out together with *S. marcescens* to establish anaerobiosis (method ii). Without ascorbate as carbon source, sulfate-reducers (but not methanogens) grew in the test media as well as in the controls (with a maximum of 50 MPN/mL at a depth of 2 m). Benzoate, however, was not attacked. Yet, as the sulfate/sulfide chemocline (Fig. 3) indicates too, distinct populations of sulfate-reducing bacteria seem to play a role in the Schwelvollert. It was not possible to conduct tests on nitrate-reduction with method ii, because *S. marcescens* reduced nitrate using sample ingredients as reductants. The nitrite assays, however, worked well compared to the controls (Table 1): the results obtained

with both reductants are quite comparable (in view of the employment of only three parallel test tubes per dilution step).

Table 1: Number of nitrite-reducing benzoate degraders (23 May 1995)

Depth	MPN/mL[a]	MPN/mL[b]
0.5 m	11	20
2 m	2.1	9
5 m	15	20
8 m	15	20
15 m	0.23	0.03
27 m	0.23	0.04

[a] Medium chemically reduced (i)
[b] Medium biologically reduced (ii)

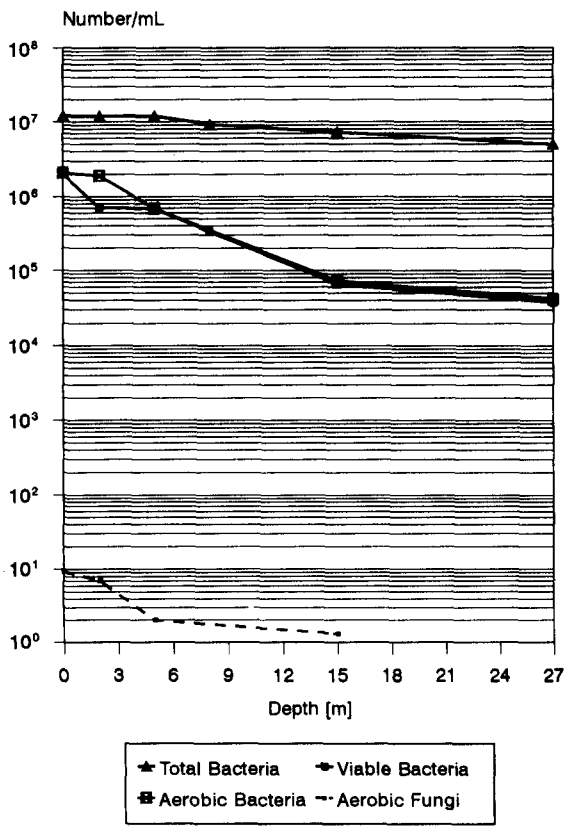

Figure 5: Cell counts of different ecophysiological groups

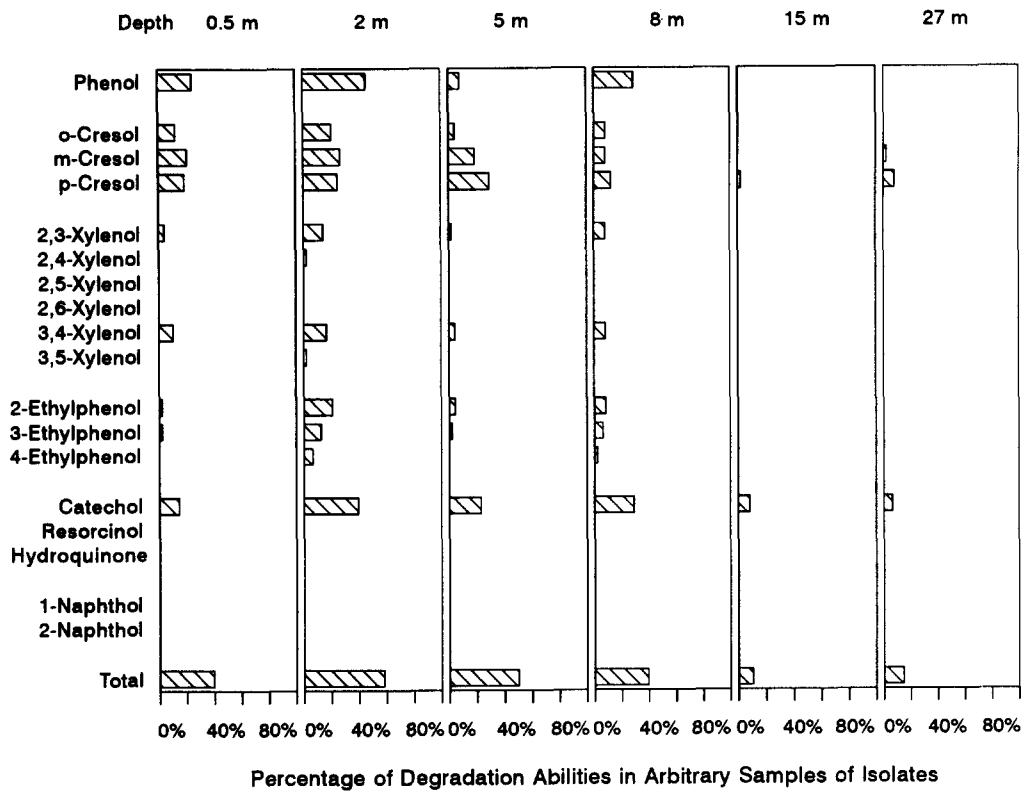

Figure 6: Degradation potentials of aerobic heterotrophic microbial communities

3.3. Aerobic Microbiota

Cell counts of aerobic heterotrophic bacteria (on R2A agar) were essentially the same as viable cell counts (hydrolysis of FDA as indicator) (Fig. 5). The number of aerobic and viable prokaryotes/mL was approximately 10^4 in the bottom water and 10^6 in the surface layer. Their proportions in total cell counts were about 1% (depth) to 10% (surface).

When testing arbitrary samples of bacteria, significant degradation potentials for some of the aromatic compounds (chosen because of their dominance among the pollutants) were found in the upper lake zone. These compounds were measured in the deepest layer of the Schwelvollert in the ppm range [1, 2]. At the surface only traces were detected, even of total volatile phenols (Fig. 2). The percentage of aromatic compound-degrading strains within the heterotrophic microbial communities was 40-60% in the upper layer, whereas in the bottom water only 10-15% of the test organisms were able to degrade phenolic compounds. (The differences in degradation patterns of 0 to 8 m samples are not to be overrated as it is just possible to manage studies on 48 isolates, but for statistical purposes this size is very small.) Different strains were able

to cleave phenol, cresols, 2,3-, 2,4-, 3,4-, 3,5-xylenol and ethyl phenols (Fig. 6).

About 70% of the aerobic heterotrophic isolates we tried to identify with the BIOLOG or API 20NE system proved to be rather inactive oligotrophic bacteria (a phenomenon which not only applies to the extreme habitat Schwelvollert but also to other environmental sites we have studied). About two thirds of these bacteria showed neither the growth velocity nor the variety of substrate assimilation required by both identification systems and hence could not be identified (owing to too few matching positive reactions). In terms of identification results, the following species were named by the BIOLOG system: *Acinetobacter calcoaceticus, Acinetobacter baumanii, Alcaligenes latus, Ancylobacter aquaticus, Aquaspirillum aquaticum, Bacillus insolitus, Bacillus megaterium, Bacillus pumilus, Curtobacterium luteum, Brucella abortus, Klebsiella pneumoniae, Lampropedia hyalina, Methylobacterium extorquens, Moraxella bovis, Pseudomonas corrugata, Pseudomonas nautica, Pseudomonas synxantha, Pseudomonas vesicularis, Rhodococcus maris, Vibrio fischeri, Xanthomonas campestris* and *Xanthomonas oryzae. Comamonas, Corynebacterium* and *Moraxella* were determined at genus level. However, only the identification of the *Acinetobacter* strains and *Bacillus pumilus* at species level and *Comamonas* at genus level were confirmed by the API 20NE system. Some strains belonging to the rather inactive bacteria were named as *Brucella* or *Moraxella* species, whose type strains are pathogenic bacteria that need complex media or certain growth factors. Strains named as *Aquaspirillum aquaticum, Lampropedia hyalina, Moraxella bovis* and *Methylobacterium* spec. differed in main features (cell morphology, oxidase reaction, pigmentation) from the type strains and thus some identifications were definitely incorrect. About 4% of the strains with a marked reaction pattern were not identified by the BIOLOG system, apparently due to the lack of corresponding reference data. One of these strains isolated from the surface turned out to belong to a hitherto undescribed taxon close to *Rhodobacter spaeroides* and *Paracoccus denitrificans*, as was stated by the DSM (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). The strain deposited as UFZ B438 (Becker 3) in the central culture collection of the UFZ Centre for Environmental Research Leipzig-Halle was able to cleave phenol and showed a different morphology when freshly isolated and after subculturing on R2A agar: the newly isolated bacteria were long and rod-like, whereas later on they became short single cells.

All in all, the BIOLOG system, though developed for the identification of environmental bacteria, proved unable to adequately name more than 60% of the isolates from the admittedly severe Schwelvollert environment. Surprisingly, we did not find any fluorescent *Pseudomonas* strain at the surface, even though these bacteria are very versatile substrate degraders and, as was shown by studies not reported here, can be identified quite well from environmental samples. However, as these bacteria prefer habitats with good oxygen supply [31], their absence might be explained by the oxygen deficiency in the Schwelvollert.

Fungi were found in similar amounts to the nitrite-reducing benzoate degraders (probably owing to the lack of readily assimilable organic carbon and/or lower turnover rates than the aerobic bacteria). When comparing different media, significantly higher numbers of fungal colony-forming units were obtained on R2A agar dosed with gentamycin sulfate than on malt extract agar. Thus, it seems that fungi from oligosaprobic sites

(oligosaprobic with regard to readily utilizable carbon sources) prefer lower substrate concentrations than media for fungal diagnosis contain as well as oligotrophic bacteria [32].

The growth of algae was detected on gentamycin-dosed R2A agar too. From samples from the surface down to a depth of 5 m, a few CFUs of *Chlorella* spec. (probably *vulgaris*) were isolated, although light penetration measured as Secchi depth was less than 3 cm. However, *Chlorella* spec. has been shown to survive harsh conditions before, and even to degrade phenol aerobically when exposed to light [33].

4. Discussion

Most physiologically active specific degraders in the Schwelvollert deposit were shown to be - at least facultatively - aerobic. Although there might be some season-dependent oxygen input and mixing in the upper part of the lake, this could not be verified during the sampling period (probably because of an immediate consumption). Hence, anaerobic metabolic capabilities seem to be a prerequisite for survival in the strictly anaerobic lake, and most of the aerobic heterotrophic bacteria are likely to ferment in the deposit, with the ten- to hundred-fold larger proportion of "inactive" biomass (low active, damaged, or perhaps strictly anaerobic cells?) in total microbiota as possible carbon and energy supply.

Although nitrifiers could be enriched from surface water (enrichment media according to WATSON [34] and ZAVARZIN and LEGUNKOVA [35]), neither nitrite nor nitrate was detectable in the Schwelvollert. These compounds are probably reduced immediately after their production. Consequently, facultatively aerobically respiring organisms are not likely to play a significant role some distance from the top, which was to some extent confirmed by the non-detectability or low cell numbers of the nitrate-/nitrite-reducing benzoate degraders.

Although cell numbers are underestimated when using enrichment techniques for detection [7] or other methods basing on cultivation, more adequate methods for the simultaneous enumeration of diverse ecophysiological bacterial groups, which may each use a variety of different degradation pathways for the degradation of distinct contaminants [cf. 36], have still to be developed [37]. However, the comparability of the data on viable (FDA-hydrolyzing) and culturable cell counts of aerobic bacteria speaks well for the use of the respective methods within this case study. Our findings may indicate a correlation between the putative fermentative metabolism of these bacteria and the esterase activity measured. Although it has been shown by various teams that both eucaryotic and many bacterial cells can be enumerated by means of FDA-hydrolysis [14, 15], strictly anaerobic bacteria can obviously not be determined in this way. Maybe the anaerobes tested were damaged by oxygen during the testing procedure, so that the uptake of FDA into the cell, which is discussed as a decisive step [38, 39], was inhibited.

The determination of aerobic heterotrophic bacteria with the BIOLOG and API 20NE identification kits proved too ineffective to be useful in our study. These findings correspond to the results of KLINGLER et al. [40], who thought enteric bacteria and those belonging to the genus *Methylobacterium* were unsatisfactorily

identified by the BIOLOG system, and RÜGER and KRAMBECK [41], who found the BIOLOG system inefficient for marine bacteria. Obviously wrong identifications such as animal pathogens like *Brucella abortus*, *Klebsiella pneumoniae* and *Moraxella bovis* were also reported by ZHENG et al. [42]. As there seems to be no stringent correlation between taxonomic diversity and the degradation abilities for hydrocarbons of aerobic heterotrophic microbial communities [7, 8, 43], for remediation purposes the knowledge of the degradation potentials and activities of the indigenous microbiota should be of primary interest. These data bear more specific information than bacterial names, which moreover are often very laborious to ascertain.

The physiological spectra within the arbitrary samples of aerobic isolates turned out to be significantly different above and below 12 m, where almost no degradation ability was detected. These data were confirmed by the results of studies of degradability under aerobic conditions: biodegradation in the bottom water only started after the addition of precultured organisms or water from upper layers as inoculum [4]. Thus, the mere sedimentation of bacteria from top to bottom of the deposit seems rather unlikely, and some adaptation to the respective layers is most probable. While the bottom layer proved stable throughout the year, meromixis governed by seasonal factors apparently led to comparable concentrations of pollutants as well as microbial potentials in the upper regions.

Recent measurements indicated the rather unchanged state of the organic pollution, featuring slight reductions at best (Fig. 1). This finding coincided with the results of the microbiological studies. Besides the failure to detect degraders of aromatic compounds significant for anaerobic *in-situ* degradation, high concentrations of contaminants indicate the limitation of biodegradation, especially at the bottom. The following turnover- and growth-limiting factors were detected: a lack of electron acceptors (oxygen, nitrate, nitrite, sulfate), the toxicity of contaminants and phenolic autoxidation products [2], a lack of phosphate, and invariably low temperatures beneath 6 m. But nevertheless, we found *in-situ* degradation potentials for aromatic compounds: both aerobic (in the top and middle layer) and anoxic degradation abilities were detected. As was already stated, using the measuring methods described above, benzoate-degrading nitrite reducers were only found in densities of some four to six powers lower compared to aerobic bacteria which were able to cleave aromatic rings. Hence, on supplying oxygen as a remediation strategy, phenolic compounds should compete with non-aromatic organic material as a carbon source for indigenous aerobic bacteria exhibiting the corresponding abilities [cf. 4]. Dosing with nitrate, nitrite or sulfate, however, would have to firstly stimulate the growth of the respective users to give higher cell numbers and thus higher activities. FLYVBJERG et al. [44] showed that phenol, the cresols, 2,4-xylenol and 3,4-xylenol were removed in a creosote-contaminated groundwater under nitrate-reducing conditions, thus confirming the results of RUDOLPHI et al. [45] on their transformability by denitrifying bacteria. Yet, 2,3-, 2,5-, 2,6- and 3,5-xylenol were resistant to biodegradation during 7 - 12 months of incubation [44]. Under sulfate-reducing conditions phenol, o-cresol and m-cresol disappeared, but 2,3-, 2,4-, 2,5- and 3,5-xylenol was not removed (7 months' incubation) [44]. SUFLITA et al. [46] found degradation of all three cresols occurring both under sulfate-reducing and methanogenic conditions. However, FEDORAK and HRUDEY, and FOX et al. thought o- and

m-cresol [45, 46], 2,4- [48], 2,5-, 2,6-, 3,4- and 3,5-xylenol [47] undegradable under methanogenic conditions. Thus, even when optimizing growth conditions, anaerobic treatments obviously have their limitations compared to the input of oxygen for the bioremediation of carbonization wastewater.

One great disadvantage of treatment with oxygen is of course the enhancement of the autoxidation of the phenolic pollutants. Because of the huge oxygen-trapping capacity of the deposit, the spreading of oxygen by diffusion will be very ineffective for decades. To overcome these difficulties, different water treatments are currently being studied both on bench and pilot scale [43] to find the most suitable remediation strategy.

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