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Adsorption, Immobilization and Activity of Cellulase in Soil: The Impacts of Maize Straw and Its Humification

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ABSTRACT

The present work aimed to study some aspects of sorption and immobilization of cellulase molecules on soil components by the analysis of the reactions of cellulase in a soil treated with different levels of maize residue and incubated for 90 days. The analysis of variance showed that the effects of the treatments of maize straw, incubation time and their interaction on cellulase adsorption, desorption and immobilization were statistically significant. The adsorption and immobilization capacities of soil by application of maize straw increased significantly. However they decreased with decreasing the soil organic matter (SOM) after 45 days of incubation. The desorption of adsorbed cellulase molecules from the soil by washing with distilled water depended on the SOM contents and its humification. The binding strength of cellulase molecule with fresh miaze straw was significantly stronger than that with humified maize straw. The immobilized cellulase activity, particularly its specific activity increased significantly by increasing the OC contents in the soil treated with maize straw.

Key words: Maize straw, Humification, Cellulase, Sorption, Immobilization, Activity

INTRODUCTION

Microbial cellulases have potential application in various industries, including pulp and paper, textile, laundry, biofuel production, food and feed industry, brewing, and agriculture (Kuhadet al. 2011). Cellulases hydrolyze cellulose polymer to simpler sugers and finally glucose. Cellulases are composed of at least three types of isoenzymes, endo-(1,4)-β-D-glucanase (EC 3.2.1.4) exo-(1,4)-β-D-glucanase (EC 3.2.1.91), and β-glucosidases (EC 3.2.1.21) acting synergistically. The exoglucanase (CBH) acts on the ends of the cellulose chain and releases β-cellobiose as the end product; endoglucanase (EG) randomly attacks the internal O-glycosidic bonds, resulting in glucan chains of different lengths, and the β-glycosidase acts specifically on the β-cellobiose disaccharides and produces glucose (Henrissat 1994; Wyman 1999; Singh 1999; Lynd et al. 2002). It was mentioned that cellulase adsorption is a necessity for the hydrolysis to occur during the conversion (Huet al. 2008; Liu and Hu 2012).

The sorption and binding of cellulase molecules to insoluble microcrystalline cellulose (Avicel) have been investigated by a number researchers and the Langmuir isotherm have been used for expressing cellulase adsorption on cellulose (Boussaid and Saddler 1999; Lynd et al. 2002; Daset al. 2012). The adsorption and activity of cellulase on raw and pretreated agricultural wastes and residues have been considered (Azevedo et al. 2000; Lu et al. 2002; Hu et al. 2008; Kumar and Wyman 2009; Boonme 2012; Liu and Hu 2012; Du et al. 2012) due to its applications in different industries.

Despite the importance of cellulases in the C-cycle in aquatic and terrestrial environments, most studies on these enzymes deal with their

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production, purification, characterization, and immobilization on natural, or synthetic adsorbent instead of with their reaction in natural environments. Therefore, bearing in mind the environmental importance of cellulases, studies to explain the reactions of cellulases in natural aquatic and terrestrial systems are required. Cellulase activities in the soils have been studied by Schinner and Von Mersi (1990) and Deng and Tabatabai (1994) who have devised methods for the measurement of their activity. The effects of some important ions and heavy metals on the cellulase activity in solution form were studied by Safari Sinegani and Emtiazi (2005). Previous study revealed that the amount and activity of immobilized cellulase on Avicel as an organic matter were significantly higher than the amounts and activities of immobilized cellulase on soil mineral components. However, coating soil components with Al(OH)x improved their adsorption and immobilization capacities. The amounts of cellulase desorbed and released from soil surfaces were quite low (about 16%) and coating them with Al(OH)x improved the retention of adsorbed cellulase molecules on soil solid surfaces. Avicel and soil solid particles with higher surface area had higher adsorption capacity (Safari Sinegani et al. 2005).

The study of adsorption of cellulase molecules on some calcareous soils sampled from the arid, semiarid and humid regions of Iran showed the importance of clay and carbonates in the soil in cellulase molecules adsorption. The maximum binding level of cellulase molecules estimated by the Langmuir model was higher in the humid soils but the association binding constant was higher in the arid soils compared to those in the other soils. The correlation tests also revealed that the adsorption capacity of the soils was significantly related to the clay and carbonates contents of the soil. In contrast, the amounts of cellulase adsorbed on the calcareous soils were not correlated with their organic carbon contents (Safari Sinegani and Hosseinpour 2006).

Different soil samples have diverse properties and the results of correlation tests need to be tested in the laboratory. Safari Sinegani and Safari Sinegani (2011) reported that the amounts of cellulase molecules adsorbed and immobilized in the soil increased significantly by increasing calcium carbonate (prepared from Merk Co) levels in a soil decabonated by acetic acid. However, the treatment of that soil with calcium carbonate decreased the activity and specific activity of immobilized celluase strongly. So, the addition of carbonates to soils had a negative effect on cellulase activity. The addition of acids and lowering soil equivalent calcium carbonate (ECC), however, can increase the cellulase activity immobilized in calcareous soil. The decrease of ECC and dissolution of native soil carbonates by the addition of acid acetic increased the activity and specific activity of immobilized celluase molecules in the soil significantly (Safari Sinegani and Safari Sinegani 2012).

In the present study, the previous result (i.e. the null effects of SOM) was examined on a specific soil sample with different levels of maize straw in laboratory condition. Thus, the objective of the present work was to understand the basic understanding of the interaction between cellulase molecules and soil organic matter by investigating the sorption, immobilization and activity of cellulase molecules in a calcareous soil treated with different levels of maize straw and incubated in field capacity for 90 days.

MATERIALS AND METHODS

Soil and maize sampling and analysis

The methods used for soil sampling and analysis were reported in Safari Sinegani and Safari Sinegani (2011). Maize straw pH and electrical conductivity (EC) were measured in a 1:10 plant residue: water extract after shaking for 2 h. Total dissolved solids (TDS) in maize straw were measured by evaporating the 1:10 water extract of plant residues and weighing its oven dried solids (American Public Health Association., 1998). Maize straw organic carbon (OC) was analyzed by dry oxidation in electrical furnace at 570^{o}C for 2 h (Matthiessen et al. 2005). Total P was determined in acid (HCl) solution of ash of plant residues spectrophotometrically as blue molybdate-phosphate complexes under partial reduction with ascorbic acid (Peperzak et al. 1959). Total nitrogen content was determined by the Kjeldahl method (Hinds and Lowe 1980).

Soil preparation and treatment with maize straw

Soil organic carbon removed by treating repeatedly with 10% hydrogen peroxide with boiling until SOM was completely decomposed. After overnight equilibration, the SOM was measured by dichromate oxidation and titration with ferrous ammonium sulfate (Walkley and Black 1934) to check that the SOM had been completely removed. After SOM removal, the soil was Ca-homoionized with 1 N $CaCl_2$ solution for three times and washed three times with 95% alcohol and then distilled water. The soil was then air-dried and mixed with different amounts of mild (d<2 mm) maize straw (0, 2, 5, 10, 20% m/m). A 100% maize straw (d<2 mm) was also used for a better understanding of the interaction between cellulase molecule and organic matter. The treated soils and maize straw were uniformly wetted with a spray to a water content near field capacity. The approximation of field capacity of each mixture of soil and maize straw was determined gravimetrically in a column saturated and drained for 48 h. The volume of water required to bring each of the soils to the approximated field capacity was determined by weighing daily. The treated and moistened soils were incubated in lab condition (20- 25^{o}C) in glass containers for 90 days. After 1, 15, 45, 60 and 90 days of incubation, a portion of each soil was taken for the study of celluase adsorption.

Cellulase molecule adsorption

The methods used for measuring the adsorption, desorption and immobilization of cellulase molecules on soil particles were according to Safari Sinegani and Safari Sinegani (2011).

Adsorption isotherms

Maize straw was humified in lab temperature separately with water content near field capacity for 45 days. Cellulase adsorption isotherms were studied in the soils treated with different levels (0, 2, 10 and 20% m/m) of the humified maize straw. Appropriate aliquots of cellulase solution (10 g L^{-1}) were added to the soil suspension (concentrations of cellulase 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 remaining in the solution was determined. The adsorbed cellulase molecule was calculated and experimental data of cellulase molecule adsorption were adjusted to linear forms of both Langmuir and Freundlich isotherms as described by Safari Sinegani and Safari Sinegani (2011).

Statistical analyses

The experiment was a completely randomized factorial design with three replicates. The factors applied were maize straw (0, 2, 5, 10, 20 and 100%) and incubation time (0, 15, 45, 60 and 90 days). Experimental data of cellulase molecule adsorption, desorption, immobilization and activity were subjected to analysis of variance and the means compared with the Duncan’s new multiple range test.

RESULTS AND DISCUSSION

The sand, silt and clay contents were 48, 31 and 21%, respectively in the soil corresponding to a loam texture. The soil was not saline (EC 0.12 dS m^{-1}); it was calcareous (with 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 contents were relatively high (77.16 and 186 µg g^{-1}, respectively). Table 1 shows some properties of the maize straw. Maize straw had neutral pH (7.62), high OC (535 g kg^{-1}) values and C/N (147.5) and C/P (187.9) ratios.

The results of cellulase adsorption on the soil treated with different levels of humified maize straw adjusted well to the Freundlich isotherm (Table 2). The amount of adsorbed cellulase molecule increased with increasing of equilibrium cellulase concentration. In contrast, the Langmuir isotherm did not exhibit significant correlation coefficients with the experimental data probably due to low concentration of cellulase molecule applied to a soil containing a large number of binding sites. The increase of humified maize straw to soil had not significant effect on the constants of the Langmuir and Freundlich isotherms. This could be related to high capacity of mineral components of the soil for adsorption of cellulase molecules. A study with several soils from the arid, semiarid 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). Their higher concentrations of cellulase molecules were used in adsorption isotherms compared to those used in this study.

Table 1 - Some physical and chemical properties of soil and maize residue used in this study.

Soil property		**maize residue property**	
Texture	Loam	pH (1:10)	7.62
Sand (%)	48	EC (1:10) (dSm^{-1})	4.06
Silt (%)	31	OC (g kg^{-1})	535.5
Clay (%)	21	TN (g kg^{-1})	3.63
CEC (Cmolc kg^{-1})	23.8	C/N	147.5
pH (1:5)	7.90	TP# (g kg^{-1})	2.85
EC# (1:5) (dSm^{-1})	0.12	C/P	187.9
ECC# (%)	3.70	Total dissolved solid (g kg^{-1})	46.72
OC# (g kg^{-1})	21.34	Dissolved OC (g kg^{-1})	33.2
TN# (g kg^{-1})	2.11		
C/N	10.11		
Available P (μg g^{-1} soil)	77.16		
Available K (μg g^{-1} soil)	186		

EC – electrical conductivity, ECC- Equivalent carbonate calcium, OC- dichromate (oxidable) organic carbon, TN- Total Kjeldal nitrogen, TP- Total phosphorus.

Table 2 - Langmuir (Q0, and K_L) and Freundlich (K_F and n) constants and their correlation coefficient for cellulase protein adsorption on a decarbonated soil treated with maize residue composted and humified for 45 days.

Applied maize straw %	***Langmuir constants***			***Freundlich constants***		
	r	**Q0**	**K_L**	**r**	**-n**	**K_F**
		mg g^{-1}	**mL mg^{-1}**			**mg g^{-1}**
0	0.75	-3.49	-4.72	0.97 **	0.54	333.75
2	0.77	-3.97	-4.58	0.98 **	0.56	292.01
10	0.74	-3.77	-4.53	0.97 **	0.55	290.12
20	0.79	-3.49	-4.62	0.98 **	0.53	320.54

* R-squared values marked by *, ** and *** are significant at the 0.05, 0.01 and 0.001 level respectively.

Table 3 shows the analysis of variance of the effects of maize straw application, incubation time and their interaction on cellulase adsorption, desorption, immobilization, activity and specific activity in soil. The effects of maize straw application, incubation time and their interaction on cellulase molecule adsorption, desorption and immobilization were significant ($p<0.01$). Maize straw application and incubation time had significant effects ($p<0.01$) on cellulase activity and specific activity in the soil. However, the interaction between the application of maize straw and incubation time had not significant effects on cellulase activity and specific activity in the soil.

Table 3 - Analysis of variance (mean square) of the effects of maize straw application (MS appl.), incubation time (IT) and their interaction (MS appl. * IT) on cellulase protein adsorption (Ads.), desorption (Des.), immobilization (Immob.), activity (Act.) and specific activity (Sp.Act.) in soil.

Source	**DF**	**Ads.**	**Des.**	**Immob.**	**Act.**	**Sp.Act.**
Maize straw appl.	5	0.67 **	0.18 **	0.91 **	0.0007 **	0.0001**
Incubation time	4	0.80 **	0.71 **	0.18 **	0.0007 **	0.0002**
MS appl.* IT	20	0.11 **	0.15 **	0.31 **	0.00004 ns	0.000008ns
Error	60	0.01	0.01	0.02	0.00006	0.00001

ns) Mean square of the treatment is not significant, *) Mean square (MS) of the treatment is significant at the 0.05 level and **) Mean square of the treatment is significant at the 0.01 level.

Table 4 shows Duncan's tests of means of cellulase adsorption, desorption and immobilization in the soil as affected by maize straw percentage applied in the soil and incubation time. The addition of 2% maize straw to soil increased the amounts of cellulase adsorbed and immobilized in the soil on the first day of incubation significantly. This finding was not in accordance to the pervious correlation tests, which revealed that the sorption capacity of the soils sampled from the arid, semiarid and humid regions of Isfahan, Hamadan and Guilan provinces of Iran, was significantly related to the clay and carbonates contents of the soil, and the amount of cellulase adsorbed on the calcareous soils was not correlated with their organic matter contents (Safari Sinegani and Hosseinpour 2006).

The amounts of cellulase adsorbed and immobilized on soil increased with increasing the maize straw in the soil. The highest amounts of cellulase adsorbed (2.33 mg g^{-1} soil) and immobilized (2.27 mg g^{-1} soil) were found when 20% maize straw was added to the soil. However, these values were significantly lower than the amounts of cellulase adsorbed and immobilized on maize straw alone as 100% treatment. On the first day of incubation, maize straw was raw and cellulase adsorption and immobilization was higher on the fresh maize straw in the soil than on the soil mineral components. The addition of 2% maize straw decreased the amounts of cellulase desorbed from the soil significantly on the first day of soil incubation. The highest amount of desorbed cellulase (0.52 mg g^{-1} soil) was measured in the untreated soil (control). This revealed that, the binding of cellulase to its substrate (here fresh maize straw) was stronger than that to mineral components in the soil.

Table 4 - Duncan's new multiple range tests of means of cellulase protein adsorption, desorption and immobilization in soil as affected by maize straw percentage applied in soil (MS) and incubation time (IT).

IT*MS Day*%	Adsorption		Desorption		Immobilization	
	mg protein g^{-1} soil		mg protein g^{-1} soil		mg protein g^{-1} soil	
	Mean	SD	Mean	SD	Mean	SD
1*0	1.04 i	0.17	0.52 defgh	0.03	0.52 j	0.18
1*2	1.92 h	0.09	0.07 l	0.00	1.85 efg	0.09
1*5	1.98 h	0.12	0.04 l	0.00	1.94 defg	0.12
1*10	2.25 fg	0.06	0.04 l	0.00	2.21 bc	0.06
1*20	2.33 efg	0.12	0.05 l	0.00	2.27 b	0.11
1*100	2.56 abc	0.20	0.04 l	0.02	2.52 a	0.21
15*0	2.33 ef g	0.07	0.48 efghi	0.06	1.85 efg	0.06
15*2	2.34 defg	0.06	0.13 kl	0.02	2.21 bc	0.08
15*5	2.38cdefg	0.14	0.42 fghi	0.10	1.96 cdefg	0.21
15*10	2.57 abc	0.01	0.55 cd	0.02	2.02 bcdefg	0.03
15*20	2.54 bc	0.10	0.36 ij	0.06	2.04 bcdefg	0.11
15*100	2.64 ab	0.11	0.54 defg	0.06	2.10 bcde	0.07
45*0	2.33 efg	0.01	0.44 fghi	0.09	1.89 efg	0.08
45*2	2.52 bcde	0.01	0.60 cd	0.18	1.92 defg	0.18
45*5	2.53 bcd	0.02	0.48 efghi	0.10	2.05 bcdef	0.11
45*10	2.55 bc	0.01	0.46 efghi	0.06	2.08 bcde	0.06
45*20	2.58 abc	0.02	0.42 fghi	0.12	2.16 bcd	0.13
45*100	2.68 ab	0.10	0.41 ghi	0.06	2.27 b	0.05
60*0	2.28 fg	0.24	0.38 hi	0.17	1.90 efg	0.38
60*2	2.55 bc	0.01	1.07 b	0.04	1.48 h	0.03
60*5	2.53 bcd	0.09	0.48 efghi	0.00	1.82 fg	0.09
60*10	2.56 abc	0.03	0.70 c	0.03	1.86 efg	0.06
60*20	2.41 cdef	0.12	0.24 jk	0.06	2.17 bcd	0.15
60*100	2.69 ab	0.10	0.48 efghi	0.00	2.21 bc	0.10
90*0	2.20 g	0.02	0.42 fghi	0.06	1.78 g	0.05
90*2	2.54 bc	0.04	1.31 a	0.06	1.23 i	0.09
90*5	2.55 bc	0.13	0.44 fghi	0.07	1.86 efg	0.10
90*10	2.56 abc	0.05	0.60 cd	0.00	1.96 cdefg	0.05
90*20	2.56 abc	0.11	0.50 efghi	0.03	2.06 bcdef	0.12
90*100	2.75 a	0.09	0.64 cd	0.12	2.11 bcde	0.14

* Means followed by the same letter in each column are not significantly different ($P < 0.05$).

The study with several soils sampled from the 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 molecules did not wash out easily from Guilan soil with lower carbonates and higher clay, OC and Al and Fe hydroxide contents (Safari Sinegani and Hosseinpour 2006). Coating soil mineral with Al(OH)x increased the adsorption capacity of Ca-homoionized soil, palygorskite, montmorillonite, illite kaolinite, and Avicel. The amounts of cellulase desorbed from the coated soil surfaces were quite low (about 6%) (Safari Sinegani et al. 2005). In addition to that, light fraction of SOM (here fresh maize straw) plays an important role in retaining of cellulase molecule from washing out.

Kumar and Wyman (2009) studied cellulase adsorption and desorption kinetics upon dilution with an equal amount of fresh buffer at 4 ^{o}C and at a loading of 400 mg cellulase/g solids for Avicel glucan and for corn stover solids pretreated by different materials. They reported that although pure Avicel cellulose contained no lignin, it desorbed the least amount of protein, while the highest percentage release was from the controlled pH pretreated solids (30%). They observed no direct relationship between the amount of lignin in the solids and enzyme desorption.

After 15 days of soil incubation the amounts of cellulase adsorbed and immobilized on the soil untreated with maize straw increased significantly. They were 1.04 and 0.52 mg g^{-1} soil on the first day of incubation, respectively, which increased to 2.33 and 1.58 mg g^{-1} soil on the 15th day of incubation and this increase continued until 45th day. Then, the amounts cellulase adsorbed and immobilized in the control soil decreased continuously. These fluctuations were related to increase and decrease of the OC contents in the control soil. Figure 1 shows the OC contents of the treated and control soil during incubation in the lab condition. Incubating the soil with low OC contents in lab condition with water content near field capacity made a suitable growth chamber for the autotrophs to increase the OC contents of the soil. However, the increase of OC contents was significant only in the control soil after 15 days of incubation. The importance of OC produced by the autotrophs was negligible in the treatments with higher maize straw and OC decomposition by heterotrophs was significant even in early stages of incubation (Fig. 1). The decrease of OC of maize straw in the treatment 100 % was significantly high in early stages of incubation and had a kinetic very similar to the first order reactions. The amounts of cellulase adsorbed and immobilized on the soil treated with 2% maize straw was similar to those in the control soil. They increased with increasing time of incubation and then decreased (after 45 days). These changes reasonably related to the OC variation in the soil.

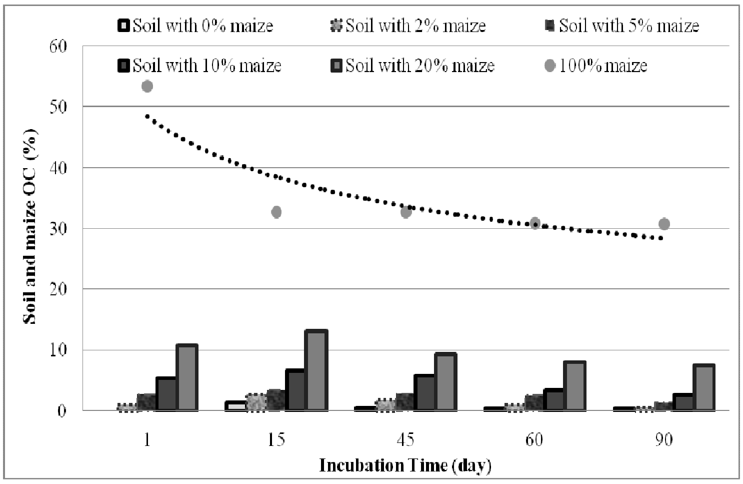

Figure 1 - Organic carbon contents of maize straw and soil treated with maize straw during 90 days of incubation.

The amounts of cellulase adsorbed in the soils treated with 5, 10, and 20% maize straw increased after 15 days of incubation similarly but they remained nearly constant in following days of incubation. In contrast to those findings, the amount of cellulase adsorbed on the maize straw (in 100 % treatment) increased continuously with increasing its humification. The highest amount of adsorbed cellulase (2.75 mg g^{-1} maize straw) was obtained for the maize straw incubated for 90 days. Cellulases from a 0.14 mg mL^{-1} solution were adsorbed in higher amounts on more humified maize straw with lower C/N ratio.

The adsorption capacity of the total proteins on the three cellulosic substrates was studied by Luet al. (2002). These included Avicel PH101 (a microcrystalline cellulose), SO_2-impregnated, medium-severity steam-exploded Douglas-fir (DF), and the water-insoluble fraction of the DF substrate extracted by hot alkali peroxide (DFP) were the three substrates. Cellulase adsorption was represented by Langmuir isotherms. The DF and DFP substrates demonstrated similar adsorption saturation kinetics. But they differed substantially from the adsorption profile observed with Avicel and the maximum protein adsorbed was much higher. The pretreated softwood substrates (DF, DFP) had a higher adsorption capacity than Avicel (Luet al. 2002). The results obtained for 100% maize straw treatment were in accordance with these findings. Fresh maize straw acts like Avicel because of higher crystalline cellulosic materials. The amount of these materials would reduce and the percentages of lignin-like materials would increase during maize humification.

Cellulase are not only adsorbed to the cellulosic part of the substrate, but also remain adsorbed to the residual material that is void of any polysaccharides and contain primarily lignin (Boussaid and Saddler 1999). Luet al. (2002) reported also that the role of lignin and its influence on cellulase adsorption have still not been fully resolved. For example, although the DF substrate contained a higher residual lignin content, its maximum protein adsorption was very similar to that of the DFP, which possessed significantly lower lignin content (~50 vs ~10%). This suggests that lignin plays a significant role in adsorbing the cellulases, while concurrently acting as a barrier to the cellulase enzymes and limiting the efficacy of hydrolysis. Although the presence of lignin contributes to this difference, it is likely that the drying and bleaching of Avicel, and consequently the limited “reswelling” of this substrate, influence the structure of the cellulose, adsorption capacity, and enzymatic hydrolysis (Luet al. 2002).

During incubation, the humification of maize straw in the treated soil and the mineralization of maize straw (i.e., reduction SOM contents) had opposite effects on the amount of adsorbed cellulase. For this reason, the amounts of cellulase adsorbed on the soils treated with 5, 10, and 20% maize straw remained nearly constant after 45 days of soil incubation. The amount of cellulase immobilized on the soils treated with 5% maize straw increased after 15 days of incubation similarly but it remained constant in the following days of incubation. In contrast to those findings, the amounts of cellulase immobilized on the soil and maize straw (in 10, 20 and 100 % treatments) decreased after 15 days of incubation. This decrease was statistically significant for maize straw (in 100% treatment). Cellulase from a 0.14 mg mL^{-1} solution was immobilized in higher amounts on less humified maize straw with higher C/N ratio. During the incubation, the humification of maize straw in the treated soil and the mineralization of maize straw (i.e. reduction SOM contents) had parallel effects on the amount of immobilized cellulase. For this reason, the amounts of cellulase molecule immobilized on the soils treated with 5, 10, and 20% maize straw decreased after 90 days of incubation. However, these decreases were not statistically significant. Although the study on the amount of desorbed cellulase molecule from the soil and maize straw incubated for 90 days confirmed that cellulase was bonded and immobilized more on less degraded maize straw, this finding should be tested with other methods.

The depletion method was used to measure cellulase adsorption. In this method, cellulase adsorption is monitored as the difference in protein concentration between the original solution and the equilibrium cellulase solution. The cellulase and substrate characteristics and the medium, or operational conditions in which the enzymatic reaction occurred could influence the results. The new analytical methods such as sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) should be used as a more accurate analytical means to measure the adsorption and recovery of different isozymes in cellulase mixtures (Huet al. 2010).

It has been reported that the sorption capacity of calcareous soils with low organic carbon for cellulase molecules was significantly related to the clay and carbonate contents (Safari Sinegani and Hosseinpour 2006). Although the amounts of cellulase adsorbed on the calcareous soils was not correlated with their organic matter contents, this study showed that the organic matter of the soil played an important role in the adsorption of cellulase in the calcareous soil. Especially the fresh plant residue and light fraction of soil organic matter have a high capability to retain and immobilize the cellulase.

Table 5 shows Duncan's test of means of immobilized cellulase activity and specific activity in the soil as affected by maize straw supplementation. The means of immobilized cellulase activity and specific activity in the soil increased with increasing maize straw in soil continuously. However, these increases were only significant for 20% maize straw to soil. The lowest means of immobilized cellulase activity and specific activity were obtained in the control soil (in 0% treatment). They were 0.004 (µm glucose min^{-1} g^{-1} soil) and 0.002 (µm glucose mg^{-1} protein), respectively. The highest means of immobilized cellulase activity and specific activity were obtained in maize straw (in 100% treatment). They were 0.022 (µm glucose min^{-1} g^{-1} soil) and 0.01 (µm glucose mg^{-1} protein), respectively.

Table 5 - Duncan's new multiple range tests of means of immobilized cellulase activity and specific activity in soil as affected by maize straw (MS) percentage applied in soil.

MS (%)	Activity µM glucose min^{-1} g^{-1} soil		Specific activity µM glucose mg^{-1} enzyme protein	
	Mean	SD	Mean	SD
0	0.004 c	0.005	0.002 b	0.002
2	0.005 c	0.006	0.003 b	0.004
5	0.006 c	0.008	0.003 b	0.004
10	0.008 c	0.006	0.004 b	0.003
20	0.016 b	0.011	0.007 a	0.005
100	0.022 a	0.015	0.010 a	0.007

*Means followed by the same letter in each column are not significantly different ($P < 0.05$).

The activity and specific activity of immobilized cellulase in the soil and maize straw increased significantly after 45 days of incubation and then they decreased (Table 6). But this decrease was only significant for the specific activity after 90 days of incubation. The activity of immobilized cellulase had the lowest value (0.004 µm glucose min^{-1} g^{-1} soil) at the first day and the highest value (0.019 µm glucose min^{-1} g^{-1} soil) at 45 days of incubation. The specific activity of immobilized cellulase had also the lowest value (0.002 µm glucose mg^{-1} protein) at the first day and the highest value (0.009 µm glucose mg^{-1} protein) at 45 days of incubation, which decreased significantly to 0.004 µm glucose mg^{-1} protein at the end of incubation time. During soil incubation, fibers in maize residue change and lignin percentage increases. Luet al. (2002) reported that lignin plays a significant role in adsorbing the cellulases, while concurrently acting as a barrier to the cellulase enzymes and limiting the efficacy of hydrolysis. Liu and Hu (2012) showed the importance of cellulose binding domains (CBDs) in the adsorption and activity of cellulases onto fibers. CBDs clearly play an important role in cracking of the crystalline region of cellulose increasing its adsorption capacity.

Table 6 - Duncan's new multiple range tests of means of immobilized cellulase activity (µm glucose min^{-1} g^{-1} soil) and specific activity (µm glucose mg^{-1} enzyme protein) in a maize residue treated soil as affected by incubation time (IT).

IT (days)	Activity µM glucose min^{-1} g^{-1} soil		Specific activity µM glucose mg^{-1} enzyme protein	
	Mean	SD	Mean	SD
1	0.004 b	0.006	0.002 b	0.003
15	0.006 b	0.007	0.003 b	0.004
45	0.019 a	0.017	0.009 a	0.008
60	0.014 a	0.008	0.007 a	0.003
90	0.007 a	0.006	0.004 b	0.003

*Means followed by the same letter in each column are not significantly different ($P < 0.05$).

According the previous discussions and the following the analysis of data the variations in activity and specific activity of immobilized celluase strongly depended on the organic carbon changes in the soil. During incubation, the OC contents decreased and it was humified. There were significant linear relations between the OC content and the activity and specific activity of immobilized cellulase in the treated soils (Figs 2 and 3). Figures 2 and 3 showed that the relation between the activity of the immobilized cellulase and SOC content was stronger than that the relation between the specific activity of the immobilized cellulase and SOC content. Because the immobilized cellulase specific activity did not depend on the amounts of cellulase immobilized on 1.0 g of the soil but the cellulase activity dose.

Safari Sinegani and Safari Sinegani (2011) reported that the addition of calcium carbonate to a soil decabonated by the acetic acid increased significantly the amounts of cellulase adsorbed and immobilized in the soil. However, the treatment of that soil with calcium carbonate decreased the activity and specific activity of immobilized cellulase in the soil strongly. It was also reported that the decrease of native soil carbonates increased the activity and specific activity of immobilized cellualse in the soil significantly Safari Sinegani and Safari Sinegani (2012). Here, the addition of maize residues and increasing SOM of the studied calcareous soil diminished considerably the inhibitory effects of soil carbonates on cellulase activity and specific activity.

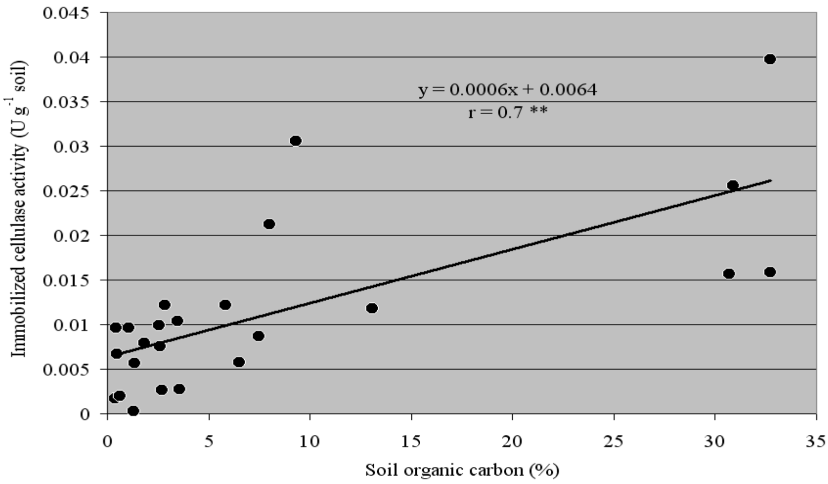

Figure 2 - Linear relation between the activities of immobilized cellulase in soil and OC contents of soil treated with maize straw.

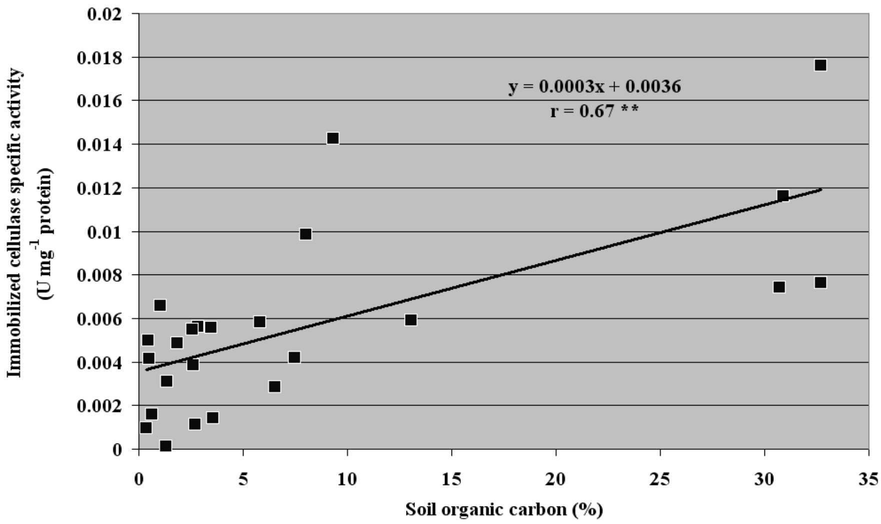

Figure 3 - Linear relation between the specific activities of immobilized cellulase in soil and OC contents of soil treated with maize straw.

CONCLUSIONS

This study showed that the increase of the SOM by the addition of maize straw to soil, especially the increase of light fraction and unhumified maize residues improved the adsorption, retaining and immobilization of cellulase in the calcareous soil. The study on the amounts of desorbed cellulase from the soil and maize straw incubated for 90 days showed that cellulase was bonded strongly and immobilized more on its substrate (i.e. fresh and less degraded maize straw). The activity of immobilized cellulase had a positive and significant correlation coefficient with the organic carbon contents of the soil. Although the adsorption and immobilization of cellulase on the solid surfaces of the soil resulted in inactivation due to conformational changes and/or reaction with the soluble and insoluble minerals (specially carbonates) of the soil, the increase of the SOC reduced cellulase inactivation in the soil because the specific activity of immobilized cellulase had a positive and significant correlation coefficient with OC contents in the soil.

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