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Determination of arylsulphatase and phosphatase enzyme activities in soil using screen-printed electrodes modified with multi-walled carbon nanotubes

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ABSTRACT

Sustainability of agricultural systems has become an important issue all over the world. The activity of enzymes is potentially an important quality bioindicator in soils. The aim of the present study was to develop a simple and convenient assay to determine the activity of arylsulphatase (AS), acid (ACP) and alkaline phosphatase (ALP) in agricultural soil. The activities of these enzymes were detected using a non-electroactive substrate, which produces an electroactive product. To this end, p-aminophenyl phosphate (pAPP) was used as a substrate which is converted to p-aminophenol (pAP) after enzymatic dephosphorylation; and 4-nitrocatechol sulphate (4-NCS) was used as a substrate for AS activity based on its catalytic effect on the hydrolysis of 4-NCS into 4-nitrocatechol (4-NC). The products of both enzymatic reactions were quantified on carbon-based screen-printed electrodes (SPCEs) modified with carbon nanotubes (CNTs), using Osteryoung square-wave voltammetry (OSWV). The determination of the reaction products allowed more sensitive determination of ALP, ACP and AS activities in soil than that obtained with a spectrophotometric method. This assay also diminishes the generation of waste, which is desirable in green analytical chemistry. The optimization of the analytical procedure in terms of the nature of electrode type, applied potential, pH of solution, and precision of measurements is reported.

1. Introduction

In soil ecosystems, phosphatase (PS) and arylsulphatase (AS) play crucial roles in phosphorus (P) and sulphur (S) cycles, respectively (Speir and Ross, 1978). The enzymes regenerate inorganic nutrients from organic materials and have been reported as the rate-limiting step in the nutrient cycling process (Chróst, 1991). Enzymatic activities have several important functions in soil. They are intimately involved in the cycling of nutrients, in the efficiency of fertilizer utilisation, reflect the microbiological activity in soil and can play a role as indicators to monitor soil change (Dick et al., 2000).

Organic P present in soil must be mineralized into inorganic orthophosphate (PO_4^{3-}) ions to be assimilated by many organisms, particularly plants. Only enzymes produced by plants and/or microorganisms are able to hydrolyze organic P into phosphates. These enzymes can be located in soil microorganisms, in root cells and in extracellular forms in soils. The general term phosphatases describes a broad group of enzymes that catalyze the hydrolysis of both esters and anhydrides of phosphoric acid (Schmidt and Laskowski, 1961). PS activity is expected to be enhanced by the application of various organic manures, which often results in enhanced P availability in soil. Among PS enzymes, acid (ACP) and alkaline phosphatase (ALP) (E.C. 3.1.3.) and phosphodiesterases (E.C. 3.1.4.) are considered as the predominant PS in most types of soil and litter (Tabatabai, 1994; Criquet et al., 2004). The activities of these phosphatases are influenced by various soil properties, soil organism interactions, vegetation cover, leachate inputs and the presence of inhibitors or activators (Juma and Tabatabai, 1977). The understanding of these factors has remained unclear, despite numerous attempts to relate phosphatase activities to P pools in soils (Turner and Haygarth, 2005). The method used to measure this enzyme activity was proposed by Tabatabai and Bremner (1969). This method is based on conversion of the synthetic substrate p-nitrophenyl phosphate to p-nitrophenol, which can be quantified by a spectrophotometric method.

AS activity is widespread in soil (Cooper, 1972; Gupta et al., 1993; Ganeshamurthy et al., 1995), and is typically measured according to the method of Tabatabai and Bremner (1971). This method is very similar to the one used for PS, but in this case the substrate is p-nitrophenyl sulphate which is converted to p-nitrophenol and quantified by a spectrophotometric method.

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The AS catalyses the hydrolysis of aromatic sulphate esters to phenols and sulphate. In soil, sulphate esters represent a large fraction (25.3–93.1%) of the total S and, therefore, arylsulphatases are important for mobilisation of inorganic SO_4^{2-} for plant nutrition (Fitzgerald, 1976).

With regards to the detection system, the formation of the product is generally followed by spectrophotometric, fluorescence or chemiluminescence detection (Girotti et al., 1994; Tillyer et al., 1994; Kawakami and Igarashi, 1995; Withold et al., 1996; Lin et al., 1997). However, in recent years, analyses via electrochemical detection constitute a methodology used extensively (Lasalle et al., 1994; Della Ciana et al., 1995; Jiao et al., 2002; Gyurcsanyi et al., 2002). Some advantages of this method include speed, accuracy, and precision. Square-wave voltammetry (SWV) is one of the electrochemical techniques more widely applied to quantitative analysis, especially due to its high sensibility, which is a consequence of the rejection of most of the capacitive currents (Garay and Lovrić, 2002; O'Dea et al., 1981). A variety of substrates were used in electrochemical assays for the quantification of ACP and ALP activities, such as phenylphosphate, p-aminophenyl phosphate (pAPP), p-nitrophenyl phosphate (pNPP), and 3-indoxyl phosphate (Wehmeyer et al., 1986; Tang et al., 1988; Abad-Villar et al., 2000; Fanjul-Bolado et al., 2004).

Carbon nanotubes (CNTs) are a novel type of carbon material and can be considered as the result of folding graphite layers into carbon cylinders. There are two groups of carbon nanotubes, multi-walled carbon nanotubes (MWCNT) and single-walled carbon nanotubes (SWCNT) (Zhao et al., 2002). The CNTs have generated great interest in future applications based on their field emission and electronic transport properties (Murakami et al., 2000), their high mechanical strength and their chemical properties (Treacy et al., 1996). The research has been focused on their electrocatalytic behaviour toward the oxidation of biomolecules and their performance has been found to be much superior to those of other carbon electrodes in terms of reaction rate, reversibility and detection limit (Li et al., 2005).

The advantages of carbon-based screen-printed electrodes (SPCEs), such as simple and low-cost fabrication and conveniently practical application in detection of biomolecules, have been extensively illustrated (Gilmartin et al., 1995; Hart and Wring, 1997; Wang et al., 1998; O'Halloran et al., 2001). The uses of CNTs for preparation of CNT-modified carbon-based screen-printed electrodes (CNTs-modified SPCEs) have been reported previously (Fanjul-Bolado et al., 2007; Sato and Okuma, 2008; Lee et al., 2007; Ye and Ju, 2005).

The aims of the present study were test and use a simplified and convenient procedure for the assay of AS, ACP and ALP activities in agricultural soil. The activities of these enzymes were detected electrochemically using a non-electroactive substrate, which produces an electroactive product. This method allows improve the sensitivity of the determination of the reaction product produced by ALP, ACP and AS activities in soil compared to the spectrophotometric method. This assay was performed in order to reduce the amount of solvents required and also to diminish the generation of wastes, which is an important requisite in green analytical chemistry (Armenta et al., 2008). The optimization of the analytical procedure in terms of the nature of electrode type, applied potential, pH of solution, and precision of measurements was studied.

2. Materials and methods

2.1. Reagents and solutions

All reagents used were of analytical reagent grade. The pNPP disodium salt hexahydrate were purchased from Fluka Chemie (Steinheim, Switzerland). pAP, pNC and pNCS were obtained from Sigma Chemical Co. (St. Louis, MO). The SPCEs was purchased from EcoBioServices & Researches s.r.l. (Fienze, Italy). All other reagents employed were of analytical grade and used without further purifications. Aqueous solutions were prepared using purified water from a Milli-Q system.

2.2. Apparatus

Electrochemical detection was performed using a BAS 100B/W electrochemical analyzer (Bioanalytical Systems, West Lafayette, IN, USA) which was used for cyclic voltammetric analysis and OSWV.

All pH measurements were made with an Orion Expandable Ion Analyzer (model EA 940, Orion Research, Cambridge, MA, USA) equipped with a glass combination electrode (Orion Research). The absorbencies were detected by a Beckman DU 520 general UV/Vis spectrophotometer (Fullerton, CA, USA).

2.3. Soil samples

The samples were obtained from the upper horizon (0–15 cm) of four Entisols soils of San Luis and two Mollisols soils of Santa Fé. The samples were from Argentinean soils used for agricultural activities. The coordinates of the obtained samples were soil 1 (33°19′ S, 66°20′ O), soil 2 (33°74′ S, 65°55′ O), soil 3 (32°32′ S, 65°14′ O), soil 4 (34°06′ S, 66°44′ O), soil 5 (32°53′ S, 60°56′ O), soil 6 (31°37′ S, 61°01′ O).

The moist soil sample was sieved (≤2 mm) after removing the plant material and roots. Soil samples were kept at 4 °C in plastic bags for a few days to stabilize the microbiological activity disturbed during soil sampling, handling. The analyses were achieved within two weeks after the sample collection. The physical and chemical characteristics of the soil are given in Table 1.

2.4. Synthesis of pAPP

Synthesis of pAPP by catalytic hydrogenation of pNPP was performed using the procedure (Gehring et al., 1996) with the following modifications. In a 100 mL glass hydrogenation vessel, 2 g of pNPP was dissolved in 30 mL of 50% ethanol containing 0.11 g of 10% palladium on charcoal catalyst. The hydrogenation reaction was conducted overnight at room temperature at an initial pressure of 1.3 atm. The resultant mixture was filtered on a Buchner funnel to remove the catalyst and the volume of solvent was reduced to 10 mL using a rotary evaporator. The oily residue was diluted to 20 mL with distilled deionised water and clarified by filtration. Cold ethanol (20 mL, 4 °C) was added to the filtrate and the precipitated product was recovered by filtration, dried under vacuum and stored at −10 °C. The pAPP product was greater than 98% pure as determined by NMR and electrochemical methods.

2.5. Preparation of the CNTs-modified SPCEs

An electrode pretreatment was carried out before each voltammetric experiment in order to oxidize the graphite impurities and to obtain a more hydrophilic surface (Wang et al., 1996), with

Table 1
Physical and chemical characteristics of soils.

Characteristic	Soil 1	Soil 2	Soil 3	Soil 4	Soil 5	Soil 6
pH	8.1	8.0	8.3	6.9	5.3	6.0
Clay (%)	5.3	6.5	8.2	9.0	29.2	35.0
Sand (%)	80	75	63	64	20	13.4
Organic matter (%)	0.63	0.84	0.97	0.59	1.63	2.07
Total N mg kg^{-1}	842	1070	1208	798	206	317