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Use of solid phase extraction in a sequential injection system for the determination of alkaline phosphatase activity in plant roots

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Phosphorus (P) is an essential nutrient to all organisms and in soils P is the most limiting nutrient that controls living processes in plants. Dissolved inorganic phosphate is readily available for assimilation by organisms such as bacteria and plants. For that reason, when dissolved inorganic phosphate is depleted compared to other nutrients both in water and soil, phytoplankton, plants and bacteria have the ability to obtain phosphorus from dissolved organic compounds due to the production of an enzyme named alkaline phosphatase. This enzyme hydrolyzes phosphate monoesters releasing inorganic phosphate and organic matter. Its production is regulated by inorganic phosphate concentrations and internal P levels so the values of alkaline phosphatase are an excellent indicator of P status.

In this work, a sequential injection methodology was developed for the determination of alkaline phosphatase activity in root plants. The enzymatic activity was assessed using *p*-nitrophenyl phosphate as substrate and measuring the absorbance of the colored product, *p*-nitrophenol. The alkaline phosphatase is an extra/intra cellular metalloenzyme, as it requires metal ions in the active site to carry out the catalytic activity. Exploring this feature of the enzyme and aiming for the low levels found, a step for the in-line pre-concentration was included. As alkaline phosphatase needs Zn^{2+} ions in the active site, a resin, Nitrilotriacetic Acid (NTA) Superflow, was charged with Zn^{2+} to retain the enzyme.

The proposed methodology allowed the determination of alkaline phosphatase activity in plant roots within a range between 0.044 – 0.441 unit cm^{-3} enzyme activity and 19 – 280 μmol dm^{-3} *p*NP. A determination rate of 17 h^{-1} and detection limits of 0.025 unit cm^{-3} enzyme activity and 1.9 μmol dm^{-3} *p*NP were obtained. The activity was assessed with a minimal incubation time (≈12 s).

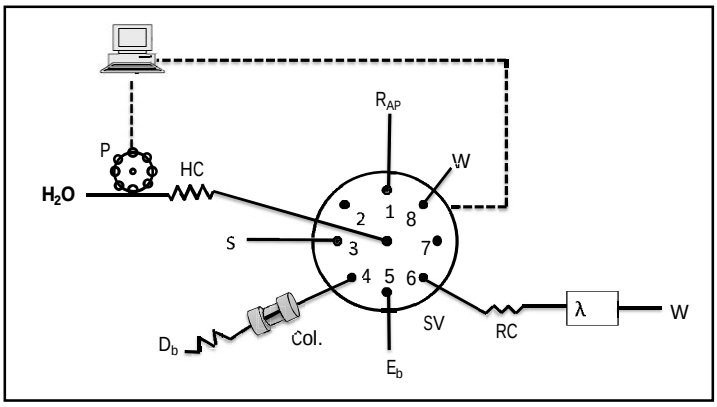

Figure 1. Sequential injection manifold for the spectrophotometric determination of alkaline phosphatase activity: P, peristaltic pump; SV, eight-port selection valve; HC, 4.25 m holding coil; S, sample or standard; Col., column with NTA Superflow resin charged with Zn^{2+} ions; R_{AP}, *p*NPP or *p*NP; E_b, eluting buffer; D_b, diethanolamine buffer; RC, 0.95 m reaction coil; λ, spectrophotometer (405 nm); W, waste.

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