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Real-time monitoring of beta-D-glucuronidase activity in sediment laden streams: A comparison of prototypes

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ABSTRACT

Detection of enzymatic activities has been proposed as a rapid surrogate for the culture-based microbiological pollution monitoring of water resources. This paper presents the results of tests on four fully automated prototype instruments for the on-site monitoring of beta-D-glucuronidase (GLUC) activity. The tests were performed on sediment-laden stream water in the Hydrological Open Air Laboratory (HOAL) during the period of March 2014 to March 2015. The dominant source of faecal pollution in the stream was swine manure applied to the fields within the catchment. The experiments indicated that instrument pairs with the same construction design yielded highly consistent results ($R^2 = 0.96$ and $R^2 = 0.94$), whereas the results between different designs were less consistent ($R^2 = 0.71$). Correlations between the GLUC activity measured on-site and culture-based *Escherichia coli* analyses over the entire study period yielded $R^2 = 0.52$ and $R^2 = 0.47$ for the two designs, respectively. The correlations tended to be higher at the event scale. The GLUC activity was less correlated with suspended sediment concentrations than with *E. coli*, which is interpreted in terms of indicator applicability and the time since manure application. The study shows that this rapid assay can yield consistent results over a long period of on-site operation in technically challenging habitats. Although the use of GLUC activity as a proxy for culture-based assays could not be proven for the observed habitat, the study results suggest that this biochemical indicator has high potential for implementation in early warning systems.

1. Introduction

Agricultural activities may cause faecal pollution in surface water and groundwater (Blann et al., 2009; Bradford et al., 2013; Buck et al., 2004; Farnleitner et al., 2010, 2011). Streams receiving agricultural runoff often contain pathogenic bacteria from manure (Hutchison et al., 2004; Jones, 1999; Mawdsley et al., 1995; Tyrrel and Quinton, 2003). Thus, the real-time detection of faecal pollution in surface waters has high potential for use-orientated protection of water resources.

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Cultivation-based standard analyses of faecal pollution typically require one to several days and are therefore not suitable for rapid water quality assessment (Cabral, 2010). Methods involving enzymatic activity have been tested in various aquatic habitats and have been suggested as surrogates for culture-based microbiological pollution monitoring (Farnleitner et al., 2001, 2002; Fiksdal and Tryland, 2008; Garcia-Armisen et al., 2005). There are various chromogenic and fluorogenic substrates for the specific detection of enzymatic activities, such as beta-D-glucuronidases (GLUC), galactosidases and esterases (Fiksdal et al., 1994; Morikawa et al., 2006; Noble and Weisberg, 2005; Rompré et al., 2002; Wildeboer et al., 2010). Although these common enzymatic activity measurements for faecal indicators require laboratory facilities and elaborate sampling methods (Lebaron et al., 2005; Rompré et al., 2002), research within the last two decades has focused on developing rapid enzymatic assays (Fiksdal et al., 1994; George et al., 2000). However, these assays still require manual sampling and laboratory analytics.

Recent technological developments have brought automated on-site measurements of enzymatic activity within the reach of real time monitoring (Koschelnik et al., 2015; Ryzinska-Paier et al., 2014; Zibuschka et al., 2010). These studies have mainly been conducted for groundwater. The measurements are more challenging for surface waters because of the larger temperature variations and potentially high sediment concentrations. In this study, a field test of instruments for automated on-site enzymatic activity detection for stream water with high suspended sediment loads resulting from runoff events was conducted to understand the strengths and limitations of the instruments and optimize the measurement setup.

2. Materials and methods

2.1. Site description

The methodological basis of the field test conducted in this study is a comparison of automated rapid on-site GLUC measurements with culture-based microbiological measurements as well as with hydrological data in the HOAL - Hydrological Open Air Laboratory (Blöschl et al., 2011, 2015). The HOAL in Petzenkirchen (Lower Austria) is operated and maintained by the Institute for Land and Water Management Research (*Federal Agency for Water Management, Austria*) and the Vienna Doctoral Programme of Water Resource Systems (*Centre for Water Resource Systems, TU Wien, Austria*).

The HOAL catchment is 0.66 km^2 in size and drained by a stream 620 m in length. Twelve point discharges contribute to the stream, including tile drains, springs and surface tributaries (Exner-Kittridge et al., 2013a, b). The mean annual precipitation during the 1990–2014 period was 823 mm/yr. The land use of the catchment is dominated by agriculture, consisting of 87% arable land, 5% grassland, 6% forested area and 2% paved land. The hydrogeology is characterized by porous and fissured aquifers consisting of clay, marl and sand. The soils exhibit medium to limited infiltration capacities. The annual sediment erosion is approximately 10 t/km^2 (Eder et al., 2010). The main source of faecal contamination of groundwater and surface water is swine manure applied to the fields. In 2014, manure was applied in March, April, August and October, with a typical rate of 20 m^3/0.1 km^2.

The stream has high discharge dynamics (Table 1) with a rapid response to rain events, causing significant peaks in the concentration of suspended sediments in the stream water. Typically, sediments re-suspended from the riverbed control the sediment concentrations early in the event, whereas sediments from the hillslopes dominate later in the event (Eder et al., 2014). A considerable proportion of sediments stem from tile drainages. Relatively brief, intense events can cause a significant increase in sediment concentrations. Thus, the site is ideal for testing measurement methods under demanding conditions with strong variations in the weather conditions, hydrology, land use management and microbiological impact.

The instrumentation of the HOAL included on-line measurements of water level for discharge determination, electrical conductivity (EC), turbidity and water temperature (Table 1) at the stream monitoring station MW (Fig. 1), which is located at the catchment outlet (see Blöschl et al., 2015 for details). The turbidity measurements were calibrated with grab samples and referenced to the total suspended solid concentrations (TSS mg/l).

Winter and spring 2014 were characterized by fairly low discharges, resulting in an annual average of 2.4 l/s for 2014. Rain events in late spring, summer and autumn caused several high discharge peaks, with a maximum (hourly average) of 73.4 l/s in May 2014 at station MW (Table 1). The minimum discharge in 2014 of 0.5 l/s was recorded in August. The stream water temperature was continuously monitored because of the importance of temperature regarding enzymatic activity in aquatic habitats (Chróst, 1989). Stream water temperature generally tracked the annual trend in air temperature. Water temperature reached a minimum of 0.2 °C in January 2014 and a maximum of 20 °C in July 2014 (Table 1). The average water temperature in 2014 was 10.3 °C. Diurnal fluctuations of water temperature (up to ± 7 °C in April 2014) exhibited maximum values in the afternoon and minimum values in the early morning. The turbidity in the monitored stream is highly event-linked, as rain events promptly cause an increase in the suspended solids in the stream water. Maximum suspended sediment concentrations of over 3 g/l TSS (Table 1) were recorded in July 2014 and January 2015.

2.2. Automated on-site GLUC measurements

At location MW (Fig. 1), two ColiMinder devices (Vienna Water Monitoring - VWM GmbH, Zwerndorf, Austria) for rapid on-site GLUC monitoring have been operating in parallel since March 2014. At the same location, two BACTcontrol devices for rapid on-site GLUC monitoring (MicroLan, Waalwjik, Netherlands) have also been operating in parallel since 2012 (only measurements after the installation of an improved sampling set-up in July 2014 were used in this study). Both devices detect beta-D-glucuronidase enzymatic activity and record and transmit the data on a continuous basis. The measurement is based on the optical detection of highly fluorescent 4-Methylumbelliferon (MU), that is produced due to the enzymatic hydrolyses of substrate 4-Methylumbelliferyl-β-D-glucuronid (MUG) at defined conditions (for details see Sigma-Aldrich assay EC 3.2.1.31). Incubation temperature of all tested prototypes was set to 44.0 ± 0.1 °C according to George et al. (2000). More information about incubation time, pH adjustment and calibration can be found in Ryzinska-Paier et al. (2014) and Koschelnik et al. (2015).

The ColiMinder is based on a flow-through photometric measurement chamber (patent: PCT/AT2011/000497), which enables a high-resolution fluorescence analysis. The shapes of the measuring chamber and fluidic system are optimized for automated water sampling, reagent dispensing and effectiveness in the cleaning process. A data correction algorithm (patent: PCT/AT2014/050036) was used to obtain accurate fluorescence readings independent of turbidity. The GLUC activity measurements were performed in batches using 6.5 ml of sample per measurement. The measurement step takes approximately 15 min, and the full measurement cycle, including cleaning and sample