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Chemical and biological characterisation of biofilms formed on different substrata in Tisza river (Hungary)

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The Plexi-glass substrate is recommended for monitoring river benthic microbiota.

Abstract

Natural biofilms were simultaneously grown on granite, polished granite, andesite, polycarbonate and Plexi-glass substrata for six weeks in the Tisza River. Biofilm production and abundance of algae were influenced by the substratum. Magnitude of the substratum effect was andesite < polished granite < Plexi-glass < granite < polycarbonate. The benthic diatom community on polycarbonate had a high population of *Achnantes helvetica*. Bacterial activity was similar among substrates for 95 different carbon sources. The concentrations of essential elements and heavy metal pollutants (Zn, Ni, Pb and Cu) were highest in biofilms on polished granite or granite. On basis of algological, bacteriological and chemical investigations, as well as literature data, the Plexi-glass substratum is recommended for biomonitoring of river benthic microbiota.

Keywords: Biofilm; Biomonitoring; Substrata; Heavy metal

1. Introduction

Biofilms formed on the surface of artificial or natural substrata in the photolytic layer of lakes or rivers are complex communities, composed mainly of photoautotrophic (algae) and heterotrophic microorganisms (bacteria, fungi, protozoa). These organisms are embedded in their extracellular polymeric substances (EPS) (Characklis and Marshall, 1990). The EPS-matrix is a dynamic system, which fills and forms the space between the cells and it is responsible for the architecture and morphology of the biofilm (Lewandowski et al., 1994). The major components of EPS are polysaccharides and proteins, in some cases lipids, nucleic acids and other biopolymers (Flemming and Wingender, 2001).

Biofilms, because of their position at the interface between the substratum and the water, play a fundamental role in the various biogeochemical cycles and dynamics of the aquatic ecosystems (Amblard et al., 1990; Schorer and Eisele, 1997). They can sorb water, inorganic and organic solutes and particles. EPS, cell walls, cell membranes and cell cytoplasm can serve as sorption sites. These sites display different sorption properties, preferences and capacities (Späth et al., 1998).

Considering the accumulation of pollutants in the biofilms and their characteristic response to major changes in water quality, these living systems were widely used in monitoring studies (Masseret et al., 1998; Vis et al., 1998; Gold et al.,

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2002; Mages et al., 2004). Biofilms possess many of the attributes required for monitoring organisms (McCormick and Cairns, 1994; Fuchs et al., 1997): (1) they are widely distributed; (2) they are sessile, therefore, they reflect the real conditions of the habitat; (3) they respond more rapidly to environmental changes than higher level organisms, because of their short life cycle; (4) these communities are rich in species, composed of many algal taxa with various environmental tolerances; (5) it is relatively easy to collect biofilm samples.

There are three main approaches to in situ biomonitoring of pollution (Calow, 1993): (1) monitoring the effects of pollution on the community (e.g. biomass, diversity, presence or absence of species); (2) monitoring the effects of pollutants on the biochemical or physiological processes of the organisms (e.g. photosynthetic assimilation, community respiration); (3) measuring concentration of pollutants in the indicator organisms or communities.

Many ecotoxicological and environmental studies investigated the effects of pollution on the community structure or community functioning. Havens et al. (1999) used HPLC pigment analysis and light microscope counts to characterise the biofilms at seven locations in a subtropical lake. Some authors studied the interactions between the physicochemical characteristic of water and the biomass, diversity, Chlorophyll-*a* content using glass (Masseret et al., 1998), plexi-glass (Vis et al., 1998) or stone substrata (Chuks Chindah, 1998). Hill et al. (1997) investigated the effects of different metals on the biofilms grown on rock surfaces using measures of primary conductivity, community respiration, water column toxicity and ICP-analysis of water. The structure and metabolic activities of biofilms were studied above and below a treated sewage outflow in the Le Raby stream applied glass microscope slides as substrata (Masseret et al., 1998).

Some authors made comparisons of biofilms grown on different artificial and natural substrata, however, their biological conclusions are contradictory. Barbiero (2000) reported that natural substrata exhibited greater species richness than artificial substrata. Danilov and Ekelund (2001) applied glass slides, glass tubes, pieces of PVC and pieces of wood as substrata and concluded that the nature of substratum can considerably affect patterns of colonisation of algae. Glass tubes turned out to be the most favourable substratum on the basis of settlement patterns of algae, while no growth of algae was observed on PVC pieces. Their results are contradictory to those summarised by Cattaneo and Amireault (1992) where plastics were considered to be favourable artificial substratum for biofilms. Lane et al. (2003) reported further contradictory results. They compared biofilms colonising glass slides and clay tiles to the naturally occurring communities in the epipelon, epilithon and epiphyton and concluded that the composition of diatom communities on artificial substrata was representative for the community composition on natural substrata. Cattaneo and Amireault (1992) explained the contradictions saying that authors evaluate their results differently in relation to the goals of their study, and the performance of substrata may depend on the environment studied and methods used.

The metal uptake of biofilms was investigated only in a few biomonitoring studies, applying mostly AAS (Ivorra et al., 1999) and TXRF (Friese et al., 1997) methods for elemental analysis. In these studies only one type of artificial substratum was applied, Ivorra et al. (1999) used glass discs, Friese et al. (1997) ceramic plates. In the comparative studies where different types of substrata were used, the authors did not investigate the element content of biofilms.

In 2000 cyanide and heavy metals containing industrial wastewater reached through tributaries the Szamos and the Tisza river from a gold overburden mine near Baia Mare (northwest Romania). These caused a serious pollution with cyanide and heavy metals (e.g. Cd, Pb, Cu and Zn) and made it necessary to monitor heavy metal pollutants of Tisza river (Kraft et al., 2002).

Considering the necessity of monitoring of heavy metal pollutants in the Tisza river and the literature data with their contradictions, we decided to investigate the applicability of biofilms as biomonitors forming simultaneously on natural and artificial substrata. In order to select the most suitable substratum the biomass production, the algological composition, the carbon source utilisation of bacterial communities and the element concentrations were determined. This paper discusses the biological and chemical characters of these biofilms and recommends a substratum for biomonitoring of the Tisza river.

2. Materials and methods

2.1. Growing of natural biofilms on different substrata

Between May 15 and June 28 of 2003 the natural biofilms were developed in the Tisza river at Tiszaörvény. The biofilms were grown on granite, polished granite, andesite, polycarbonate and plexi-glass substrata vertically submerged into the water (at depth of 20 to 30 cm) in five plexi-glass holders fixed to a plastic buoy (Fig. 1) which was connected to a landing-stage at 6 m distance from the riverside. Each plexi-glass holder contained 10 pieces of a given substratum with a surface area of with a surface area of 10 cm^2. The position of the plexi-glass holders was parallel to the water-stream. After six weeks the substrata were removed from the plexi-glass holders and the biofilms were divided into two parts. For elemental analysis one part of the wet biofilms was scraped into sterile plastic vessels using a ceramic knife and the other part of the biofilms was put into physiological saline solution for biological investigations. Samples were transported to the laboratory in a cold box.

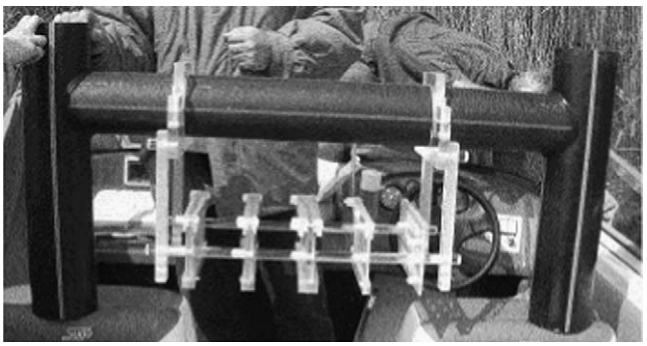

Fig. 1. Biofilm sampling system.