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Evaluation of 2D enzyme activity in soils based on 3-d modeling

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Quantitative interpretation of soil zymography requires an accurate assessment of rates and distances, at which substrates and products can diffuse in and out of, as well as within, soil and a substrate-saturated polyamide membrane. The goals of this study were: (i) to collect experimental data on diffusion rates and diffusion distances of the fluorogenic substrates and products of enzyme catalysis in soil; (ii) to model the diffusion and substrate catalysis in the zymography membrane/soil surface system; and (iii) to assess the spatial distribution and activity of the enzymes based on experiments and modeling. The studied enzyme was β-glucosidase. Dynamics of 4-methylumbelliferone (MUF) production and redistribution in the membrane were measured in a set of membrane incubation experiments. The areas of contact between the membrane and the soil surface, as well as the distances from the membrane to the soil surface, were obtained based on laser scanning and X-ray computed micro-tomography. The diffusion of the substrate and the product was modeled using the HP2 program of HYDRUS-2D/3D software. The Michaelis-Menten equation was introduced into the HP2 program to model MUF production by β-glucosidase.
Diffusion experiments demonstrated that β-glucosidase itself did not diffuse from the soil into the membrane; only diffusion of the substrate and the product took place during zymogram incubations. The cleavage of MUF-β-D-glucopyranoside by the enzyme occurred on the water film, which was in the hydraulic contact with the membrane and the soil surface. It appears that a certain portion of fluorescent areas on zymography membranes, which are commonly attributed to presence of enzyme active zones, might actually be a result of MUF diffusion from soil to the membrane and its diffusion inside the membrane. The incubation time affected the spread of MUF in the membrane and the size of fluorescent areas. Thus, fluorescent brightness patterns on the zymography membranes could be associated both with high enzyme activity and with distances between the soil and the membrane. Therefore, 1) assessment of distances between the soil surface and the membrane and 2) modeling diffusion and catalysis processes are the prerequisites for accurate quantitative measurements of the enzyme activities from zymography membranes.