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FLUORESCEIN DIACETATE HYDROLYSIS AS A MEASURE OF TOTAL BIOFILM ACTIVITY IN STREAM BED SEDIMENTS: OPTIMIZATION AND APPLICATION OF THE METHOD

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ABSTRACT

A sensitive method to measure hydrolytic activity of intact stream sediment biofilm, using fluorescein diacetate (FDA), has been developed and evaluated. Non-specific esterases transform FDA to fluorescein which can be measured spectrophotometrically. Improvements of the method include the use of blanks, short incubation times (30 min) at in situ temperature and the extraction of fluorescein with acetone and sonication. The sensitivity and accuracy of the method were tested with cultivated biofilm and in a series of field measurements in the hyporheic zone of a headwater stream (Oberer Seebach, Ritrodat, Austria). The method was sensitive enough to detect a fine scale vertical profile of hydrolytic activity in the hyporheic zone. Good congruence was found between spatial patterns detected in a multiple assay comparison. FDA hydrolysis significantly correlated with the electron transport system activity (iodonitrotetrazolium formazan method, $r = 0.72$), bacterial activity ([^{3}H] thymidine incorporation, $r = 0.67$), extracellular enzymatic activity (α-glucosidase, $r = 0.69$; β-glucosidase, $r = 0.79$), extracellular enzymatic polymeric substances ($r = 0.84$) and chlorophyll a ($r = 0.94$). I therefore suggest the FDA method as an alternative or complement to the tetrazolium salt method (INT) to estimate total biofilm activity in stream sediments.

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