

ACCUMULATION OF FAECAL INDICATOR BACTERIA IN RIVER BIOFILMS

Mathias Balzer, Nina Möller, Hans-Curt Flemming, Jost Wingender

Biofilm Centre, Aquatische Mikrobiologie, Universität Duisburg-Essen, D-47057 Duisburg, Germany,

(Email: mathiasbalzer@web.de; ninamoeller1@gmx.de; hanscurtflemming@compuserve.com; jost.wingender@uni-due.de)

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Surface waters influenced by human activities can become contaminated with faecally derived bacteria, for example from sewage effluents and agricultural run-off. *Escherichia coli* and enterococci are widely used as microbiological parameters indicating both faecal pollution and the possible presence of pathogenic organisms, while total coliforms which are of faecal but also of environmental origin can serve as a parameter to provide basic information on surface water quality. Routine monitoring of microbiological surface water quality is usually limited to testing of the water phase. The aim of the present study was to investigate the occurrence of faecal indicator bacteria in river biofilms as possible reservoirs for hygienically relevant organisms of faecal origin.

Ten samplings of water and biofilms (epilithic biofilms, sediments) were performed in different German streams. Suspensions of biofilm bacteria were prepared by dispersal of the cells using a stomacher (epilithic biofilms) or an ultrasonic bath (sediments). Total cells counts were determined by microscopic enumeration of bacteria stained with 4',6-diamidino-2-phenylindole. The heterotrophic plate count (HPC) was determined using R2A medium incubated at 20 °C for 7 d. The most probable number (MPN) determination of coliform bacteria and *E. coli* was performed using the Colilert-18/Quanti-Tray/2000 system (IDEXX) incubated at 36 °C for 20 h. Colony-forming units (cfu) of enterococci were quantitated on Slanetz and Bartley medium incubated at 36 °C for 44 h followed by incubation at 44 °C for 2 h on bile-aesculin-azide agar (ISO 7899-2).

The levels of all microbiological parameters determined were always higher in biofilms compared with the corresponding water samples. Total cell counts of water samples varied between 1×10^6 and 1×10^7 cells/mL, while biofilms displayed cell counts between 2×10^9 and 5×10^{10} cells/g wet weight. The HPC percentages of total cell

counts were 0.2 to 17.2 % for water and 0.04 to 8.2 % for biofilms with a trend towards slightly lower culturability of organisms in biofilms compared with the water phase. Both *E. coli* and enterococci were detected in all water and biofilm samples. The concentrations of *E. coli* ranged from 4×10^1 to 1×10^3 MPN/100 mL of water and from 1×10^3 to 2×10^5 MPN/100 g wet weight of the biofilms. There were wide variations of the *E. coli* fraction among the total coliform bacteria, ranging from 3.2 to 45.4 % in the water samples and from 0.9 to 83.3 % in the biofilms. Most coliform bacteria were identified as members of the genera *Citrobacter*, *Enterobacter*, *Klebsiella* and *Serratia*. The levels of enterococci in water samples varied between 4×10^0 and 5×10^2 cfu/100 mL, while in biofilms the values ranged from 3×10^3 to 3×10^5 cfu/100 g wet weight. Enterococcal species in the biofilms were identified as *E. faecalis*, *E. faecium*, *E. hirae* and *E. durans* which are considered mainly of faecal origin.

The amounts of total coliforms, *E. coli* and enterococci were approximately one to three log units higher in biofilms than in the bulk water phase. These results suggest that faecal indicator bacteria and thus possibly bacterial pathogens of faecal origin can accumulate in river biofilms. Events of biofilm mobilization contribute to the deterioration of water quality by release of faecally derived pathogens. This can be of health significance in surface waters which are used for recreational purposes or as source waters for drinking water production.