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Factors affecting cellulase sorption in soil

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The sorption of cellulase proteins of *Trichoderma viride* on calcareous soils was investigated, and the soil factors affecting cellulase sorption were also studied. The study on some arid, semiarid and humid soils of Isfahan, Hamadan and Gilan provinces of Iran revealed that the sorption of cellulase on the calcareous soils obey both the Freundlich and the Langmuir isotherms. The maximum binding levels of cellulase on the Isfahan, Hamadan and Gilan soils estimated by the Langmuir model were 58.72, 41.64 and 74.07 g kg⁻¹, respectively. The association binding constants were 3.32, 2.17 and 1.86 ml mg⁻¹ for cellulase sorption on the Isfahan, Hamadan and Gilan soils, respectively. The observed differences may be due to the deferent clay and carbonate contents of soils, because the correlation tests revealed that the sorption capacity of soils was significantly related to the soil clay and carbonates contents. Cellulase sorbed on the soils was not correlated with soil organic mater contents. Sorbed cellulase washed out more easily from the Hamadan soils than from the other soils, probably due to their lower clay contents.

Key words: Sorption, calcareous soils, cellulase.

INTRODUCTION

Cellulases hydrolyze cellulose polymer to smaller oligosaccharides and glucose, and include three major types of enzymes: endoglucanases (EC 3.2.1.4) which randomly attack the cellulose polymer by endoaction, cellobiohydrolases (EC 3.2.1.91) which act as exoenzymes and remove cellobiose or glucose from the non-reducing end of the cellulose chain, and -glucosidases (EC 3.2.1.21) which hydrolyze cello-oligosaccharides and cellobiose into glucose (Enari, 1983; Ilmen et al., 1997; Lynd, et al, 2002). These enzymes can either be free, particularly in aerobic microorganisms, or grouped in a multi- component enzyme complex, cellulosome, such as in anaerobic cellulolytic bacteria (Bayer et al., 1998).

Extracellular enzymes, often involved in breaking up of polymers and complex organic molecules, are themselves subject to sorption and deactivation by soil colloids (Skujins, 1967). Enzymes are able to be sorbed and immobilized by clay minerals and humic colloids in soil environments (Burns, 1986; Dick 1997; Safari Sinegani et al, 2005). Adsorption of enzymes at solids can result in both inactivation due to conformational changes or enhancement due to the increased

concentration of enzyme and substrate at the solid- water interface (Boyd and Mortland, 1990; Staunton and Quiquampoix, 1994). Naidja and Huang (1996) found that the large molecules of aspartase (MW=180,000) was intercalated between the montmorillonite layers.

Cellulases in soils have been extensively studied by Schinner and Von Mersi (1990) and Deng and Tabatabai (1994b) who have devised methods for the measurement of their activity. Despite the importance of cellulases in the C cycle in environments, most studies on these enzymes deal with their production, purification, characterization, and immobilization on natural or synthetic adsorbent (namely cellulose) instead of with its reaction in natural environments. Therefore considering the environmental importance of cellulases, studies to elucidate reaction of cellulases in natural systems are required. The objectives of the present study were to verify the sorption model of cellulase in soil environment and to recognize the factors affecting its sorption in soil.

MATERIALS AND METHODS

Soil sampling and analysis

Thirty surface-soil samples (0-30 cm) representing a wide range of pH values, organic matter, cation exchange capacity (CEC), electrical conductivity, equivalent calcium carbonate, and clay con-

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Table 1. Mean of selected chemical and physical characteristics of 10 soils of each province.

Soil properties	Gilan province	Hamadan province	Isfahan province
Sand (g kg ⁻¹)	230.9 a	326.7 a	318.2 a
Silt (g kg ⁻¹)	197.3 a	308.8 a	268.9 a
Clay (g kg ⁻¹)	472.7 a	364.5 a	412.9 a
CEC (CmolcKg ⁻¹)	15.92 a	13.19 ab	9.59 b
Eq.CaCO ₃ (g kg ⁻¹)	31.5 c	166.8 b	425 a
OC (g kg ⁻¹)	16.53 a	7.79 b	7.07 b
EC (dSm ⁻¹)	0.388 c	0.78 b	1.75 a
PH	5.06 c	7.37 b	7.77 a

Values followed by different letters in each row are significantly different at the 0.05 probability level.

tent were obtained from fields in different locations of the provinces of Isfahan, Hamadan and Gilan in Iran. Ten soils sampled from each province. The climate of these provinces was arid, semiarid and humid, respectively.

Air-dry soil was crushed and sieved to pass a 2-mm mesh screen for particle-size analysis using the hydrometer method (Gee and Bauder, 1986). Calcium carbonate equivalents (CCE) were measured by back titration procedure. Soil pH and electrical conductivity (EC) were measured in a 1:2 soil: water extract after shaking for 30 min (Sparks 1996). Organic carbon (OC) content was determined by dichromate oxidation and titration with ferrous ammonium sulfate (Walkley and Black, 1934). Soils cation-exchange capacity (CEC) was measured as described by Bower et al. (1952). Means of some selected physical and chemical properties of ten sampled soils from each province are given in Table 1.

Cellulase sorption by soils

Soils with different physical and chemical properties were washed with distilled water and Ca-homoionized soils were prepared using 1 N CaCl₂, repeatedly. The samples were Ca-homoionized and autoclaved. Sterilized vessels were used for all experiments that were conducted in aqueous solution in the presence of toluene (1 ml l⁻¹). The suspensions of Ca-homoionized soils were dispersed by ultrasonification. Fluca prepared cellulase (with 1.4 Uml⁻¹ activity) was used in this study. For application of different cellulase concentration appropriate aliquots of 10 g l⁻¹ of cellulase solution were added to each soil suspension. After shaking for 1 h, they were centrifuged at 15000 x g. The amount of the cellulase protein remaining in solution was determined by the method of Bradford (1976) by spectrophotometer using bovine serum albumin as the standard. The amount of protein sorbed was obtained using the following formula:

$$(x/m)_a = (C_i - C_e) * V_a / W$$

Where $(x/m)_a$ is the amount of protein sorbed per unit weight of soil (mg mg⁻¹), C_i is the initial concentration of protein (mg ml⁻¹), C_e is the equilibrium concentration of protein (mg ml⁻¹), V_a is the solution volume (ml) and W is the soil weight (mg).

The cellulase-soil complexes in centrifuge tube were washed with distilled water until no protein was detected in the washings, using the method of Bradford 1976, and the amount of cellulase protein in the washings was also determined.

Statistical analyses

Experimental data of cellulase sorption on soils were transformed for linear analysis. The Lineweaver-Burk linearization has been used for linearized Langmuir isotherm. The natural logarithm of data has been used for linearized Freundlich isotherm. Pearson linear correlation was performed to ascertain whether the investigated cellulase sorption was correlated with soil physical and chemical properties.

RESULTS AND DISCUSSION

The sampled soils had high clay contents and the textures of them were often determined as clay to clay loam. There are significant differences between soil CEC, equivalent CaCO₃, OC, EC and pH of the provinces (Table 1). The CEC and OC contents of Gilan soils were significantly higher than those of the other soils. On the other hand, the equivalent calcium carbonate content, EC and pH of Isfahan soils were significantly higher than those of Gilan and Hamadan soils. Hamadan soils exhibited intermediate properties. These differences may be due to the soil forming factors. The climates of Gilan, Hamadan and Isfahan provinces are humid, semiarid and arid respectively.

Cellulase sorption isotherms on soils showed that the amount of sorbed cellulase protein increased by increasing equilibrium cellulase concentration (Figures 1, 2 and 3). The experimental data show that both the Langmuir and Freundlich isotherms yielded good curve fits for cellulase sorption on calcareous soils. Both models exhibited significant correlation coefficients with experimental data. However, the correlation coefficients of the Freundlich isotherms in Isfahan and Hamadan soils were higher than those of the Langmuir isotherms. The correlation coefficient of the Langmuir isotherm in Gilan soils was higher than that of the Freundlich isotherm.

The parameters of the Langmuir and Freundlich isotherms were estimated by linear least square method. The binding of cellulase by Isfahan and Hamadan soils is described by Freundlich isotherm better than Langmuir

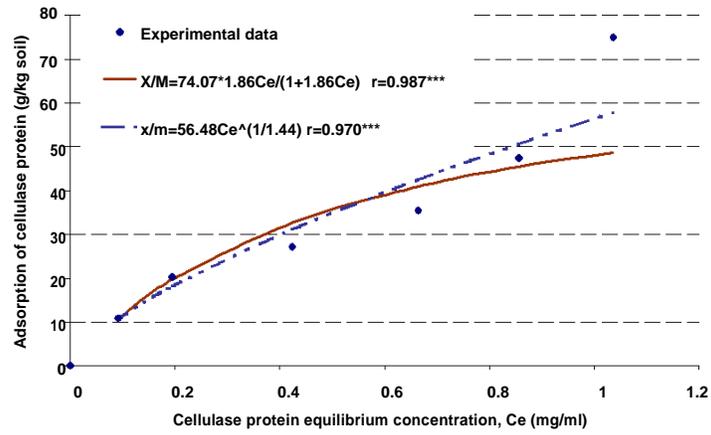


Figure 1. Equilibrium sorption isotherm of cellulase proteins on Gilan soils at 20°C and their Langmuir and Freundlich models.

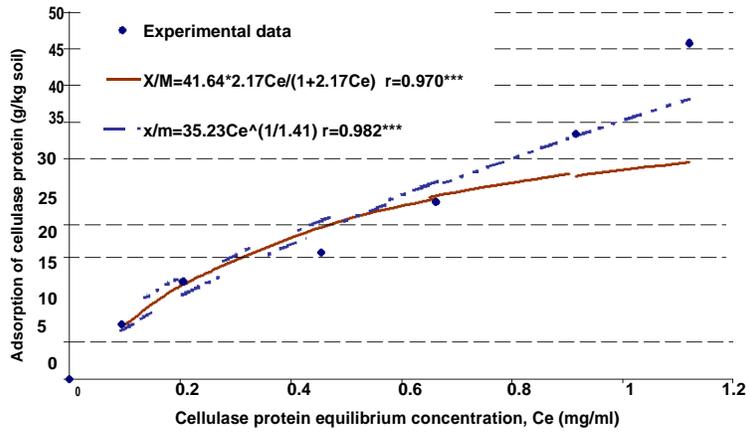


Figure 2. Equilibrium sorption isotherm of cellulase proteins on Hamadan soils at 20°C and their Langmuir and Freundlich models.

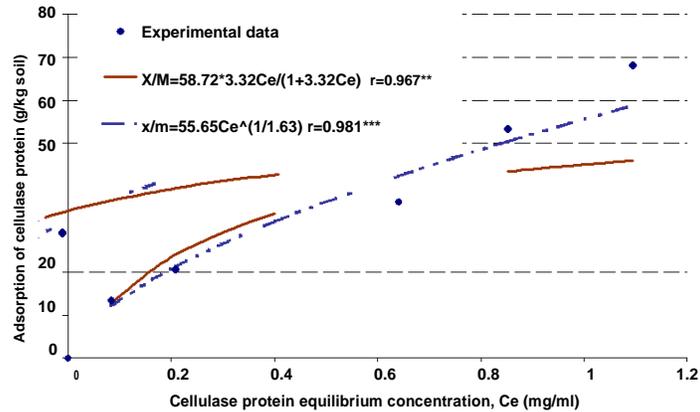


Figure 3. Equilibrium sorption isotherm of cellulase proteins on Isfahan soils at 20°C and their Langmuir and Freundlich models.

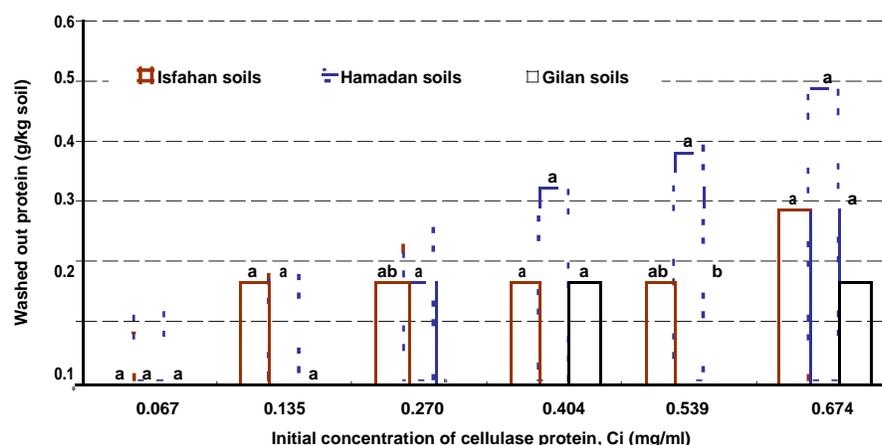


Figure 4. Washed out cellulase protein adsorbed on soils in different applied concentrations.

Table 2. Pearson correlation coefficients between sorbed cellulase and some physical and chemical soil properties.

Applied cellulase (mg ml^{-1})	Clay	Silt	Sand	Eq. CaCO_3	pH	CEC	EC	OC
0.067	0.379*	-0.14	-0.11	0.414*	-0.072	0.07	0.233	-0.2
0.135	0.447*	-0.19	-0.11	0.156	-0.22	0.045	0.057	0.153
0.27	0.465**	-0.03	-0.25	0.221	-0.11	0.08	0.168	0.066
0.404	0.281	-0.02	-0.15	0.191	-0.11	0.03	0.107	0.081
0.539	0.286	-0.04	-0.14	0.175	-0.1	0.01	0.142	0.134
0.674	0.42*	-0.24	-0.05	0.006	-0.24	0.062	-0.16	0.048
Mean	0.436*	-0.15	-0.14	0.161	-0.18	0.027	0.042	0.081

Correlation coefficients marked by *, ** and *** are significant at the 0.05, 0.01 and 0.001 level respectively, (2-tailed).

isotherm. The opposite is observed for Gilan soils due to higher correlation coefficient with the Langmuir isotherm. The maximum binding amounts of cellulase on the Isfahan, Hamadan and Gilan soils estimated by the Langmuir model were 58.72, 41.64 and 74.07 g kg^{-1} , respectively. The association binding constants were 3.32, 2.17 and 1.86 ml mg^{-1} for cellulase sorption on the Isfahan, Hamadan and Gilan soils respectively.

The sorbed cellulase protein was washed out from Hamadan soils in higher amount than from Isfahan and Gilan soils (Figure 4). The amount of sorbed cellulase protein washed from Gilan soils was considerably low.

Pearson correlation coefficients between sorbed cellulase (in each applied concentration) and some physical and chemical soil properties are shown in Table 2. In most cases the sorption capacity of soil was significantly related to its clay contents. The effect of carbonates on the soil sorption capacity markedly decreased by increasing applied cellulase concentration. In high concentrations the correlation coefficients between sorbed cellulase and soil carbonate contents were not significant.

The interaction of organic molecules with mineral surfaces is a subject of interest in a variety of disciplines. Aspartase adsorption on montmorillonite was found to obey the Langmuir isotherm. The binding of cellulases to insoluble cellulose have been investigated by a number researchers and the Langmuir isotherm have been used for expressing cellulase adsorption on cellulose (Bader et al., 1992; Bothwell and Walker, 1995; Boussaid and Saddler, 1999; Lynd, et al., 2002). The Langmuir model is derived using the primary assumptions that the rate of adsorption and desorption are in equilibrium and that the energy of adsorbed species is equal over the entire adsorbent's surface. The resulting model predicts the amount of bounded species as a function of free species concentration and two binding parameters, the association binding constant and maximum binding level of the enzyme. Despite some evidence that cellulase binding dose not comply with Langmuir assumption, its use is popular because it is an excellent fit to the data and it provides researchers with a simple mechanistic model to compare the kinetic properties of various adsorbent-cellulase systems.

It has been suggested that a close relationship between enzyme activity and organic matter content of soil and that enzymes are strongly associated with organic complexes (Safari Sinangani et al., 2001; Lynd et al., 2002). However there was no significant correlation between cellulase sorption and organic carbon content of these calcareous soils. These soils are relatively poor in organic matter and the cellulase sorption was mainly correlated with their clay and carbonates contents. Sorbed cellulase washed out more easily from the Hamadan soils than from other soils, probably due to their lower clay contents. Gilan soils with higher clay contents and OC showed the highest maximum binding levels of cellulase estimated by the Langmuir model.

The association binding constant for cellulase sorption on the Isfahan soils was considerably high. It decreased for cellulase sorption on the Hamadan and especially Gilan soils. This finding may be due to the difference of soils in carbonate contents. It may be concluded that cellulase sorbed on soil carbonates is immobilized and cannot be separated easily.

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