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Determination of poly-β-hydroxyalkanoate in Peat

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ABSTRACT

Poly-β-hydroxyalkanoate (PHA) is a prokaryotic energy reserve material which has been used as an indicator of environmental stress in aquatic bacteria. The following technique has been used to quantify PHA in peatland microorganisms. Peat samples were dried, digested in sulphuric acid to convert PHA into crotonic acid, and the resulting acid determined using organic acid high performance liquid chromatography (HPLC) and ultraviolet (UV) detection at 214 nm. This technique is suggested to have potential value as an indicator of environmental stress on peatland microorganisms, such as that caused by summer drought, or changes in soil nutrient availability, which are predicted consequences of climatic change.

KEYWORDS: High performance liquid chromatography; Microbes; Peatland; Poly-β-hydroxyalkanoate.

INTRODUCTION

Poly-β -hydroxyalkanoate (PHA) is an energy reserve polymer found in almost all genera of the bacterial kingdom and can accumulate to levels as high as 90% of the cell dry weight (Madison and Huisman 1999; Kadouri et al. 2005). The ability to produce PHA is an adaptation to environments where nutrients are scarce or exhibit temporal fluctuations (Koller et al. 2011). Many bacteria deposit energy reserve material when their growth is restricted in the presence of excess carbon and/or an energy source, which is then utilized when the energy supply from exogenous sources is no longer sufficient for optimal maintenance of the cell (Dawes 1976). For this reason, PHA has been used as an indicator of changes in nutrient availability, and particularly nutritional stress, on microorganisms. PHA can be used as an indicator of nutritional stress because: (i) increasing the carbon (C): nitrogen (N) ratio of microbial growth media increases the PHA content of certain bacteria (Macrae and Wilkinson 1958), and (ii) certain bacteria metabolize their PHA store when starved of energy and nutrients (Malmcrona-Friberg et al. 1986). Furthermore, estuarine bacteria can accumulate PHA following disturbance to the redox potential of the sediment (Herron et al. 1978), whereas biofilms in rivers can use up their PHA reserves following a change in environmental conditions until the biofilm acclimates to the new conditions (Freeman et al. 1993a).

In soils, changes in temperature or water availability can result in changes to nutrient availability and soil redox potential, which may place bacteria under stress. Because water is the most important factor regulating the biogeochemistry of wetland soils (Fenner et al. 2009), and the frequency of severe droughts in upland UK is reported to be increasing (Worrall et al. 2006), this effect is currently particularly important for study. Although measurement of PHA has been undertaken in a saline wetland substrate (Ramezani et al. 2014), a number of wetland analogues,

such as intertidal and marine sediments (Findlay and White 1983; Herron et al. 1978) as well as in rivers (Freeman et al. 1993a; Freeman et al. 1994; Lòpez et al. 1995) and lakes (van Gemerden et al. 1985; Mas-Castellà and Guerrero 1995), as yet, PHA has not been measured in peat. This paper presents the results of a preliminary experiment aimed at adapting a technique used to determine the PHA content of laboratory cultures of bacteria (Karr et al. 1983) for the determination of the PHA content of microorganisms in peat soils. The technique, which involves the digestion of peat samples in sulphuric acid to convert PHA into crotonic acid, which is quantified using organic acid high performance liquid chromatography (HPLC) and ultraviolet (UV) detection at 214 nm, was used on peat samples from a minerotrophic fen.

MATERIALS AND METHODS

Collection of Samples

Peat was collected from a minerotrophic fen at Cors Goch, on Anglesey, Wales, UK (NGR SH 496813). The site is an M13a mire, dominated by *Festuca rubra* and *Juncus acutiflorus*. The pH of the pore water 5 cm below the peat surface was 5.4. The top layer of vegetation was removed and peat collected from the top 5 cm of the profile. This peat was used for method development purposes. Subsequently, the method was also tested on samples of drained and pristine peat, collected using the same procedure, from an area of tropical peat swamp near Bukit Kemuning, Selangor, Malaysia, approximately 80 km northwest of Kuala Lumpur. The pristine site was dominated by peat forest trees, such as *Koompassia malaccensis*, *Shorea uliginosa,* and *Santiria* spp. The drained site has been used principally for mixed agriculture, including the growing of pineapples, following drainage approximately 10 years previously.

PHA Analysis

Samples were analysed using a modification of the method of Karr et al. (1983), whereby PHA is converted to crotonic acid by sulphuric acid digestion at 90°C. A heating block enabled samples to be digested in batches. During preliminary studies, samples of the fen peat were either digested wet or oven-dried at 60°C to determine whether water reduces the efficiency of the sulphuric acid digestion. Peat samples (0.01 g) were digested in 1, 2, 3, 4, and 5 mL of sulphuric acid and over a range of digestion times up to 2 hours to determine the optimal digestion conditions at 90°C. After cooling the digested samples for 30 minutes, samples were diluted 1:20 with deionized water and analysed by HPLC, based on the method of Karr et al. (1983), also used by Freeman et al. (1993a). A DIONEX AI-450 (Dionex Corp., Sunnyvale, CA) chromatograph with a VDM-II variable wavelength detector set at 214 nm was fitted with an Aminex HPX-87H organic acid HPLC column (Bio-Rad, Hemel Hempstead, UK). Sulphuric acid (0.028 M) was used as the eluent at a flow rate of 0.7 mL min^{-1}. Crotonic acid had a retention time of 26.5 minutes. Using this detector, the crotonic acid standard was linear up to at least 50 mg L^{-1} (r^2 = 1.000).

Statistically significant differences in the conversion of PHA to crotonic acid were investigated using ANOVA on Minitab, version 9.2 (Minitab Inc., Coventry, UK).

RESULTS AND DISCUSSION

Using a digestion volume of 1 mL sulphuric acid, significantly more PHA was converted to crotonic acid using dried peat samples compared with waterlogged peat samples (Figure 1; ANOVA; $p < 0.001$). However, for all other sulphuric acid volumes no difference was observed between the dried and waterlogged samples. In the waterlogged peat samples, maximum

89 conversion of PHA to crotonic acid was observed using 4 mL of sulphuric acid. No more PHA
90 was converted to crotonic acid using 5 mL of digestion mixture. In the dried peat samples,
91 maximum conversion of PHA to crotonic acid was observed using 3 mL of sulphuric acid. Though
92 there was no significant difference between using dry peat with 3 mL of sulphuric acid, or wet peat
93 with 4 mL of sulphuric acid, it is preferable to use dried peat with less acid, because larger volumes
94 of acid entail a greater sample dilution with water, decreasing the HPLC detection limit. Using 4
95 mL of sulphuric acid and waterlogged peat, 30 minutes was demonstrated to be the optimal
96 digestion time (Figure 2). After 30 minutes, conversion of PHA to crotonic acid fell slightly before
97 appearing to plateau.

98 Using the optimum conditions established for measuring PHA concentration in waterlogged
99 samples (digestion in 4 mL of sulphuric acid for 30 minutes), PHA measurement of a Malaysian
100 peat was undertaken. The method was sufficiently sensitive to identify a significant difference
101 between the pristine (1.76 ± 0.40 mg L^{-1} crotonic acid g^{-1} peat) and drained site (2.38 ± 0.18 mg
102 L^{-1} crotonic acid g^{-1} peat). These values were both substantially higher than the equivalent
103 measurement for the fen peat (0.62 ± 0.06 mg L^{-1} crotonic acid g^{-1} peat) and may be related to
104 higher productivity in the tropical peat.

105 The status of wetland systems as carbon sinks is dependent on the prevalence of waterlogged
106 (anaerobic) conditions, since this acts to suppress the microbial degradation of organic material
107 (Freeman et al. 2001). Increased drought in wetlands, a predicted consequence of climatic change
108 (Worrall et al. 2006), may result in lowered water table levels, which can widen the aerobic zone
109 at the surface of the peat and result in significant changes to, for example, the rate of nutrient
110 release from the peat (e.g. Heathwaite 1990; Freeman et al. 1993b). Nutrient availability may
111 determine the rate of other biogeochemical processes in soils. For example, nitrate release

following water table drawdown (Freeman et al. 1993b) can result in significant increases in nitrous oxide production from peatlands by microbial denitrification (Dowrick 1998). Since PHA can act as an indicator of nutritional stress in microbial communities (Koller et al. 2011), it could offer a useful means of assessing the impact of climate change on the microbial response of peatlands, which may have important consequences for the stability of peatland carbon stocks and greenhouse gas emissions.

CONCLUSIONS

The present study demonstrates for the first time, the potential for direct measurement of PHA concentrations in peat samples. We recommend further validation work using the method presented here including measurement of PHA concentrations over a wider range of experimental conditions, at different substrate depths and in additional peatland environments. Measurements of PHA over a range of nutritional conditions could also help to assess the value of PHA as an indicator of nutritional stress in peatland environments.

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