Studies on Cellulase from Trichoderma Reesei and Its Effect on Pretreated Cellulosic Materials

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Synopsis

The stability of locally produced cellulase from *T. reesei* was studied. The enzyme solution was stable at temperatures up to 45°C over which a gradual inactivation occured until 55–60°C. A rapid loss of enzymatic activity was observed over 60°C and complete inactivation occurred at 70°C after only 1 h. The maximum enzymatic saccharification was obtained at 50°C and pH 6.5. The pretreatment of cellulosic wastes, bagasse, and sawdust from soft and hard wood was carried out in different solvents, chloroform, dioxan, dichloroethane, ethylacetate, and ethanol, for 8 h. These treatments increased the susceptibility of the cellulosic wastes to enzymatic saccharification. The bagasse was highly affected, followed by the sawdust from soft and hard wood, respectively.

INTRODUCTION

The cellulase enzymes produced by different microorganisms have a variable composition and fluctuate in their effeciency for degrading the cellulosic materials. Many investigators studied the production of cellulases by different microbes and the effect of these enzymes on pure and native celluloses. Enari and Markhanen[1] studied the cellulases producing microorganisms and the mode of enzyme function. Halliwell and Griffen,[2] Skinner,[3] and Suzuki and Hachikubo[4] reported that cellulase is a series of enzymes that possess various activities.

The properties of cellulases were studied by Halliwell and Griffen[2]; they found that the highest activity for cellulase from rumen microorganisms at pH 6.6–6.8, but at pH 5.9 the enzymatic process was stopped. The optimum pH for cellulase from *Trichoderma viride* was pH 6.0. In 1964 Flora[5] reported that the optimum pH for hydrocellulase activity was 4.7–4.8, while the most favorable pH for cellulase from *Poronia oedopus* was 4.4–5.0.[6] Johnson et al.[7] mentioned that the maximum activity of cellulase from *Clostridium thermocellum* was displayed at 70°C and at pH 5.7–6.1.

The effect of pretreatments of native cellulosic materials on the effeciency of its enzymatic hydrolysis was investigated by Yoshito et al.[8]; they mentioned that the swelling of sawdust with 50% phosphoric acid for 2 h at 30°C increased cellulose hydrolysis. Sasaki[9] reported that enzymatic destruction of crystalline cellulose alone was not practical, while in combination with chemical treatments, which convert the crystalline cellulose to the amorphus type, a higher saccharification rate was obtained. In ad-

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dition, the removal of admixtures bounded with cellulose in the native cellulosic wastes permits a direct contact between cellulose and the enzyme, which results in a higher enzymatic activity.

The aim of the present work is to study the stability of the locally produced cellulase from *Trichoderma reesei* and its effect on pretreated cellulosic wastes which could be commercially used for the production of sugars for human nutrition.

MATERIALS AND METHODS

Cellulosic Materials. Bagasse was obtained from sugar and distillates company at Hawamdia. The raw material was washed with water to remove all residual sugar, dried, and milled to 20 mesh powder. Sawdust of hard and soft wood was supplied from a local workshop. Cellulase solution was the filterate of the fermentation mother liquor of *Trichoderma reesei.*

Chemical Treatments. Cellulosic materials were treated in different solvents, dioxan chloroform, ethanol, ethylacetate and dichloroethane for 8 h, then delignified, washed with water and dried.

Enzymatic Saccharification. The mixture used was 0.1 g cellulosic material plus 9.9 ml citrate buffer pH 5.0 plus 0.1 mL enzyme solution containing 1.7 cellulase units. The mixture was maintained for 1,3,5, and 7 h at 50°C in a water bath. The reducing sugars (mg glucose) were estimated in the reaction mixture after each of the mentioned periods by the method of Lavinson and Reese.[10]

Thermostability. The enzyme solution was attained for 1 h in a water bath at increasing temperatures from 20 to 75°C. After each treatment the enzyme units were estimated according to the amount of reducing sugars, and the percentage loss was calculated.

Optimum Temperature for Cellulase Activity. As mentioned for saccharification but the mixture was maintained for 1 h at each of the experimental temperatures (25–70°C).

Effect of pH. The enzyme solution containing 17.5 U/mL was adjusted to different pH values from 2.0 to 10.0 and kept for 24 h at 10°C. At the end of the treatment the enzymatic activity was determined.

RESULTS AND DISCUSSION

Thermal Stability

The enzyme solution was attained for 1 h at increasing temperatures from 20 to 75°C at 5°C intervals. The enzymatic activity and percentage loss occured are presented in Table I. The data showed that temperatures up to 45°C have no effect on the enzyme activity; at higher temperature the enzyme gradually loses its potency. The destruction of the enzyme markedly increased over 60°C. The temperature up to 55°C did not severly affected the enzyme; only 7.5% of its activity was lost. A higher temperature (70–75°C) showed a drastic effect, and complete inactivation of the enzyme occured within only 1 h. Similarly, Ryu and Mandels[11] reported that cel-

TABLE I
Thermal Stability of Cellulase Enzyme from *T. reesei*

Incubation temp (°C)	Cellulase activity (U/mL)	Loss in enzyme activity (%)
20	17.5	0.0
25	17.5	0.0
30	17.5	0.0
35	17.5	0.0
40	17.5	0.0
45	17.3	1.14
50	16.2	7.5
55	15.8	9.8
60	15.1	13.8
65	9.1	48.0
70	0.6	96.6
75	0.0	100.0

lulase enzymes are slowly inactivated under reactor conditions at 50°C and more rapidly inactivated at higher temperatures.

Optimum Temperature for Cellulase Enzyme

The enzymatic hydrolysis of cellulose was carried out at different temperatures between 25 and 70°C. Table II presents the resulting reducing sugars (mg glucose/mL) at the end of each treatment. The data show that the optimum temperature which gave the highest amount of reducing sugars was 50°C. The resulting reducing sugars increased gradually with the increase of temperature up to 50°C, while over 55°C a sharp decrease occurred as a result of enzyme inactivation. Saunders et al.[12] and Kassim[13] mentioned that the optimum temperature for the cellulases from *M. verrucaria* and *A. niger* were around 40°C, and above 45–50°C the enzyme activity was rapidly inactivated. With regard to cellulase from *T. reesei,* Ryu and Mandels[11] mentioned that it was stable at 50°C for 48 h. At higher temperatures a rapid inactivation occurred.

TABLE II
Effect of Temperature on the Enzymatic Activity of *T. reesei* Cellulase

Incubation temp (°C)	Reducing sugars as glucose (mg/mL)
25	0.1
30	0.3
35	0.6
40	0.7
45	1.0
50	1.2
55	1.0
60	0.3
65	0.1
70	0.0

Effect of pH

The cellulase solution was adjusted to different ph values and kept for 24 h at 10°C, after which the enzyme activity was determined. Results in Table III show that the highest enzymatic activity was at pH 6.5, at which no loss was observed. A gradual destruction of the enzyme occurred on both acidic and alkaline sides in the range of pH 5.0–8.0. From data it is clear that a sharp decrease in enzyme activity occurred at the pH values below 5.0 and over 8.0 and at pH 2.0 there was complete inactivation. Many investigators 2,5,7 studied the effect of pH on the activity of cellulase produced by different microorganisms; the optimum range was between pH 4.8 and 6.8 according to the microbe used.

Effect of Pretreatment of Cellulosic Wastes

The chemically treated bagasse, untreated (control) and pure cellulose powder (standard cellulosic material) were enzymatically hydrolyzed. Table IV presents the resulting reducing sugars after 1,3,5, and 7 h. The pure cellulose powder gave the highest yield of reducing sugars, followed by bagasse treated in ethylacetate, dichloroethane, chloroform, ethanol, and dioxan, respectively. The difference between treated bagasse samples may be due to the effect of each solvent on the crystalline cellulose structure. Mandels et at.[14] reported that the rate of saccharification depend on the nature and pretreatment of the substrate. The untreated bagasse gave the lowest saccharification effeciency because of the hard structure of native crystalline cellulose.

Generally, it is clear that the pretreatment showed a marked effect on the susceptibility of bagasse for enzymatic saccharification. In case of the ethylacetate treatment, the resulting sugars were approximately the same as the reference cellulose powder (7.0 and 7.2 mg/mL respectively).

TABLE III
Effect of pH Value on the Stability of *T. reesei* Cellulase Solution

pH values	Cellulase activity (U/mL)	Loss of activity (%)
2.0	0.00	100.00
3.0	3.25	81.50
4.0	8.60	50.86
4.5	10.00	42.86
5.0	13.50	22.85
5.5	14.75	15.75
6.0	15.25	10.30
6.5	17.5	00.00
7.0	16.75	4.29
7.5	16.00	8.58
8.0	14.25	18.60
8.5	12.25	30.00
9.0	4.50	74.29
9.5	2.00	88.58
10.00	0.50	97.15

TABLE IV
Effect of Chemical Pretreatment of Bagasse on the Enzymatic Saccharification

Treatment	Reducing sugars produced (mg/mL) after			
	1 h	3 h	5 h	7 h
Untreated bagasse	1.1	1.3	1.6	1.8
Dioxan	3.2	4.0	4.8	5.2
Ethanol	4.3	4.6	5.1	5.6
Ethylacetate	4.0	4.8	6.2	7.0
Chloroform	3.1	4.5	5.9	6.5
Dichloroethane	3.4	4.7	6.2	6.9
Cellulose powder[a]	5.2	6.0	6.8	7.2

[a] Pure cellulose powder as standard cellulosic material.

The effect of pretreatment on the ensymatic hydrolysis of sawdust from soft and hard wood was investigated. The data are presented in Tables V and VI respectively. The sawdust was generally less affected by chemical treatments than bagasse, and sawdust from soft wood was more susceptible to pretreatment than that from hard wood, This may be due to the difference in degree of crystallinity and the content of admixtures; this conclusion is in agreement with that mentioned by Ryu and Mandels[11] and Sasaki.[9]

All treated sawdust samples gave lower reducing sugars than the reference cellulose powder but markedly higher than the untreated saw dust, which indicates that the used solvents had affected the hydrogen bonding of the crystalline form of cellulose and encouraged the enzymatic saccharification.

According to these results we can conclude that:

1. Cellulase from *Trichoderma reesei* was stable at temperatures up to 45°C after which rapid inactivation occurred.
2. The optimum temperature for saccharification was about 50°C.
3. The highest enzymatic activity was obtained at pH 6.5.
4. The pretreatment of native cellulosic wastes with solvents increased the susceptibility for enzymatic hydrolysis. In this way the large amounts of cellulosic wastes could be used as a promising carbohydrate, after enzymatic conversion to sugars, in many fields of human nutrition.

TABLE V
Effect of Chemical Pretreatment of Sawdust from Soft Wood on the Enzymatic Saccharification

Treatment	Reducing sugars produced (mg/mL) after			
	1 h	3 h	5 h	7 h
Untreated sawdust	0.3	0.6	1.2	2.4
Dioxan	2.5	3.7	5.0	6.0
Ethanol	0.8	1.2	1.8	2.9
Ethylacetate	2.0	3.0	4.2	5.2
Chloroform	1.4	2.2	3.6	4.5
Dichloroethane	1.0	1.4	2.0	3.2
Cellulose powder[a]	4.8	5.5	6.8	7.0

[a] Pure cellulose powder as standard cellulosic material.

TABLE VI
Effect of Chemical Pretreatment of Sawdust from Hard Wood on the Enzymatic Saccharification

Treatment	Reducing sugars produced (mg/mL) after			
	1 h	3 h	5 h	7 h
Untreated sawdust	0.2	0.4	0.7	0.9
Dioxan	1.8	2.1	2.6	3.7
Ethanol	0.8	1.2	1.5	1.9
Ethylacetate	0.9	1.9	2.8	3.6
Chloroform	0.7	0.9	1.3	2.7
Dichloroethane	0.8	1.3	1.6	2.1
Cellulose powder[a]	4.8	5.5	6.8	7.0

[a] Pure cellulose powder as standard cellulosic material.

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