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Effect of Pretreatments and Utilization of Lignocellulosic Material for the Microbial Production of Cellulase

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Abstract: Fungal strains were isolated and cultured to produce cellulase using lignocellulosic materials i.e. rice husk, wheat bran and bagasse as substrates- untreated, steam treated and alkali treated at room temperature and 100°C. Appreciable cellulose activity was measured with steam treated and alkali heated at room temperature for rice husk and wheat bran. The untreated and steam treated bagasse showed measurable activity while it was very low in bagasse-alkali heated at room temperature and almost negligible activity was detected when bagasse-alkali treated at 100°C was used.

Key words: Lignocellulosic substrates, biomass, pretreatments.

Introduction

Lignocellulosics are the main sources of the naturally occurring cellulose. It is also the largest waste component generated in agriculture, lumber, food processing industry and municipal services [1]. Due to protection of lignin sheath and crystalline structure, cellulose is not degraded by extracellular enzymes. Pretreatment of the substrate is carried out to increase its susceptibility to hydrolysis which will affect not only the degree of saccharification but also the economics of the process [2]. There are different methods of pretreatment [3-7] to increase the surface area and to reduce the degree of polymerization to increase the susceptibility of lignocellulosics for enzymatic degradation [8-11]. Cellulolytic enzyme systems can be produced by a number of microorganism [12-16] such as aerobic and anaerobic bacteria, white rot fungi, soft rot fungi and anaerobic fungi. Cellulase preparations have mostly been based on expensive high purity cellulose as carbon source. However, promising results obtained for enzyme production using several lignocellulosic substrates [17-20] such as wheat straw, bagasse, rice husk, agrowaste, waste paper sludge etc. With this aim in view this study investigates the effect of alkali and steam pretreatment of lignocellulosic materials on cellulose activity and fungal growth.

Materials and methods

The fungal strains *Phanerochaete chrysosporium* and *Cladosporium* sp., were isolated from the soil of paddy field by enrichment with 1.0 % carboxymethyl cellulose as carbon source and 0.5 % yeast extract as growth supplement. The mineral base medium consisted of (g/l): NaCl, 10; KH_2PO_4, 1.0; $MgSO_4$, 0.25 and $FeSO_4$, 0.01. Single colonies were isolated and maintained on PDA slants at 4°C.

Lignocellulosic wastes i.e. rice husk, wheat bran and bagasse were used for the production of cellulase. These substrates were subjected to steam treatment and alkali treatment at two different temperatures by the methods described elsewhere [3,21] prior to its utilization.

Cells were grown aerobically at 30°C in the de-

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fined media [13] of pH 4.8 for 72 h and used as the inoculum for the fermentative production of enzyme. The carbon source of the inoculum medium was replaced by pretreated lignocelluloses substrate for the production of enzyme by fungal degradation. The Erlenmeyer flasks employed for the production were incubated at 30°C in shaking condition at 200 rpm for 8 days. The samples were withdrawn aseptically at different time intervals and analyzed for enzyme activity [22,23].

Results and discussion

In order to increase enzyme susceptibility the cellulosic material was subjected to various pretreatments to expose and to separate elementary micro fibrils. Table 1 shows the percent composition of cellulose, hemicellulose and lignin after various pretreatments. The data show that the contents are comparable in all three lignocellulosic sources. At optimum performance parameters i.e. temperature 30°C, pH 4.8, substrate concentration 2 % (w/v) and inoculum size 8 % (v/v), the pure cellulosic substrates induced high level of cellulase. Instead, low levels of cellulase enzyme were induced by other sugars (Table 2). The cellulase appears to be partially constitutive [24,25]. The xylose and xylan sugar neither support growth nor cellulase production. However, the pretreated lignocellulosic substrates supported both growth and cellulase enzyme production.

Table 3 & 4 show the production of cellulase enzyme obtained from pretreated rice husks, wheat bran and bagasse by both the isolated fungal cultures at the different culture conditions. From the data it is observed that the maximum amount of cellulase enzyme activity with rice husk and wheat bran, attained by steam treatment and it was almost comparable to the activity obtained with alkali treated at room temperature and untreated substrates. Very less activity was detected during fermentation with alkali treatment at 100°C. This may be due to the fact that intense alkaline treatment may reduce the enzyme production [3,9,19]. However, the biomass production for alkali treated at high temperature was more than the other pretreated substrates. Thus it is clear that cellulase enzyme must have been produced. It could be attributed to the fact that microorganisms were

Table 1. Percentage composition of components of lignocellulosic substrate after various treatments

Source	Steam treatment (ST)			Alkali treatment at room temperature (ATRT)			Alkali treatment at 100°C (ATHT)		
	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)	Hemi-cellulose (%)	Cellulose (%)	Lignin (%)
Rice husk	18.25	52.52	27.50	18.25	48.35	27.25	18.00	48.00	28.50
Wheat bran	23.75	49.00	23.40	21.50	48.50	23.00	20.50	46.00	24.40
Bagasse	34.35	50.60	9.90	33.50	50.60	11.90	33.50	46.00	11.50

Table 2. Effect of different carbon sources on cellulase production after 8 days of incubation

Carbon source	*Phanerochaete chrysosporium* Growth	*Phanerochaete chrysosporium* Cellulase (FPU/ml)	*Cladosporium sp.* Growth	*Cladosporium sp.* Cellulase (FPU/ml)
Xylose	-	Nil	-	Nil
Arabinose	+	0.9	-	Nil
Glucose	+	0.6	+	0.48
Maltose	+	0.36	+	0.48
Lactose	+	0.61	+	0.64
Sucrose	+	0.84	+	0.91
MN-Cellulose	+	2.06	+	2.24
FP-Cellulose	+	2.16	+	2.36
CM-Cellulose	+	2.46	+	2.64
Xylan	-	Nil	-	Nil
Wheat bran	+	1.96	+	1.84
Rice husk	+	1.84	+	1.92
Bagasse	+	2.48	+	2.24

Table 3. Production of cellulase by utilizing different lignocellulosic substrates in shake flask experiments by *Phanerochaete chrysosporium*

Substrate	Conc. of substrate (g/l)	Cellulose Conc. (g/l)	Lignin Conc. (g/l)	Hemi-cellulose (g/l)	Biomass Conc. (g/l)	Activity on day 8 (FPU/ml)	Yield (FPU/g of cellulose)
Rice husk (UT)	20	9.65	5.22	3.25	3.60	2.04	211.39
Rice husk (ST)	20	10.5	5.50	3.68	3.24	2.5	238.09
Rice husk (ATRT)	20	9.67	5.45	3.68	3.38	2.25	230.61
Rice husk (ATHT)	20	9.60	5.70	3.60	4.15	0.63	65.62
Wheat bran (UT)	20	9.65	4.50	3.95	3.45	1.92	198.96
Wheat bran (ST)	20	9.80	4.68	4.75	3.65	2.37	241.83
Wheat bran (ATRT)	20	9.70	4.60	4.30	3.14	2.01	207.21
Wheat bran (ATHT)	20	9.20	4.88	4.10	3.96	0.75	81.52
Bagasse (UT)	20	10.65	2.10	6.52	3.26	2.42	227.23
Bagasse (ST)	20	10.12	1.98	6.87	3.65	1.96	193.67
Bagasse (ATRT)	20	10.12	2.38	6.70	3.48	0.87	85.96
Bagasse (ATHT)	20	9.32	2.30	6.70	4.53	0.09	9.65

UT: Untreated; ST: Steam treated; ATRT: Alkali treated at room temperature
ATHT: Alkali treated at 100°C; FPU: Filter Paper Unit

hydrolyzing cellulose but not producing measurable cellulase enzyme in the broth [25].

In the case of bagasse the highest cellulase enzyme activity was observed in untreated bagasse, it was found to be quite close to that of bagasse steam treated. However low activity was detected with bagasse alkali-treated at room temperature while the activity almost attained zero value with bagasse treated at 100°C by both the fungal cultures. Since untreated bagasse was kept in pure water for 60 minutes while washing, it is possible that the presence of water in fiber capillaries

Table 4. Production of cellulase by utilizing different lignocellulosic substrates in shake flask experiments by *Cladosporium* sp.

Substrate	Conc. of substrate (g/l)	Cellulose Conc. (g/l)	Lignin Conc. (g/l)	Hemi-cellulose (g/l)	Biomass Conc. (g/l)	Activity on day 8 (FPU/ml)	Yield (FPU/g of cellulose)
Rice husk (UT)	20	9.65	5.22	3.25	4.04	1.87	193.78
Rice husk (ST)	20	10.5	5.5	3.68	3.26	2.25	214.28
Rice husk (ATRT)	20	9.67	5.45	3.68	3.68	2.04	210.96
Rice husk (ATHT)	20	9.6	5.7	3.6	3.6	0.63	65.62
Wheat bran (UT)	20	9.65	4.5	3.95	3.96	1.8	186.52
Wheat bran (ST)	20	9.8	4.68	4.75	3.84	2	204.08
Wheat bran (ATRT)	20	9.7	4.6	4.3	4.06	1.62	167.01
Wheat bran (ATHT)	20	9.2	4.88	4.1	4.13	0.37	40.21
Bagasse (UT)	20	10.65	2.1	6.52	3.26	2.25	211.26
Bagasse (ST)	20	10.12	1.98	6.87	3.46	1.76	173.91
Bagasse (ATRT)	20	10.12	2.38	6.7	3.96	0.63	62.25
Bagasse (ATHT)	20	9.32	2.3	6.7	4.12	Nil	Nil

UT: Untreated; ST: Steam treated; ATRT: Alkali treated at room temperature
ATHT: Alkali treated at 100°C; FPU: Filter Paper Unit

maintains dilations of the fiber walls reducing the inter-chain associations through a cellulose-HOH-cellulose hydrogen bonding arrangement [7], in turn making it more available for cellulase activity. These results were in agreement to the previous workers [11,14,26,27] where three stage treatment strategies, physical/chemical pretreatment enhance the availability of the surface area of the cellulose micro-fibrils for the microorganism for to act upon and enhance the production of cellulolytic enzymes.

Intense alkaline treatment at higher temperature causes various structural changes. The three major components of lignocellulose undergo hydrolytic and degradative reactions resulting in depolymerization and solvation of hemicellulose through peeling reaction [3,8,9,10]. Cellulose also degrades following enolisation of carbonyl groups, finally decomposing the end units and partly solubilizing lignin, accumulating toxic substances. The loss of activity could be due to the absorption of cellulase on cellulose and lignin or due to the washings to which bagasse alkali treated at 100°C was subjected not sufficient to extract toxic substances. These accumulated substances might have been formed rendering the microorganisms to excrete the appreciable amount of cellulase enzymes in broth [19,28,29].

Conclusion

Steam treated and alkali treated at room temperature and to a certain extent untreated rice husk and wheat bran seem to be suitable for cellulase enzyme production. However, especially untreated bagasse will likely to be more economical. The overall feasibility is certainly dependent on the efficient utilization of all components. The present status of feasibility studies are under process, considering all important features of utilization of lignocellulosic material, enormous and renewable resources.

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