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Ion exchange mechanisms and the entrapment of nutrients by river biofilms

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Abstract

Biofilms envelop all surfaces in aquatic ecosystems. They possess an extremely efficient nutrient entrapment mechanism which is widely believed to be mediated through ion exchange processes. During a field experiment, potassium and bromide were transported along a 105 m reach at different rates. The distance between the two solute pulses increased with increasing distance downstream. And, in a laboratory experiment, changing the ionic composition of waters overlying the biofilm influenced the retention of (phenolic) material by that biofilm. An analogy was drawn with ion chromatography (IC): In IC, different ions show different rates of progress through the column (retention times), and also show increasing separation between peaks with increasing distance from the point of injection (column length). Likewise, the affinity of a given ion for the column can be modified by manipulation of the ionic composition of overlying waters (eluent). The observed similarities between IC columns and the biofilm-coated stream channel may therefore represent a degree of experimental support for the putative involvement of ion exchange in the biofilm nutrient entrapment mechanism.

Introduction

River biofilms are a complex assemblage of bacteria, fungi and algae embedded within a polysaccharide matrix. They ubiquitously coat every wetted surface within aquatic systems (Lock, 1993), where they act as a trophic link between dissolved nutrients in the water column and the higher trophic levels of the ecosystem (Hynes, 1970). The polysaccharide matrix is believed to confer on the constituent micro-organisms a number of advantages over their freeliving counterparts (Lock *et al.*1984; Lock, 1993). Perhaps the most important of these, is the ability to act as a retention/trapping system for dissolved nutrients. This increases nutrient availability in the vicinity of the constituent micro-organisms to a much higher level than that present in the overlying waters. A study of dissolved organic carbon (DOC) retention in streams has shown that biofilm covered stones possess the most efficient DOC sequestration mechanism of any river-bed substratum (Mickleburgh *et al.*, 1984). It has become widely accepted that the entrapment mechanism is dependent upon ion exchange processes (Costerton *et al.*, 1987; Hamilton, 1987; Blenkinsopp & Costerton, 1991). However, we are unaware of any studies which have demonstrated this mechanism to occur in biofilms within the natural environment. The following study was designed to investigate the assumed involvement of ion exchange in nutrient entrapment by biofilms. We postulated that ion exchange, if present, would lead stream-bed biofilms to act in a similar manner to the ion exchange resins present in an ion chromatography (IC) column. More specifically, we proposed that:

Hypothesis 1. Following the addition of a mixture of ions to the headwaters of a stream channel, the different components of the mixture would leave the end of the channel at different times – in much the same manner that different ions injected onto an IC column

would be eluted at different retention times.

Hypothesis 2. With increasing distance down-stream, the separation between the added ions would increase. In IC this could be compared with increasing the column length, which increases the resolution between peaks.

Hypothesis 3. Changing the ionic composition of the waters overlying the biofilm, should cause desorption of materials which had previously been immobilised on the surface of the biofilm. Likewise, changing the ionic concentration over an ion exchange resin may release strongly retained solutes – a property often used in gradient elution techniques (Calmon & Kressman, 1957).

Materials and methods

Investigations of hypotheses 1 & 2 were conducted in the field, while hypothesis 3 was tested in the laboratory.

The field study was conducted in the headwaters of the River Wye at Plynlimon, mid-Wales (52 °26′N 3 °44′W), and involved adding a mixed solution of potassium (as KNO_3) and bromide (as sodium bromide) to the stream during baseflow conditions. The work was carried out in summer, on 17 August 1992, and then repeated during winter, on 8 December 1992. Solutions were prepared in stream water taken from the stream head on the day of the experiment, and released into the stream from a mariotte bottle at a continuous rate for 3-4 hours (Table 1). The solution was added at a site where the stream was shallow and fast flowing to ensure thorough mixing upstream of the first sampling point 16 m, below the point of addition. The conditions during each addition are presented in Table 1.

Samples of concentrated solution were taken at the beginning of the experiment to determine the exact concentration of potassium and bromide added (Table 1). Stream water samples were collected in polypropylene bottles a) above the point of addition (background samples) and b) at each of 3 sampling locations downstream; 16 m, 53.5 m and 105 m below the point of addition. Samples were collected at 15 minute intervals at location 1 and half-hourly at sampling locations 2 and 3, and were then returned to the laboratory for filtration and analysis. Bromide was analysed by ion chromatography using a Dionex 2000i IC system using an AS4A anion column with 1.7 mM $NaHCO_3$/1.8 mM Na_2CO_3 eluent at 2 ml min^{-1} flow rate and 25 mN H_2SO_4 regenerant. Potassium was determined by flame atomic emission spectroscopy using a Perkin Elmer 280 AAS flame system, with an air/acetylene mixture.

Table 1. Experimental conditions during the summer and winter field experiments.

	17.8.92	8.12.92
Discharge	1.8 l/s	3.0 l/s
Temp. of water (°C)	12.5-13.8	4.1-4.3
K added	363 ppm	390 ppm
NO_3 added	659 ppm	664 ppm
Br added	229 ppm	92 ppm
Rate of injection	0.0065 l/s	0.0062 l/s

Hypothesis 3 was tested in the laboratory using biofilms which had been cultured in stream water containing naturally high levels of phenolic materials. Phenolics were selected due to their ease of detection and their perceived importance as a microbial energy source (Tranvik, 1988) and possible inhibitor (Freeman *et al.*, 1990; Wetzel, 1992). Biofilms were cultured on 1300 glass beads of 1.5 mm diameter (total colonised area = 85 cm^2) strung on a 2 m length of nylon monofilament supported on a plexiglass plate. These were allowed to colonise naturally in the River Conwy for 8 weeks, before return to the laboratory. Upon return, the beads were washed with deionised water, and 50 beads transferred to a series of replicate 20 ml glass vials containing 15 ml of either deionised water, or 10 mM solutions of either KH_2PO_4, $NaNO_3$, NaCl or $CaCl_2$. Biofilms were retained in the solutions for 14 d at 9 °C, in order to determine the extent to which the solutes influenced phenolic-desorption. Afterwards, the concentration of desorbed phenolics was determined using the Folin Ciocalteu method of Box (1983).

Results

The transportation of potassium and bromide along the stream channel proceeded at substantially different rates (Figs 1, 2). Unfortunately, in the first experiment, sampling was inadvertently terminated prior to concentrations returning to pre-addition levels, as we underestimated the time required for solutes to pass through

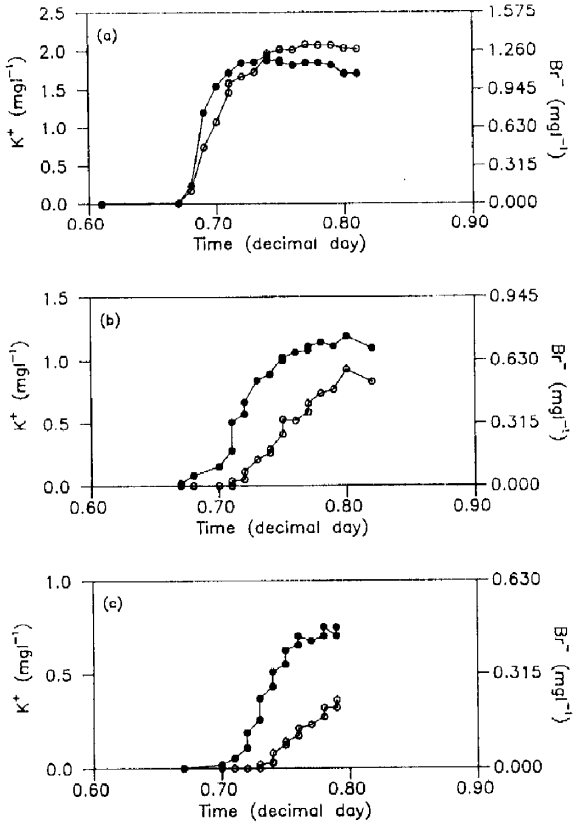

Fig. 1. Transport of potassium (◯) and bromide (•) along the headwaters of the river Wye on 17 August 1992. a) = 16 m downstream, b) = 53.5 m downstream and c) = 105 m downstream, measured from the point of addition.

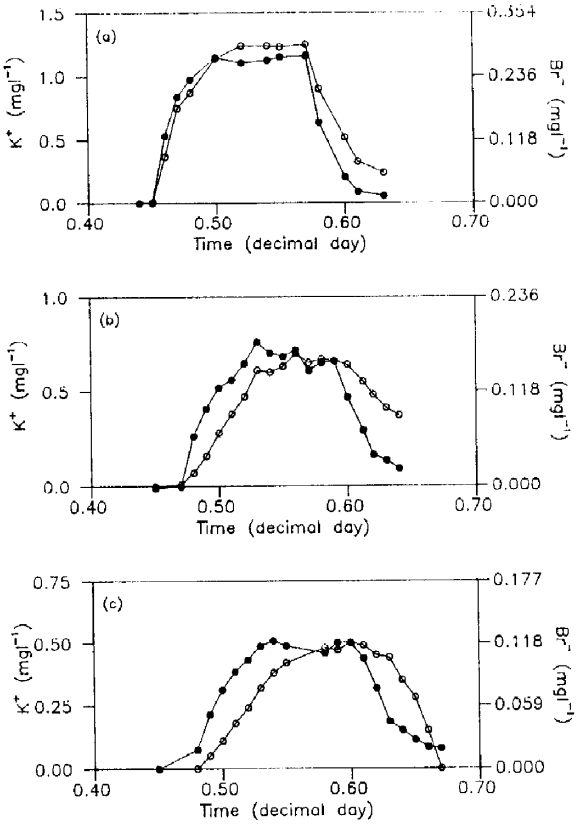

Fig. 2. Transport of potassium (◯) and bromide (•) along the headwaters of the river Wye on 8 December 1992. a) = 16 m downstream, b) = 53.5 m downstream and c) = 105 m down-stream, measured from the point of addition.

the experimental reach (Fig. 1). Thus, the retardation of potassium is best illustrated in the winter study (Fig. 2), where the longer period of sample collection allows a distinct pulse of solutes to be seen travelling down the stream. Measurement of the area beneath each of the peaks showed that all of the added potassium passed through the stream, but that it was delayed relative to the speed at which bromide was transported. The difference between the times at which potassium and bromide reach their peaks can be seen to increase with distance downstream. At site A (16 m downstream), both ions peak at approximately the same time; at site B (53.5 m downstream) there was a 40 minute separation, while at site C (105 m downstream) there was a substantial 85 minute separation between peaks. Although less clear, there was still a detectable slowing in the rate of potassium transport relative to bromide in the summer experiment. This can be seen as a gradual reduction in the rate of increase in potassium concentration (relative to bromide) with increasing distance downstream from the point of addition (Fig. 1).

Manipulation of the ionic composition of the waters surrounding the biofilms influenced the desorption of phenolics from the polysaccharide matrix (Fig. 3). The addition of KH_2PO_4 and $NaNO_3$ to biofilms from the river Conwy significantly (*t*-test) increased the release of phenolics from the biofilm surface compared with deionised water by 126% & 45% ($p<0.05$) respectively. Addition of NaCl decreased the release of phenolics by 41% ($p<0.05$), while addition of $CaCl_2$ had no significant effect.

Discussion

An investigation has been made, under field and laboratory conditions, into putative ion exchange processes associated with the biofilms that coat all wetted sur-

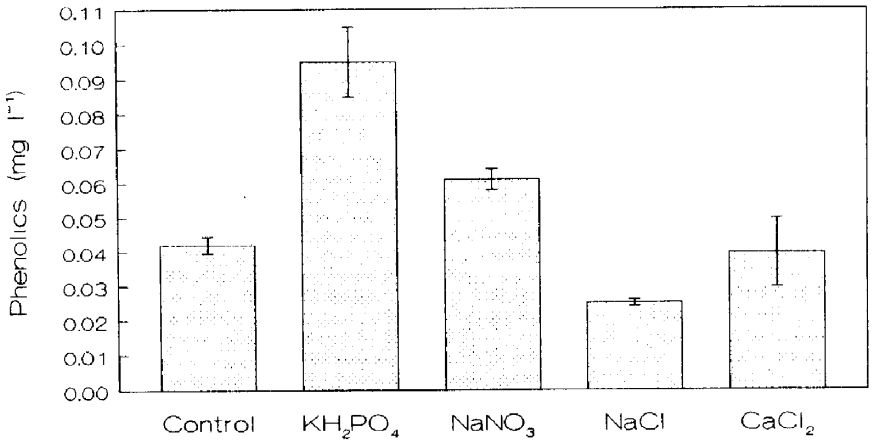

Fig. 3. Phenolics desorbed from biofilms subjected to control (de-ionised water) or 10 mM solutions of KH_2PO_4, $NaNO_3$, NaCl or $CaCl_2$ over 14 days.

faces in rivers and streams. In the field, two ions were transported through a stream channel at differing rates, leading to an increased separation between the two solute-pulses with increasing distance downstream. The observation cannot be attributed with absolute certainty to ion exchange processes: It could be suggested, for example, that the differences were hydrodynamic in origin, and caused by differences in the rate of diffusion of the two ions. However, in the dynamic environment of flowing waters, processes associated with diffusion are considered of far less importance than those of dispersion (Parker, 1961; Smettem, 1986). Furthermore, dispersion is commonly investigated using extremely large fluorescent tracer molecules (White, 1976), and we are unaware of any experiments where the ionic radius of tracers, uncoupled from adsorption processes, has influenced the results of tracer experiments on the large scale. In contrast, retardation of solute migration by ion exchange processes in flowing waters is a commonplace phenomenon, albeit on a small scale in analytical laboratories throughout the world. Clearly, ion exchange processes may also have been involved in the retardation of solutes in our field experiment.

In the laboratory experiment, manipulation of the ionic composition of the solution in which the biofilms were bathed affected the release of phenolic materials from the biofilm. It could be argued that this too cannot be attributed with absolute certainty to ion exchange processes: It is possible that the desorption was due to solubilisation of humic materials which had been freshly deposited on the surface of the biofilm. However, that contention seems unlikely as loosely adherent material would be removed during the washing of the biofilm prior to experimentation. Similarly, it could be argued that manipulation of the ionic composition of the waters above the biofilm may have influenced pH, with a concomitant impact on phenolic solubility. However, even this possibility does not negate the suggestion that biofilms respond in a manner that can be compared with ion chromatography columns: Changes in ionic composition and pH are widely used to influence the affinity of ions for IC columns in both organic and biochemical analysis (Calmon & Kressman, 1957).

Clearly, we are unable to state with absolute certainty that the observed responses were directly attributable to ion exchange processes. However, there was a remarkable degree of similarity between the properties of a biofilm-coated stream channel and those of an ion chromatography column. Likewise, nothing within our findings conflicts with the hypothesis that ion-exchange was involved in the biofilm nutrient entrapment mechanism. We therefore propose that our findings lend at least partial support for that hypothesis.

The existence of an ion exchange entrapment mechanism would be of substantial benefit to the biofilms on occasions where nutrients were added to the stream channel on an episodic basis. For example, events such as Autumn leaf fall can lead to temporary increases in nutrient concentrations (Dahm, 1981). Ion exchange could retard the progress of these nutrients, increasing the time available for microbial metabolism and hence allowing increased biofilm productivity. However, we have also noted that radical changes to the ionic composition of stream water can potentially cause the

release of (nutrient) materials that had previously been adsorbed to the biofilm surface. These findings attest to the possibility of intentionally reducing the affinity of nutrients for biofilms by manipulating the ionic composition of the overlying waters. The consequent nutrient deprivation could prove of great potential value in the control of fouling biofilms in many industrial and medical situations (Costerton *et al.* 1987; Blenkinsopp & Costerton, 1991). However, dramatic hydrochemical changes can also arise naturally in streams as a consequence of stormflow (Muscutt *et al.*, 1990; Chapman *et al.*, 1993), and clearly these events could adversely affect the putative ion-exchange retention mechanism. This is of particular concern because it has been proposed that storm events could soon arise more frequently as a consequence of climatic change (Katz, 1992). Should this occur, then in the long-term, stream biofilm productivities might potentially be compromised as a result of an impaired nutrient entrapment mechanism.

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