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An evaluation of cellobiose as an alternative reference substance for isothermal microcalorimetry measurements of soil microbial activity

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ABSTRACT

Cellobiose biodegradation in soil samples containing different doses of phosphogypsum was investigated by isothermal microcalorimetry. The results were compared with glucose biodegradation data in identical soil samples. A similar tendency was observed in cellobiose and glucose biodegradation processes. The rate of decomposition of the analyzed saccharides decreased with a rise in phosphogypsum doses in soil samples. During cellobiose and glucose biodegradation, the rate of heat production and the apparent growth rate constant decreased, whereas peak time and generation time increased with a rise in the phosphogypsum content of the investigated soil samples. The heat of cellobiose and glucose biodegradation increased in response to higher phosphogypsum doses in the soil.

1. Introduction

Microcalorimetric analyses are carried out to evaluate soil microbial activity [1]. Organic substances are a source of carbon for the growth of soil microorganisms [2]. The addition of cellobiose or glucose stimulates microbial development, and the effects of those processes can be detected by microcalorimetry. In nature, organic substances reach the soil environment mainly as remnants of plant and animal tissue [3] which stimulate soil microbial biomass and soil activity [4]. Carbon released by decomposing cellulose contributes to a significant increase in microbial counts in soil [3]. Single molecules of glucose are released during cellulose biodegradation. This process involves cellulases, hydrolyzing enzymes such as endoglucanases, exoglucanases (cellobiohydrolases) and β-glucosidases. Cellobiose is an intermediate product of cellulose biodegradation [5]. The effectiveness of cellulose degradation can be varied. Endocellulases are able to disrupt cellulose anywhere along the chain. Therefore, they can divide the molecule into several parts and reduce its molecular weight. Exocellulases act on one end of the cellulose chain to release cellobiose [6]. In the final stage of cellulose biodegradation, cellobiose is hydrolyzed to glucose. This process takes place in the presence of β-glucosidases [7].

Glucose is a common organic substance in microcalorimetric measurements [8–10]. The biodegradation of other saccharides is less frequently analyzed. Yamano and Takahashi [11] investigated the decomposition of D-galactose, D-fructose, sucrose, D-mannose, lactose and glucose at a temperature of 25 °C and reported the highest rate of biodegradation for glucose. Glucose is an easily available food substrate for soil microbes. Cellulose can also be used as an additional source of energy [12].

The greater significance of using cellobiose instead of glucose can be explained by a better stimulation of cellulose degradation in some cases. Cellulose due to the presence of strong β-1,4-glycosidic bonds and hydrogen bonds in fibrils is difficult to degrade by microorganisms [13]. The stimulation of metabolic activity of microorganisms and biodegradation of cellulose may take place after the introduction of an additional energy source in the form of glucose or cellobiose. Mechanisms of cellulose biodegradation may be different depending on the group of microorganisms carrying out these processes. Microorganisms can secrete extracellular enzymes that get into the soil environment or remain on the surface of cells. Bacteria can produce the group of cellulolytic enzymes, which are held on the surface of cell walls or in the case of anaerobic bacteria it can create cellulosomes. The mechanism of cellulose biodegradation by actinomycetes, as well as by fungi is based on a synergistic interaction between secreted endocellulases and

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exocellulases. The synthesis of these enzymes by the cells of actinomycetes and fungi can be stimulated by the presence of cellobiose and cellulose, and inhibited by addition of glucose [14]. According to the literature, the activity of cellulolytic enzymes can be induced by cellobiose [15]. Ahmed et al. [16] reported that the presence of glucose inhibits the cellulase activity. Therefore, the use of cellobiose may be desirable to stimulate the microorganisms and in addition, it may be more important in the case of testing of soils containing high amounts of cellulose. In the soil, microorganism, which prefer the use of cellobiose than glucose can be found. *Ruminococcus albus* is an example of such bacteria. The greater grown was found with the use of cellobiose than glucose. Additionally, an affinity for cellobiose were higher nad microoganisms devoted less energy to cellobiose uptake. As a result, the maintenance coefficient of cellobiose-grown cells was significantly lower than in the case of glucose [17]. Bacteria *Ruminococcus* spp. as cellulolytic organisms are very important in the cellulosic material degradation in the soil. They contribute to biodegradation the composts and municipal solid wastes [18].

The aim of this study was to demonstrate the usefulness of cellobiose as a reference substance in determinations of soil metabolic activity. Cellobiose was compared with glucose, the most popular reference substance in microcalorimetry research.

2. Experimental

2.1. Materials

Soil samples were collected at a depth of 0–20 cm (organic surface layer) at the Research Station in Tomaszkowo, Poland (53°43′N, 20°24′E), operated by the University of Warmia and Mazury in Olsztyn. The experimental soil was brown silt loam (Cambisol). Soil was stored in a laboratory (20–22 °C) to produce air-dry samples. It was passed through a 2 × 2 mm mesh sieve and stored in polyethylene bags at 4 °C.

Phosphogypsum (PG) samples were obtained from a phosphoric acid production line. Phosphogypsum was a 70:30 mixture of Syrian and Egyptian phosphorite.

The organic substrates were cellobiose and glucose:

- cellobiose: D-(+)-Cellobiose, $C_{12}H_{22}O_{11}$ (Sigma-Aldrich). Enthalpy of combustion: ΔH° (25 °C) = 450 kJ C mol^{-1}, M = 342.297 g mol^{-1} [19],
- glucose: D-(+)-Glucose, $C_6H_{12}O_6$ (Sigma-Aldrich). Enthalpy of combustion: ΔH° (25 °C) = 468.9 kJ C mol^{-1}, M = 180.16 g mol^{-1} [20].

2.2. Instruments

A calorimetry set composed of two isothermal microcalorimeters and the KRM macrocalorimeter (as a thermostat) with a high-precision temperature controller (Fluke 2100) was used to determine the microbial activity and thermokinetic parameters of cellobiose and glucose biodegradation in soil samples. Each microcalorimeter consisted of four measuring cells and one reference cell. Reference cell contained a vessel filled with Al_2O_3 and water whose heat capacity was matched to the heat capacity of samples in measuring vessels. One of the four measuring cells contained a vessel filled with thermally inactive soil, which was dried for 24 h at 105 °C and additionally amended with $HgCl_2$ in order to eliminate the microorganisms. The first isothermal microcalorimeter contained the HDPE vessels with a volume of 30 mL and a diameter of 31 mm. The vessels were sealed at the top with HDPE screw caps. The mass of the samples in the vessels was 6 g. The second microcalorimeter included glass ampoules with a volume of 20 mL and a diameter of 22.5 mm. The ampoules were sealed at the top with aluminum screw caps. Samples of 4 g were placed in the ampoules. After placing the samples in the HDPE vessels and glass ampoules, available air occupy about 2/3 of the volume of these vessels and ampoules. As a result, it was possible to maintain aerobic conditions during the investigated processes. The results of measurements, which constituted thermoelectric signals from thermoelectric modules, were recorded with an ADC-24 data logger (Pico Technology).

The calibrations of calorimetric cells were performed before carrying out measurements. The calibrations were carried out for each of the measuring vessels. An electrical method with the use of 100 Ω isolated resistor placed inside the measuring vessel with the soil material, which was dried for 24 h at the temperature of 105 °C, used for calibration. Resistance of the calibration resistor was precisely determined using the 4-wire (4-R) method with the use of 2700 Digital Multimeters (DMM)/Data Acquisition System from the Integra Series (Keithley). Current intensity was recorded under steady-state conditions using a 2700 Digital Multimeters (DMM). During calibration, the measurement of a baseline was carried out and afterwards the constant current was turned on. After reaching a constant value of signal, the current was turned off and the curve returned to baseline level. The coefficient of heat losses α [W V^{-1}] for each of the measuring cell was determined after reaching steady-state voltage output for generated constant thermal power. The thermal power applied in the measurement vessel was calculated according to the following formula:

$$P = RI^2 \quad (1)$$

where P [W] is the thermal power, R [Ω] is the resistance and I [A] is the current intensity.

The values of α were quotient of thermal power P [W] and electric voltage U [V] according to the following equation:

$$\alpha = \frac{P}{U} \quad (2)$$

In the case of the first microcalorimeter, the values of α were: 7.06, 7.12 and 7.10 W V^{-1}, respectively, for individual measuring cells and for the second microcalorimeter, the values of α were: 4.10, 4.08 and 4.08 W V^{-1}.

The time constant τ [s] of the calorimetric cells was calculated based on the curves obtained during cooling of measuring vessels containing soil, which was dried for 24 h at 105 °C and water in an amount corresponding to 60% of water holding capacity. Interval between the measurements was 100 s. The value of τ was calculated for a selected period of time from t_1 to t_2, for which the best curve fit on a logarithmic graph has been found. The following formula was used:

$$\tau = \frac{(t_1 - t_2)}{\left(\ln \Theta_1 - \ln \Theta_2\right)} \quad (3)$$

where τ [s] is the time constant, t [s] is time and Θ [μV] is the thermoelectric power.

The values of τ were: 293.8, 295.0 and 294.2 s for the cells in first microcalorimeter, and 251.1, 247.6 and 245.4 s for the cells in second microcalorimeter.

2.3. Measurements

The control sample contained soil without any additives. Phosphogypsum (PG) at doses of 5, 10, 20 and 30% by weight of the control sample was added to the remaining soil samples. A water solution of ammonium sulfate (1 mg of ammonium sulfate per 1 g of soil dry mass) was introduced to samples 12 h before measurements. After incubation of 12 h, the reference substances were added to the samples directly before measurements. In the first