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The effects of $CaCO_3$ on adsorption, immobilization and activity of cellulase in a decarbonated soil

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Abstract

The interaction of organic molecules with mineral surfaces is a subject of interest in a variety of disciplines. The present study was done to elucidate some aspects of sorption and immobilization of cellulase on soil components by analysis of the sorption, desorption, immobilization and activity of cellulase on a decarbonated soil treated with different levels of $CaCO_3$ (0, 2.5, 10 and 20 %). Applied concentrations of cellulase protein on Ca-homoionized soil suspensions were 0, 0.014, 0.028, 0.070, 0.140, 0.280, 0.701, 0.981 and 1.402 mg mL^{-1}. After shaking for 1 h in sterile conditions, they were centrifuged and the amount of the cellulase protein remaining in solution was determined. The adsorbed cellulase protein was calculated. The immobilized and desorbed cellulase proteins were calculated after washing soil suspensions thrice with distilled water. Analysis of variance showed that the effects of the enzyme concentration, $CaCO_3$ level and their interaction on cellulase protein adsorption and activity were statistically significant. The adsorption and immobilization capacities of the decarbonated soil increased by application of $CaCO_3$. However, these effects of $CaCO_3$ were only significant when high concentrations of cellulase protein were added to the soil. The desorption of cellulase protein from the decarbonated soil did not depend on the amount of cellulase adsorbed on the soil and the $CaCO_3$ level in the soil. The immobilized cellulase activity, and particularly its specific activity, decreased considerably by increasing $CaCO_3$ levels in the soil. This negative effect of $CaCO_3$ on the cellulase specific activity in the decarbonated soil was significant even for low levels.

Keywords: $CaCO_3$ application, Decarbonated soil; Cellulase; Sorption; Immobilization; Activity.

1. Introduction

Soil enzymes participate in the biological cycles of elements and play an important role in the transformation of organic and mineral compounds. Cellulases hydrolyze the cellulose polymer to smaller oligosaccharides and glucose, and include three major types of enzymes: endoglucanases (EC 3.2.1.4) which cut internal bonds randomly, 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 β-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 multicomponent enzyme complex, cellulosome, such as in anaerobic cellulolytic bacteria (Bayer *et al.*, 1998).

All proteins are subject to more or less rapid deterioration in solution. Extracellular enzymes, often involved in breaking up polymers and complex organic molecules, are themselves subject to sorption and deactivation by soil colloids (Skujins, 1967). The interaction of organic molecules with mineral surfaces is a subject of interest in a variety of disciplines. Enzymes can be sorbed and immobilized by clay minerals and humic colloids in soil environments (Burns, 1986; Fusi *et al.*, 1989; Geiger *et al.*, 1998; Gianfreda and Bollag 1994; Dick, 1997; Safari Sinegani *et al.* 2005; Safari Sinegani and Hosseinpour, 2006). Adsorption of enzymes can result in both inactivation, due to conformational changes, or enhanced activity due to increased concentrations of the enzyme and substrate at the solid-water interface (Burns, 1978; Boyd and Mortland, 1990; Gianfreda and Bollag, 1994; Staunton and Quiquampoix, 1994). Naidja and Huang (1996) found that the large molecules of aspartase (MW=180000) could intercalate between montmorillonite layers. Aspartase adsorption on montmorillonite was found to obey the Langmuir isotherm. The binding of cellulases to insoluble cellulose has been investigated and the Langmuir isotherm used to express cellulase adsorption on cellulose (Converse, *et al.* 1988; Bader, *et al.* 1992; Bothwell and Walker, 1995; Boussaid and Saddler, 1999; Lynd, *et al.* 2002). The Langmuir model assumes that the rates 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 the concentration of the free species and two binding parameters, the association binding constant and maximum binding capacity of the enzyme. Despite some evidence that cellulase binding does not comply with Langmuir assumptions, its use is popular because it generally fit well to the data and it provides researchers with a simple mechanistic model to compare the kinetic properties of various adsorbent-cellulase systems.

Cellulases in soils have been extensively studied by Schinner and Von Mersi (1990) and Deng and Tabatabai (1994) who have devised methods for the measurement of their activity.

Despite the importance of cellulases in C cycling, most studies only deal with their production, purification, characterization, and immobilization on natural or synthetic adsorbents (namely cellulose), rather than its behaviour in natural environments.

Previous studies revealed that soil coating with Al(OH)x increased the adsorption capacity of Ca-homoionized soil, palygorskite, montmorillonite, illite kaolinite, and Avicel. The amount of sorbed cellulase desorbed from soil surfaces was small (about 16%), especially in coated samples (about 6%). X-ray diffraction analysis of K-montmorillonite and Ca-montmorillonite showed that Al(OH)x intercalated

between the montmorillonite layers but cellulase protein was not immobilized on the internal surfaces of the sorbents. It was mainly immobilized on the external surfaces of minerals, and soil particles with higher surface area had higher adsorption capacity (Safari Sinegani *et al.*, 2005).

The study of cellulase protein adsorption on some calcareous soils sampled from arid, semiarid and humid regions of Isfahan, Hamadan and Guilan provinces of Iran revealed the importance of soil clay and carbonate contents in adsorption. Cellulase adsorption obeyed both the Freundlich and the Langmuir isotherms. The maximum binding level of cellulase estimated by the Langmuir model was higher in soils from humid regions but the association binding constant was higher in arid soils. The sorption capacity of soils was correlated with soil clay and carbonates contents (Safari Sinegani and Hosseinpour, 2006). However, soils have different properties and the results of correlation tests needed to be confirmed. The objective of the present work was to gain a basic understanding of the interaction between cellulase and soil carbonates by investigating the sorption and immobilization of cellulase protein on a decarbonated soil treated with different levels of $CaCO_3$.

2. Materials and Methods

2.1. Soil sampling

A surface-soil (0-30 cm) was sampled from an agricultural field in the Hamadan province, in northwest Iran, an area with a semi-arid climate (annual rainfall of 300 mm; annual average temperature 13^{o}C).

2.2. Soil physical and chemical analyses

The soil sample was air dried and ground to pass a 2 mm sieve. Selected soil properties were determined according to standard methods (Sparks, 1996). Particle-size was measured using the hydrometer method (Gee and Bauder, 1986). Equivalent calcium carbonate (ECC) was measured by back titration (Loeppert and Suarez, 1996). Soil pH and electrical conductivity (EC) were measured in a 1:5 soil: water extract after shaking for 30 min (Hesse, 1971). Organic carbon (OC) was analyzed by dichromate oxidation and titration with ferrous ammonium sulfate (Walkley and Black, 1934). Total nitrogen was determined by the Kjeldahl method (Hinds and Lowe, 1980). Cation-exchange capacity (CEC) and available K were measured according to Bower *et al.*, 1952. Available phosphorus was extracted with 0.5 M $NaHCO_3$ (pH 8.5) and determined spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid (Jackson, 1958).

2.3. Cellulase protein adsorption

Soil carbonates were removed as follows: 500 g of soil were placed in a 800 mL beaker. Five hundred milliliters of 1 M HOAC (acetic acid) were added to the soil. The beaker was maintained at 70 – 80°C for 120 min and stirred occasionally until all carbonates have been removed (when CO_2 bubbles were no longer evident). After overnight equilibration, equivalent calcium carbonate (ECC) was measured by back titration (Loeppert and Suarez, 1996) to check that carbonates had been completely removed. After that, soil was Ca-homoionized with 0.5 M $CaCl_2$ solution. The soil was then air-dried.

The soil then received different amounts of calcium carbonate (0, 2.5, 10, 20 % m/m). Soil suspensions (1 % m/v in distilled water) were prepared and sterilized in an autoclave for 15 min at 121^{o}C. Sterilized soil and vessels were used for all experiments which were conducted in aqueous solution in the presence of toluene (1 ml L^{-1}). The suspensions of Ca-homoionized soils (1 %) were dispersed by ultra-

sonification after autoclaving. Appropriate aliquots of cellulase solution (10 g L^{-1}) were added to each soil suspension. Applied concentrations of cellulase protein were 0, 0.014, 0.028, 0.070, 0.140, 0.280, 0.701, 0.981 and 1.402 mg mL^{-1}. After shaking for 1 h, they were centrifuged at 15000 *g. The amount of cellulase protein remaining in solution was determined by the Bradford method (1976) using bovine serum albumin as the standard. The amount of protein adsorbed was obtained using the formula of Thomas *et al.*, (1983):

$$(x/m)a = (Ci - Ce) * Va /W \quad (1)$$

Where (x/m)a is the amount of protein adsorbed (mg mg^{-1} of soil), Ci is the initial concentration of protein (mg mL^{-1}), Ce is the equilibrium concentration of protein (mg mL^{-1}), Va is the solution volume (mL) and W is the soil weight (mg).

The cellulase–soil complexes in the centrifuge tubes were washed with distilled water until no protein was detected in the washings, using the Bradford method (1976), and the amount of the cellulase protein in the washings was determined. The amount of cellulase immobilized was obtained using the formula

$$(x/m)b = (Ci - Ce) \times Va - (Cw \times Vw)/W \quad (2)$$

where (x/m)b is the amount of cellulase immobilized per unit weight of sorbent (mg mg^{-1}), Ci is the initial concentration of cellulase (mg mL^{-1}), Ce is the equilibrium concentration of cellulase (mg mL^{-1}), Va is the solution volume (mL), Cw is the concentration of cellulase in the total washings (mg mL^{-1}), Vw is the total washing volume (mL), and W is the sorbent weight (mg).

The activity of immobilized cellulase on soil particles was determined by measuring the amount of reducing sugars released using Avicel as substrate (Mandel and Weber 1969). The specific activity of cellulase (µM glucose min^{-1} mg^{-1} protein) was determined by dividing the cellulase activity (µM glucose min^{-1} g^{-1} soil) by immobilized cellulase (mg protein g^{-1} soil).

2.4. Adsorption isotherms

Experimental data of cellulase protein adsorption were adjusted to linear forms of both Langmuir and Freundlich isotherms. The Langmuir equation may be written as (Langmuir, 1916):

$$Ce/(x/m) = 1/Q0\ K_L + Ce/Q0 \quad (3)$$

where *x/m* is the amount of solute adsorbed per unit weight of adsorbent (mg g^{-1}), *C*e, the equilibrium concentration of the solute in the bulk solution (mg mL^{-1}), *Q*0, the monolayer adsorption capacity (mg g^{-1}) and K_L is the constant related to the energy of adsorption.

The Freundlich equation may be written as (Zeldowitsch, 1934):

$$\log(x/m) = \log K_F + 1/n \log Ce \quad (4)$$

where x/m is the amount of solute adsorbed per unit weight of adsorbent (mg g^{-1}), Ce, the equilibrium concentration of solute in the bulk solution (mg mL^{-1}), K_F, the constant indicative of the relative adsorption capacity of the adsorbent (mg g^{-1}) and 1/n is the constant indicative of the intensity of the adsorption.

2.5. Statistical analyses

The experiment was considered a completely randomized factorial design with three replicates. The factors were applied cellulase protein (0, 0.014, 0.028, 0.070, 0.140, 0.280, 0.701, 0.981 and 1.402 mg mL^{-1}) and $CaCO_3$ application (0, 2.5, 10 and 20 %). Experimental data of cellulase protein adsorption, desorption, immobilization and activity were subjected to analysis of variance and the means compared with the Duncan's new multiple range test.

3. Results and discussion

Some physical, chemical and biological properties of soils are shown in table 1. The content of sand, silt and clay was 48, 31 and 21 %, respectively corresponding to a loam texture. The soil was not saline (EC 0.12 dS m^{-1}), it was calcareous (equivalent calcium carbonate of 3.7 % and pH 7.9), with relatively low cation exchange capacity (CEC 23.8 cmolc kg^{-1}), organic matter (OC 21.34 g kg^{-1}) and total nitrogen (TN 2.11 g kg^{-1}). Soil available P and K were relatively high (77.16 and 186 µg g^{-1}, respectively).

Table 1. Some physical and chemical properties of the soil used in the experiment.

Soil property		Soil property	
Texture	Loam	ECC# (%)	3.70
Sand (%)	48	OC# (g kg^{-1})	21.34
Silt (%)	31	TN# (g kg^{-1})	2.11
CEC (Cmolc kg^{-1})	23.8	Available P (mg g^{-1} soil)	77.16
pH (1:5)	7.90	Available K (mg g^{-1} soil)	186
EC# (dS m^{-1})	0.12		

EC – electrical conductivity, ECC- Equivalent carbonate calcium, OC- organic carbon, TN- Total Kjeldal nitrogen

The experimental data adjusted well to the Freundlich isotherm (Figure 1). The amount of adsorbed cellulase protein increased with increasing of equilibrium cellulase concentration. In contrast, the Langmuir isotherm did not exhibit significant correlation coefficients with experimental data probably due to low concentration of cellulase protein applied to a soil containing a large number of binding sites (Table 2).

The relative cellulase adsorption capacity of the soil estimated by the Freundlich model (Kf) was higher in soils treated with higher levels of $CaCO_3$. It increased from 174.72 mg g^{-1} in untreated decarbonated soil to 802.23 mg g^{-1} in soil treated with 10 % $CaCO_3$. The intensity of the cellulase adsorption estimated by the Freundlich model (1/n) was also higher in soils treated with higher levels of $CaCO_3$ (Table 2).

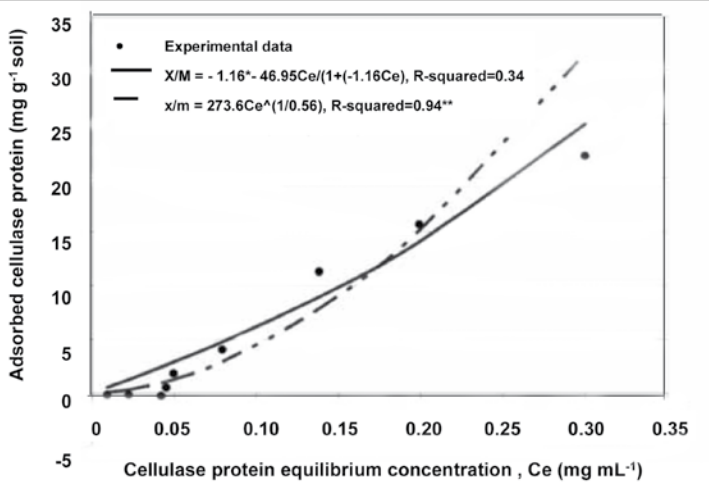

Figure 1. Equilibrium sorption isotherm of cellulase proteins on a decarbonated soil treated with 2.5 % $CaCO_3$ and their Langmuir and Freundlich models.

Table 2. Langmuir (K_L (mL mg^{-1}) and Q_L (mg g^{-1})) and Freundlich (K_F (mg g^{-1}) and n) constants and the respective R^2 for cellulase protein adsorption on a decarbonated soil treated with different levels of $CaCO_3$.

Applied CaCO3 %	Langmuir constants			Freundlich constants		
	R^2	Q_L	K_L	R^2	-n	K_F
0	0.002	-833.33	-0.07	0.87 **	0.60	174.72
2.5	0.34	-46.95	-1.16	0.90 **	0.56	273.61
10	0.33	-41.15	-1.40	0.71 **	0.44	802.23
20	0.55	-24.81	-2.17	0.85 **	0.52	485.90

*, ** and *** are significant at the 0.05, 0.01 and 0.001 p level, respectively.

A previous study with several soils from arid, semi-arid and humid areas in Isfahan, Hamadan and Guilan provinces of Iran showed that the sorption of cellulase on calcareous soils could be adjusted to both the Freundlich and the Langmuir isotherms (Safari Sinegani and Hosseinpour, 2006). This may be related to application of a higher initial cellulase protein concentration in that study.

Table 3 shows the analysis of variance of the effects of enzyme concentration and $CaCO_3$ application on cellulase protein adsorption, desorption, immobilization and activity in a decarbonated soil. The effects of enzyme concentration, $CaCO_3$ application and their interaction on cellulase protein adsorption were significant ($p < 0.01$). However, the desorption and release of cellulase protein adsorbed on the decarbonated soil was not affected by applied enzyme concentration and $CaCO_3$ application.

Table 3. Analysis of variance of the effects of enzyme concentration (Enz. C.), $CaCO_3$ application (Car. appl.) and their interaction (Enz. C. * Car. appl.) on cellulase protein adsorption, desorption, immobilization and activity in soil.

Source	DF	Adsorption	Desorption	Immobilization	Activity
Enzyme C.	8	808.49 **	3.93 ns	858.89 **	0.004 **
$CaCO_3$ application	3	1.64 **	5.50 ns	12.21 **	0.0003 **
Enz. C.* Car appl.	24	0.42 **	2.64 ns	2.80 ns	0.0005 **
Error	72	0.02	2.26	2.30	0.000005

ns- not significant, *, ** significant at the 0.05 and 0.01 p levels, repectively.

The effects of enzyme concentration and $CaCO_3$ application on immobilization of cellulase protein in the decarbonated soil were also significant. Immobilization of cellulase on the decarbonated soil was not affected by interaction between enzyme concentration and $CaCO_3$ application.

The effects of enzyme concentration, $CaCO_3$ application and their interaction on the immobilized cellulase were significant ($p < 0.01$).

When the initial concentration of applied cellulase protein was below 0.03 mg mL^{-1}, the study of adsorption, desorption, immobilization and activity was difficult due to low measurements and relatively high errors in estimates. The increases of initial cellulase protein concentration increased significantly its immobilization on the decarbonated soil treated with different levels of $CaCO_3$ (Table 4). The highest cellulase protein immobilization (20.94 mg g^{-1} soil) was obtained in soils treated with the highest level of cellulase concentration (1.402 mg ml^{-1}).

Table 4. Cellulase protein immobilization (mg protein g^{-1} soil) in soil as affected by the initial concentration of applied cellulase protein.

Applied cellulase protein (mg ml^{-1})	Immobilization	
	mean	SD
0	-	-
0.014	-	-
0.028	-	-
0.070	-	-
0.140	1.38 d	0.21
0.280	2.19 d	0.31
0.701	9.40 c	0.34
0.981	13.83 b	0.62
1.402	20.94 a	1.22

* Means followed by the same letter in each column are not significantly different ($p < 0.05$); SD- standar deviation.

The amounts of cellulase protein immobilized on decarbonated soil treated with 0, 2.5, 10 and 20 % $CaCO_3$ were 3.37, 4.52, 4.79 and 4.77 mg g^{-1}, respectively (Table 5). The means of immobilized cellulase protein on $CaCO_3$ treated soil were not significantly different. However these means were significantly higher than that obtained for soil untreated with $CaCO_3$.

Table 5. Cellulase protein immobilization (mg protein g^{-1} soil) in soil as affected by $CaCO_3$ application.

CaCO3 application (%)	Immobilization	
	Mean	SD
0	3.37 b	8.54
2.5	4.52 a	7.95
10	4.79 a	7.99
20	4.77 a	8.56

* Means followed by the same letter in each column are not significantly different ($p < 0.05$); SD- standar deviation.

Statistical analysis of means of cellulase protein adsorption and activity in the decarbonated soil treated with different levels of $CaCO_3$ in each initial concentration of applied cellulase is shown in table 6. Results showed that the positive effect of $CaCO_3$ on cellulase protein adsorption was only significant in application of higher cellulase initial concentration (Table 6). The insignificance effect of $CaCO_3$ on cellulase adsorption may be related to relatively high standard errors in estimates This shows that the decarbonated soil untreated with $CaCO_3$ has considerably high adsorption capacity for cellulase protein molecules.

Table 6. Cellulase protein adsorption (mg protein g^{-1} soil), desorption (mg protein g^{-1} soil), and activity (μM glucose min^{-1} g^{-1} soil) in soil as affected by initial concentration of applied cellulase and $CaCO_3$ application.

Initial concentration of cellulase protein and $CaCO_3$ application	Adsorption		Desorption		Activity	
	Mean	SD	Mean	SD	Mean	SD
0.070*0	0.47 l	0.12	0.36 a	0.18	-	-
0.070*2.5	0.50 l	0.12	0.39 a	0.14	-	-
0.070*10	0.79 k	0.10	0.48 a	0.11	-	-
0.070*20	0.83 k	0.12	0.45 a	0.22	-	-
0.140*0	2.80 j	0.09	0.67 a	0.12	0.009 d	0.000
0.140*2.5	2.80 j	0.10	0.41 a	0.13	0.007 d	0.000
0.140*10	2.80 j	0.09	0.56 a	0.11	0.005 de	0.000
0.140*20	2.80 j	0.12	0.49 a	0.14	0.005 de	0.000
0.280*0	3.98 i	0.01	1.10 a	0.17	0.011 d	0.000
0.280*2.5	4.02 i	0.08	1.96 a	0.20	0.010 d	0.000
0.280*10	4.09 i	0.07	1.74 a	0.21	0.008 d	0.000
0.280*20	4.13 i	0.03	1.64 a	0.26	0.003 e	0.000
0.701*0	11.16 h	0.05	2.08 a	0.08	0.011 d	0.000
0.701*2.5	11.24 h	0.05	1.85 a	0.17	0.011 d	0.000
0.701*10	11.58 g	0.32	2.12 a	0.37	0.009 d	0.000
0.701*20	11.63 g	0.41	1.97 a	0.13	0.009 d	0.000
0.981*0	15.26 f	0.16	1.82 a	0.24	0.011 d	0.000
0.981*2.5	15.65 e	0.02	1.85 a	1.05	0.011 d	0.000
0.981*10	15.72 e	0.17	1.76 a	0.56	0.010 d	0.000
0.981*20	15.83 e	0.13	1.70 a	0.08	0.009 d	0.000
1.402*0	20.64 d	0.20	1.43 a	0.19	0.079 a	0.000
1.402*2.5	22.04 c	0.15	1.23 a	0.10	0.052 c	0.000
1.402*10	22.64 b	0.20	1.22 a	0.17	0.066 b	0.000
1.402*20	23.47 a	0.32	1.14 a	0.19	0.051 d	0.000

* Means followed by the same letter in each column are not significantly different ($p < 0.05$); SD- standar deviation.

The adsorbed cellulase protein on the decarbonated soil did not release easily. The effect of $CaCO_3$ applications on protein desorption from the decarbonated soil was not significant. The release of adsorbed cellulase protein from soil did not increase significantly with increasing level of applied cellulase protein. The amounts of desorbed cellulase protein obtained for soil treated with 0 % $CaCO_3$ was higher than those obtained for soils treated with $CaCO_3$. However, the addition of $CaCO_3$ to soil did not change significantly the retention potential of the decarbonated soil. A previous study with several soils from arid, semiarid and humid areas in Isfahan, Hamadan and Guilan provinces of Iran showed that the adsorbed cellulase washed out more easily from the Hamadan soils than from Isfahan and Guilan soils, probably due to their lower clay contents. The adsorbed cellulase protein did not wash out easily from Guilan soil with lower carbonates contents (Safari Sinegani and Hosseinpour, 2006).

Immobilized cellulase activity decreased with increasing $CaCO_3$ level in soil (Table 6). The decarbonated soil untreated with $CaCO_3$ compared to those treated with $CaCO_3$ had relatively higher cellulase activity. The highest immobilized cellulase activity was obtained in application of 1.401 mg cellulase protein g^{-1} soil untreated with $CaCO_3$. It was 0.076 µM glucose min^{-1} g^{-1} soil. The negative effect of $CaCO_3$ on immobilized cellulase activity was significant in application of high levels of cellulase protein (table 6).

The specific activity of immobilized cellulase was calculated by dividing the amont of immobilized cellulase activity to the amont of immobilized cellulase protein. It was higher in application of low level of cellulase protein (Figure 2). The decrease of cellulase activity after immobilization on soil particle was higher in application of higher level of cellulase protein. The negative effect of $CaCO_3$ on soil cellulase activity became more obvious by calculating the specific activity of immobilized cellulase. The immobilized cellulase specific activity was clearly lower in higher application of $CaCO_3$ in the decarbonated soil (Figure 2).

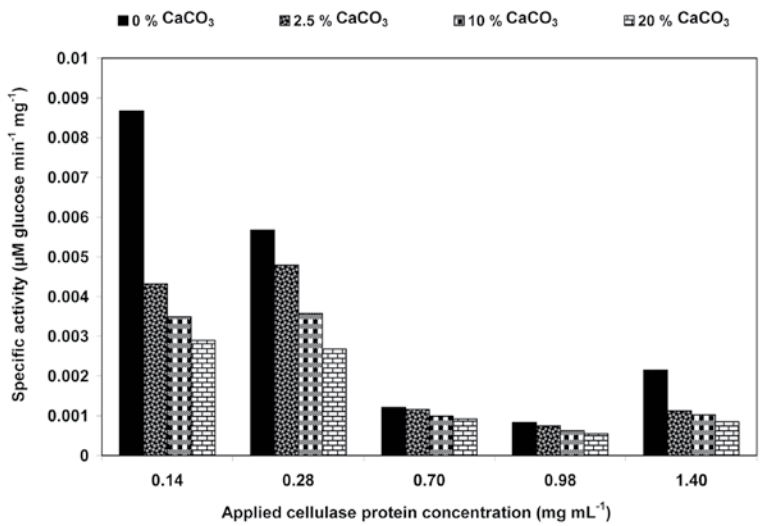

Figure 2. The effect of $CaCO_3$ levels on immobilized celluase specific activity (µM glucose min^{-1} mg^{-1} protein) in soil in different applied cellulase protein concentrations.

4. Conclusions

It was showed that the Ca-homoionized samples (soil, palygorskite, montmorillonite, illite, kaolinite and Avicel) showed a significantly higher cellulase sorption and immobilization capacities than the K-homoionized samples. Among adsorbents, soil sample showed the highest sorption and immobilization capacities (Safari Sinegani *et al*, 2005). Besides the existence of some clay minerals in soil, this may be related to the soil $CaCO_3$, organic matter, and humic substances. Sorption capacity of calcareous soils with low organic carbon was significantly related to the soil clay and carbonate contents (Safari Sinegani and Hosseinpour, 2006). This study showed that the decarbonated soil had considerably high sorption and immobilization capacities. Although application of $CaCO_3$ increased those capacities in soil, but these increasing effects of $CaCO_3$ on sorption and immobilization capacities of soil were only significant in application of high levels of cellulase protein. On the other hand, the immobilized cellulase activity and particularly its specific activity decreased with increasing $CaCO_3$. The negative effect of $CaCO_3$ on the specific activity of cellulase was more obvious in application of low levels of cellulase protein. So it may be concluded that the increasing effects of $CaCO_3$ on the adsorption and immobilization of cellulase protein in soil may not be as important as the decreasing effect of that on the immobilized cellulase activity due to low concentration of cellulase protein in soils.

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