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CHARACTERIZATION OF BIOFILMS ON CORRODED CONCRETE SURFACES IN DRINKING WATER RESERVOIRS

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ABSTRACT

The surface of concrete in drinking water reservoirs is frequently covered with a mineral coating. Since the beginning of the 1980's, there has been an increasing number of reports of brown spots within which the coating matrix was weakened. The diameter of the spots ranged from a few millimetres up to 10-20 centimetres. Cleaning measures resulted in removal of the spot material revealing that shallow pits had formed in the coating. The deeper underlying concrete body was usually unaffected. Although the removed material was shown to contain a substantial microbial biomass, there was no indication of an elevated microbial contamination in the actual drinking water as detectable by the German standard drinking water testing method. The microbial biomass from the damaged sites of six reservoirs was quantified using ester-linked phospholipid fatty acids (PLFA). Population densities were shown to range between 5×10^7 and 5×10^8 cells g^{-1} in samples collected from non-chlorinated reservoirs and 10^6 cells g^{-1} in samples from a chlorinated one (assuming 0.5 fM PLFA equivalent per cell). The recovery of PLFA indicated that physiologically active populations were present in all of the spots sampled and an analysis of the PLFA profiles revealed that the microbial community contained a large percentage of gram negative aerobic heterotrophs. Differences were found between the PLFA patterns of samples from different reservoirs. The fact that a consistent PLFA profile was not recovered from each spot sampled indicates the absence of a single dominant organism. Methyl cellulose (MC) was identified as a possible nutrient source based on the successful growth of bacterial strains isolated from the damaged areas on hydrolyzed coating material. How MC may becomes bioavailable in the reservoirs remains still unclear.

KEYWORDS

Biocorrosion; drinking water; microbial community structure; phospholipid fatty acid analysis; methyl cellulose.

INTRODUCTION

Drinking water reservoirs in Germany, especially in Bavaria, are frequently coated with a mineral layer at a thickness of 3-4 mm. The coating provides protection for the underlying concrete and is a smooth surface allowing for more efficient cleaning. The coating is considered to be stable over a long period of time and,

according to the manufacturers, has not changed in chemical composition throughout the 1980's. However, over this same time period brown spots have been observed with an increasing frequency. Approximately 2-5% of all coated reservoirs show the formation of these spots which usually develop within 1-2 years of coating application. Spot sizes range from a few millimetres to 20 centimetres in diameter. They generally occur below the water table on the bottom of the reservoir in and around crevices. Upon cleaning of the coating shallow pits were revealed indicating that the stability of the coating material had been compromised. Rarely does the underlying concrete show evidence of pitting corrosion.

The pitting phenomenon was first reported by Labitzky and Gierig in 1992. To date, at least 80 reservoirs show similar damages, approximately 50 in Bavaria and 30 in the rest of Germany as well as cases reported in Switzerland and Austria. Monetary damages are estimated to be at least 30 Mio DM.

A comparative evaluation of the case histories revealed no correlation between the size of the reservoirs, their design (spiral and rectangular), or the location of the spot within the reservoir. There was also no determinable relationship between spot formation and water quality, reservoir geographical location, coating manufacturer or coating applier.

The detection of spot associated microorganisms in all cases led to the supposition that the bacteria might play a significant role in the corrosion process. Three hypotheses were developed for testing.

(i) The presence of bacteria in the spots will lead to an enhanced bacterial contamination from the coating material.
(ii) The bacteria present in the spots will derive nutrition from the coating material.
(iii) The microflora associated with the spots will be consistent and dominated by a single organism.

The drinking water showed no increase in the microbial content when associated with the presence of the brown spots. Microbial content was determined by the measurement of colony forming units (cfu) in accordance with the German Drinking Water Regulation (Trinkwasserverordnung, 1990). Thus, question (i) can be clearly answered with "no". This paper describes the investigations made toward answering questions (ii) and (iii).

MATERIALS AND METHODS

Sampling

For this study, 6 reservoirs were selected. Approximately 10 g of material were scraped from the brown spots using a sterilized spatula, collected into sterile petri dishes, and lyophilized.

Lipid extraction

All glassware was thoroughly cleaned and heat-treated at 450°C for a period of 4 hours. Other than glass, only teflon lined screw caps (precleaned in acetone) came in contact with the samples. The lyophilized sample material was extracted using a modification of the method of Bligh and Dyer (1959) consisting of a single phase chloroform-methanol-phosphate buffer (White *et al.*, 1979a) mixture. The lipid extract was further fractionated into neutral-, glyco- and polar lipids by silicic acid column chromatography (Tunlid *et al.*, 1989). The methanol fraction, containing the polar-lipids, was then exposed to a alkaline solution resulting in the transesterification of the fatty acid moieties into fatty acid methyl esters for gas chromatographic analysis (Tunlid *et al.*, 1989). Fatty acid structural verification was done by mass spectrometry/gas chromatography as described in Ringelberg *et al.* (1994).

Microbial biomass determination

Phospholipid fatty acids (PLFA) do not occur as storage products and are rapidly degraded upon cell death (White *et al.*, 1979 b) thereby providing a quantitative measure of the viable microbial biomass. A number

of authors have used PLFA to quantify viable biomass in environmental matrices such as soil (Zelles *et al.*, 1992), sandstone (Palmer *et al.*, 1991), sediments (Manusco *et al.*, 1990) and concrete (Kerger *et al.*, 1987). PLFA abundance was related to cell numbers using a factor of 0.5 fmol PLFA $cell^{-1}$ (Tunlid and White, 1990).

Microbial community composition

The pattern of PLFA present in cell membranes has been shown to vary greatly between different groups of organisms. In some instances, specific groups or species of organisms have been shown to contain uniquely characteristic PLFA (Kerger *et al.*, 1987; Ringelberg *et al.*, 1994). An overview of PLFA composition in different organisms has been provided in Vestal and White 1989. PLFA are described by the total number of carbons followed by the number of double bonds present with the position of the double bond indicated from the methyl (w) end of the molecule in either the *trans* or *cis* configuration. For example, 18:1w9c contains 18 carbons with one double bond located 9 carbons from methyl end of the molecule in the *cis* configuration. In addition, the prefixes i and a refer to *iso* and *anteiso* methyl branching, 1 or 2 carbons from the ω end of the fatty acid, respectively. Mid-chain methyl branching is numbered from the acid end, i.e. 10me16:0, whereas an unknown position of a methyl branch is indentified with the prefix '*br*'. Cyclopropyl PLFA are identified by the prefix '*cy*'.

Microbial growth on coating components

The growth of microorganisms originating from the coating material was tested in liquid culture. Ten grams of the coating material was treated with 100 ml of HCl (pH 2) for a period of 30 min. The mixture was then filtered with a 0.45 mm polycarbonate filter, 10 ml of a mineral medium (5 mM KNO_3, 1 mM Na_3PO_4) was added, and the pH adjusted to 7.

The mixture was then filtered through a 0.2 mm pore size and inoculated with 6 isolates previously recovered from the reservoir damaged sites and cultivated on R2A agar (Reasoner and Geldreich, 1985). Kinetics of cell growth were determined by sampling at different times and counting cfu post stationary phase of growth.

RESULTS

Microbial biomass

Biomass in the corroded material was determined from the phospholipid fatty acid content, as described above. In all samples, phospholipids were detected indicating the presence of viable organisms. Estimated cell numbers ranged between 5×10^7 and 5×10^8 cells g^{-1} in samples recovered from non-chlorinated reservoirs and 10^6 cells g^{-1} in a sample collected from a chlorinated reservoir. The chlorine content of this reservoir was maintained at approximately 0.3 ppm free chlorine. This indicates that the biofilm form of life as it occurs in the corroded material protects the cells against the biocidal effect of chlorine as it has been demonstrated in other habitats (LeChevallier *et al.*, 1988).

Figure 1 illustrates cell number estimates derived from phospholipid fatty acid content in six reservoirs (A-F). Reservoir C was chlorinated.

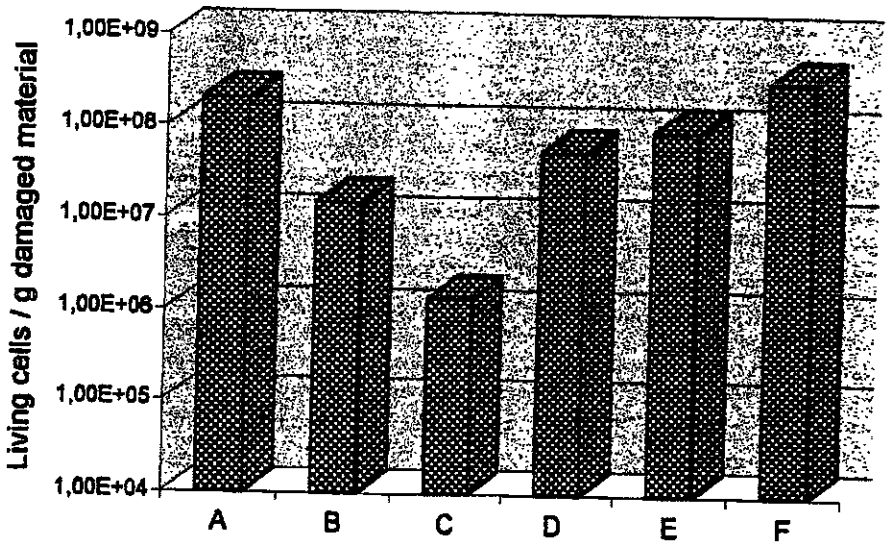

Figure 1. Estimation of the microbial biomass within corroded areas of six reservoirs.

Microbial community composition

The composition of the biomass can be derived from the analysis of the lipid profile (Tunlid and White, 1990). In Fig. 2, the fatty acid profiles are divided into six functional groups. The differences between profiles from the six reservoirs suggests that the composition of the biocoenosis is specific to a particular site and not uniform across sites.

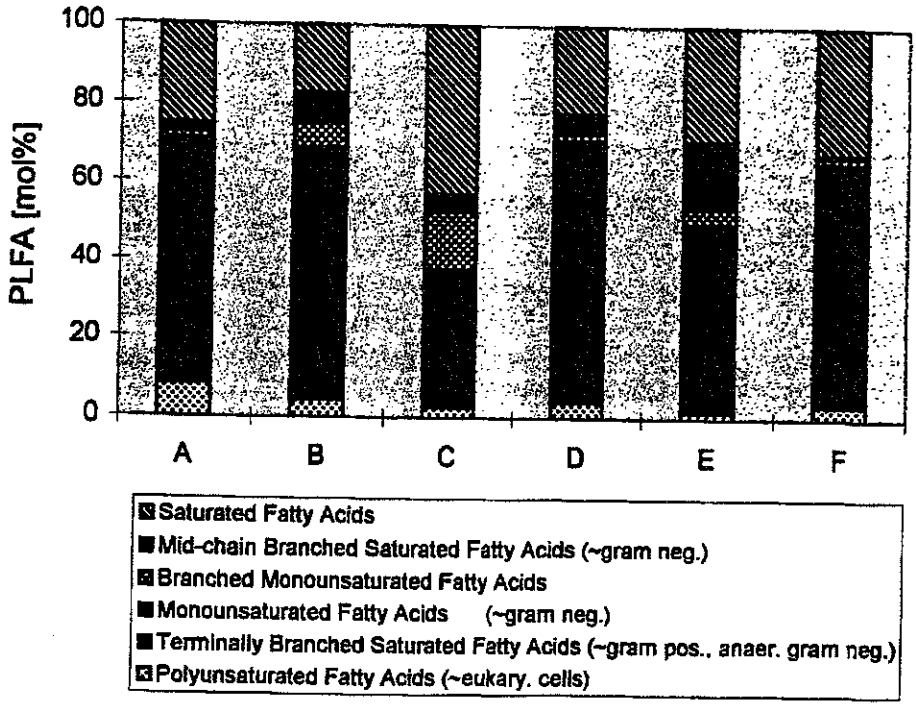

Figure 2. Ester-linked phospholipid fatty acid profiles from the same samples described in Fig. 1.

All samples contained large amounts of monounsaturated PLFA such as 16:1w7c and 18:1w7c, which are descriptive of aerobic gram-negative bacteria.

Sample A exhibited the greatest abundance of terminally branched saturated PLFA which are characteristic of the gram-positive classification of bacteria but are also present in the cell membranes of other bacteria, such as sulfate reducers (Taylor and Parkes, 1983). The ratio of i17:0 to a17:0 PLFA is 0.25, which suggests that this functional group of PLFA (the terminally branched saturates) likely belong to gram positive bacteria which, generally, exhibit more anteiso PLFA than iso (Kohring *et al.*, 1994). Sample A was also found to contain the greatest percentage of polyunsaturated PLFA which are characteristic for the most eukaryotic organisms.

Sample C was collected from a chlorinated reservoir and was shown to contain the lowest microbial biomass of the six reservoirs. This sample also showed the greatest percentage of normal saturated PLFA (40%). An increase of saturation coincides with a decrease in membrane fluidity which may have been a response to the presence of the xenobiotic, chlorine.

Sample D exhibited the greatest percentage of the PLFA 10me16:0 which describes the sulfate reducing bacteria, in particular the *Desulfobacter* species (Vestal and White, 1989).

Microbial growth on coating components

After hydrolyzing the damaged coating material and incorporating it into a liquid culture inoculated with extent microorganisms, viable counts were determined over a 30 day period. Figure 3 illustrates the growth kinetics obtained from two damaged coatings, two different original coating materials and sterilized water. Extracts from both the damaged and the original coatings were capable of supporting microbial populations indigenous to the reservoir.

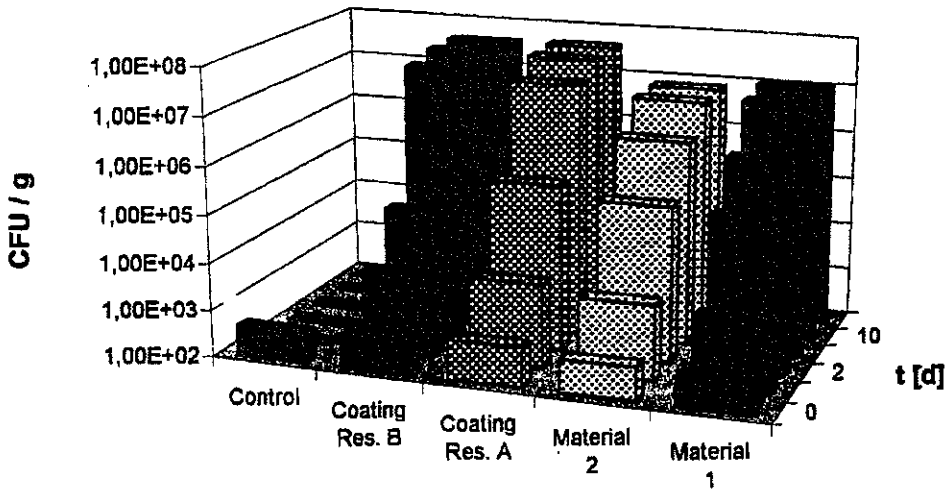

Figure 3. Support of microbial growth by extracts from two different uncorroded areas of damaged reservoirs (Res. A and B), two different original coating materials (Mat. 1 and 2) and a control.

DISCUSSION

The results demonstrate that viable microbial communities colonize the brown spots. Undamaged coatings carry biofilms as well. However, the colonization density there is not higher than 10^3 cfu cm^{-2} whereas damaged spots contain 10^5-10^6 cfu cm^{-2}. Thus, the release of bacteria from the biofilms into the water is likely, bacterial levels in the water were very low and did not represent a health hazard. The finding of

viable cells in the reservoir containing 0.3 ppm free chlorine suggests a protective advantage of biofilm formation which has been described for other habitats (LeChevallier *et al.*, 1988).

Even though the brown spots appear visually similar, differences in microbial community composition of the spots were apparent in the six reservoirs sampled. Thus, there was no clear indication of a single 'concrete attacking' organism which could be identified as responsible for the repeated pattern of damage. Since the distribution of microbial growth on the coated surface of a reservoir was not random but concentrated in corroded areas, it is unlikely that the drinking water itself was the nutrient source for these organism. Since non-corroded sites of the coating material were found to be sparsely colonized, then it is likely that the organisms derived nutrition specifically from the corroded areas.

No organic material was reported to be present in the coatings by the coating manufacturers who claimed the material was composed "exclusively of minerals". A suspected nutrient source from the coating was methyl cellulose (MC), which has been shown to be associated with microbial colonization and deterioration of the material (Morgenstern, 1982). This compound is typically used as an additive in concrete to increase viscosity and to retain water. A mass spectrometric analysis of the coating material revealed that MC was present. Coating manufacturers have recently confirmed that MC has been used as an additive at 0.1 to 0.5% (w/w). The MC was, however, found to reside primarily in the underlying highly alkaline (pH 12) concrete matrix. A pH of 7-9 is necessary for most water organisms in order for growth. It is unlikely that the extant microbiota was capable of forming enough acid from MC to neutralize the highly alkali concrete underlayment in order to sustain growth. The results of this study do, however, clearly indicate that extracts from the coating surface support microbial growth. Therefore, it appears that abiotic mechanisms in association with a possible biofilm mediated acceleration of the growth kinetics may be responsible for the pit formation.

Acidic cleaners have been identified as a possible culprits in initial damages (Schoenen, 1994), however, some of the reservoirs have never been subjected to acidic cleaners and still show the formation of brown spots. Some reservoirs have been cleaned with acidic solutions for 20 years and have never shown the formation of the brown spots. As a result, it is believed that acidic cleaners are only one number of contributing factors to the spot formations. An other concept relates concrete corrosion to the effects of electric fields (Menzel and Aktas, 1991; Müller and Tanner, 1993). Gerdes and Wittmann (1994) showed that they could reproduce the coating damage in the laboratory using electrical fields. The role of microorganisms in this process is still unclear since the experiments were carried out under non-sterile conditions.

At this point in time, it can only be recommended that the use of methyl cellulose and acidic cleaners be avoided and that the potential for the generation of electrical fields be minimized. The identification of MC as a possible nutrient source for the extant microbiota does not preclude the possibility of other sources. It is hoped that additional research will further clarify the potential role of MC in biotic processes an that relationships between abiotic and biotic processes will be identified.

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