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Soil solid materials affect the kinetics of extracellular enzymatic reactions

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INTRODUCTION

Soil solid materials affect the degradation processes of many organic compounds by decreasing the bioavailability of substrates and by interacting with degraders. The magnitude of this effect in the environment is shown by the fact that xenobiotics which are readily metabolized in aquatic environments can have long residence times in soil.
Extracellular enzymatic hydrolysis of cellobiose (enzyme: beta-glucosidase from Aspergillus niger) was chosen as model degradation process since it is easier to control and more reproducible than a whole cell processes. Furthermore extracellular enzymes play an important role in the environment since they are responsible for the first steps in the degradation of organic macromolecules; beta-glucosidase is key enzyme in the degradation of cellulose and therefore it is fundamental in the carbon cycle and for soil in general.
The aims of the project are: 1) quantification of solid material effect on degradation, 2) separation of the effects of minerals on enzyme (adsorption →change in activity) and substrate (adsorption →change in bioavailability).
Our hypothesis is that a rate reduction in the enzymatic reaction in the presence of a solid phase results from the sum of decreased bioavailability of the substrate and decreased activity of enzyme molecules. The relative contribution of the two terms to the overall effect can vary widely depending on the chemical nature of the substrate, the properties of the enzyme and on the surface properties of the solid materials.
Furthermore we hypothesize that by immobilizing the enzyme in an appropriate carrier the adsorption of enzymes to soil materials can be eliminated and that therefore immobilization can increase the overall reaction rate (activity loss caused by immobilization < activity loss caused by adsorption to soil minerals).

MATERIALS AND METHODS

Enzymatic kinetic experiments are carried out in homogeneous liquid systems and in heterogeneous systems where solid materials (bentonite, kaolinite, goethite, activated charcoal) are suspended in a mixed liquid (standard experimental conditions: 66 mM phosphate buffer, pH 5, 25°C, 20 mg solid/ml buffer). The enzyme in an immobilized form (covalent bonding to oxirane groups on the surfaces of macroporous Eupergit® C particles) is used to exclude a direct effect of soil solid materials on the enzyme without excluding their effect on the availability of the substrate.The progress of the reactions is determined by measuring the accumulation of the product (i.e. glucose) in the systems at different times (after destroying enzymatic activity by boiling the samples) with a coupled enzymatic assay and an automatic microplate spectrophotometer. A regression analysis on the data points is performed to calculate the initial reaction rates, which is the parameter that allows to compare the different systems.

RESULTS AND DISCUSSION

The results show that, under the standard experimental conditions, cellobiose is not adsorbed by the clay minerals bentonite and kaolinite and by the iron oxyhydroxide goethite. In the case of activated charcoal a rapid adsorption phase in the first 20' is followed by a much slower process; after 4h 30' approximately 98% of cellobiose was adsorbed.
The results from the adsorption experiments of beta-glucosidase to bentonite, kaolinite, goethite and activated charcoal show that, under the standard experimental conditions, the adsorption process is rapid in all cases (more than 80% of the adsorption takes place in the first 20 minutes). After 1h 20min the following fractions of enzyme were adsorbed: 30 % to bentonite, 60% to kaolinite, 67% to goethite, 100% to activated charcoal.
The effect of kaolinite on the reaction rate was quantified: under the standard experimental conditions the initial reaction rate in presence of the mineral was 22% less then in the control. The fraction of enzyme molecules which are adsorbed to kaolinite (60%) loses 37% of its activity.

CONCLUSIONS

The results from the adsorption experiments lead to the conclusion that, among the solid materials tested, only activated charcoal may affect the reaction rate by limiting the substrate bioavailability while all the materials tested may affect the reaction rate by limiting the enzymatic activity.

The results from the experiments on the effect of kaolinite on the reaction rate lead to the conclusion that even the degradation of substrates with unrestricted bioavailability can be affected by soil solid materials.

The next experiments will show how much activity is lost by beta-glucosidase when adsorbed to bentonite and goethite and how activated carbon affects the reaction by limiting the substrate bioavailability and the enzymatic activity. In this last case beta-glucosidase will also be used in the immobilized form to eliminate the effect of activated carbon on the enzyme.