

Production and Kinetics of Cellulase Enzyme from saw Dust Hydrolysate using *Trichoderma reesei* 992 6a

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Abstract

Cellulase is a multi component enzyme most widely used in food processing, paper and textile industry. Since cellulase is an inducible enzyme, high yields are obtained only when substrates containing large amount of cellulose are used. Cellulase was produced by fermenting the Saw Dust Hydrolysate residues using the organism *Trichoderma reesei*. The work was carried out for the period of seven days. The cellulase activity, amount of substrate and proteins were estimated by standard methods. Saw dust hydrolysate was chosen as the substrate due to high content of cellulose (40-55%), low cost and easy availability. Experimental work shows high growth rate and yield of cellulase by this method. The kinetic study were also made for both growth and product formation.

Keywords: Cellulase, *Trichoderma reesei*, Cellulose, Saw dust.

Introduction

Cellulase refers to a family of enzymes, which act in concert to hydrolyze cellulose. At least two steps in cellulose degradation by microorganisms begin with the preparatory prehydrolytic first step involving an enzyme (C1), which swells and/or Hydrates anhydroglucose chains. The second step uses hydrolytic enzymes (CX) and beta Glucosidase. The fungus *T. reesei* is an efficient producer of cellulase enzyme. Strains of *T. reesei* have been studied in great detail for more than two decades for the ability to Produce cellulase protein. Montenecourt and Eveleigh (1977) developed an agar plate screening technique to isolate a mutant of *T. reesei* capable of synthesizing cellulase. Ghose and Sahai(1979) produced cellulase by *T. reesei*

QM-9414 in fed-batch and continuous flow culture. Mukhopadhyay and Malik(1980) enhanced the cellulase production by adopting the strategy of pH cycling and temperature profiling using *T. reesei* MCG-77. Alimohagheghi *et al.*(1988)studied the cellulase production by the Mutant of fungus *T. reesei* on mixtures of xylose and cellulose. Schaffner and Toledo (1992) produced the cellulase in continuous culture by *T. reesei* on xylose-based media. Tholudur *et-al.* (1999) developed a mathematical model and optimized the cellulase Protein Production using *T. reesei* RL-P37 on soluble sugar lactose. Harikrishna *et-al.* (2000) used the high yielding strain,*T.reesei* QM-9414 for the cellulase enzyme production. Oinomen and Suominen(2002) identified the filamentous fungus *T. reesei* strains is an efficient producer of cellulase. Mach and

Zeilinger(2003) established the genus *T. reesei* widely used in industrial applications in the production of extra cellular hydrolase and heterologous protein. In this work wood residue was utilized for the production of cellulase.

Materials and Methods

Microorganisms and Growth conditions

The fungus *Trichoderma reesei* 992 6a was obtained from MTCC, Chandigarh and Cultured in growth medium containing malt extract 20g/lit, agar 20.0g/lit, water 1lit, sterilized at 121°C for 20 min and maintained at a pH 7.5 and incubated at 20°C for 7 days. The wood was pretreated to increase the crystallinity of cellulose, removing lignin and other inhibitors. The pretreatment was as follows, 100g of washed wood residues were treated with 2000ml of 4% solution of sodium hydroxide, autoclaved at 121°C for 30 min. It was filtered, washed until neutrality and dried at 65°C to constant weight. One portion of leaf obtained before washing was neutralized with phosphoric acid and filtered. The material recovered was then dried at 65°C to constant weight. To the leaf obtained, the same volume of distilled water was added and heated at 121°C for 30 min. The suspension was filtered and solid material was dried at 65°C to constant weight. The pretreated wood with trace elements was taken in the conical flask and analysis was conducted for every 8 hours. A 5 ml of production medium was taken then

2 ml of 70% Ammonium sulphate solution and 2 ml of 0.5 M sodium citrate buffer solution were added into it. Then it was centrifuged and the supernatant was taken for the analysis. The production medium containing treated wood residues 5 gm/l, CaCl₂ 0.3 gm/l, MgSO₄ 0.3 g/l, KH₂PO₄ 2.0, (NH₄)₂SO 1.4, Urea 0.3, trace elements FeSO₄.7H₂O 5.0 (mg/l), MnSO₄.H₂O 1.6 (mg/l), ZnSO₄.7H₂O 1.4 (mg/l), CoCl₂ (mg/l) was used.

Analytical Techniques

Fermentation was carried out on Saw Dust Hydrolysate with trace elements. The cellulase activity, amount of protein, reducing sugar and biomass were determined every 12 hours for 7 days. Cellulase activity was measured by using the filter paper, 0.5 ml culture filtrate was diluted to an appropriate concentration in sodium citrate buffer and then incubated with a 32 mg dry whatman No.1 filter paper for 1 hr at 50°C. After incubation, reducing sugar liberated was measured by the DNS method. Cellulase protein was measured by modified Lowry method using bovine serum albumin as a standard substrate. Concentration was determined by measuring the reducing sugar (DNS method) in the filtered fermentation medium culture filtrate was diluted prior to the addition of DNS reagent in sodium citrate buffer to yield an absorbance in the range 0.0 to 0.5 at 540nm. Substrate concentrations were ascertained using standard curves. For biomass estimation, 5 ml portion of culture broth was centrifuged for 20 minutes and the supernatant was discharged. The resulting pellets were dried and dry weight was estimated. (Muthuvelayudham *et al*; 2006)

Results and Discussion

The *Trichoderma reesei* 992 6a obtained from MTCC was sub-cultured on malt extract slants and petri plates and it was grown in batch culture with pretreated wood residues for the period of 7 days. Biomass, reducing sugar, amount of protein and cellulase activity was shown in fig1 & fig2. pretreated wood residues were used as substrate which contains large amount of cellulose. cellulose is a polysaccharide, thus it require more time

Table .1 Experimental data's recorded for assay with wood residue as a substrate.

Time (days)	Substrate (S)g/l	Biomass (X)g/l	Protein (V)	Cellulase activity (IFPU/ml)
0	1	-	-	
1	0.68	3.0	0.8	0.6
2	0.72	4.0	1.2	1.0
3	0.60	5.2	2.6	2.4
4	0.52	12.2	4.0	3.6
5	0.42	13.4	5.6	5.0
6	0.36	15.6	6.2	5.8
7	0.28	16.2	6.4	6.0

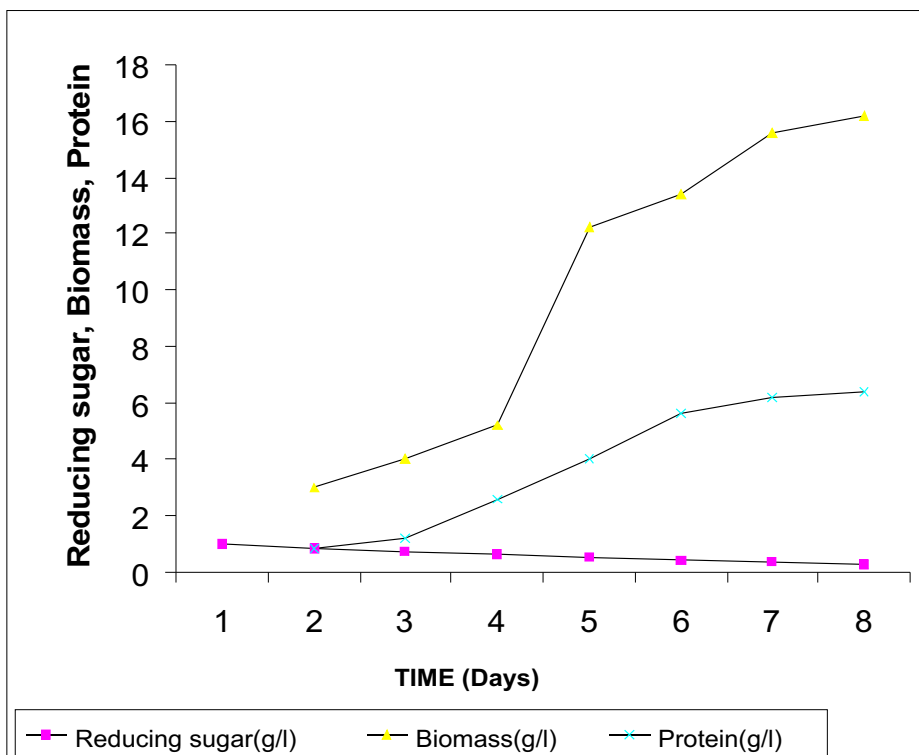


Fig 1: Effect of Reducing Sugar, Biomass, Protein on Saw Dust Hydrolysate as a Substrate

to break down and the production rate of enzyme was found to be very high. Results indicate higher growth rate and higher protein and shows higher cellulase activity.

Kinetics

The growth kinetics and production kinetics were developed for the fermentation carried

out at temperature 30°C and pH 6.5 the production kinetics was done by

- Michealis Menten kinetics
- Leudeking Piret kinetics

Michaelis Menten kinetics

$$V = V_{max} S / K_m + S \quad \dots\dots\dots(1)$$

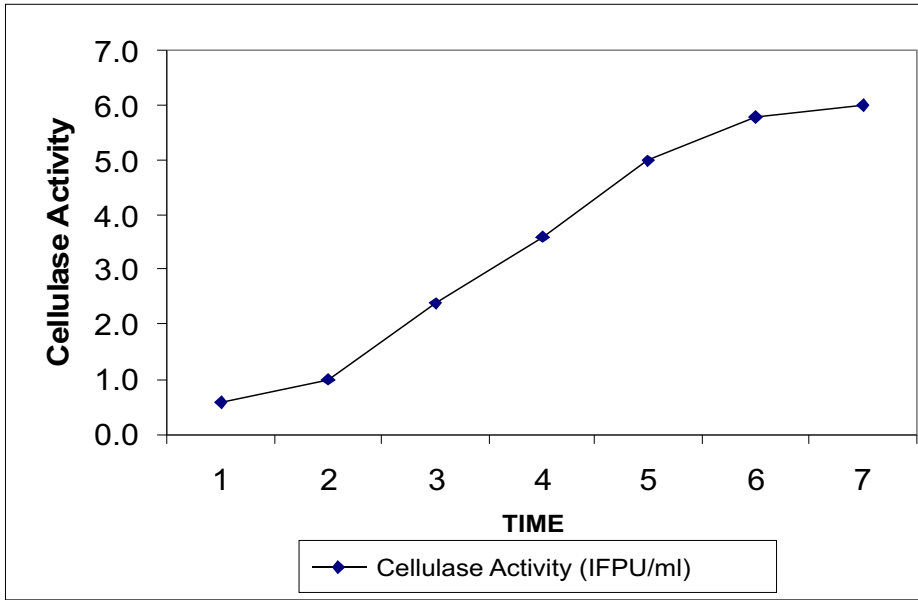


Fig 2: Effect of Cellulase Production on Saw Dust Hydrolysate

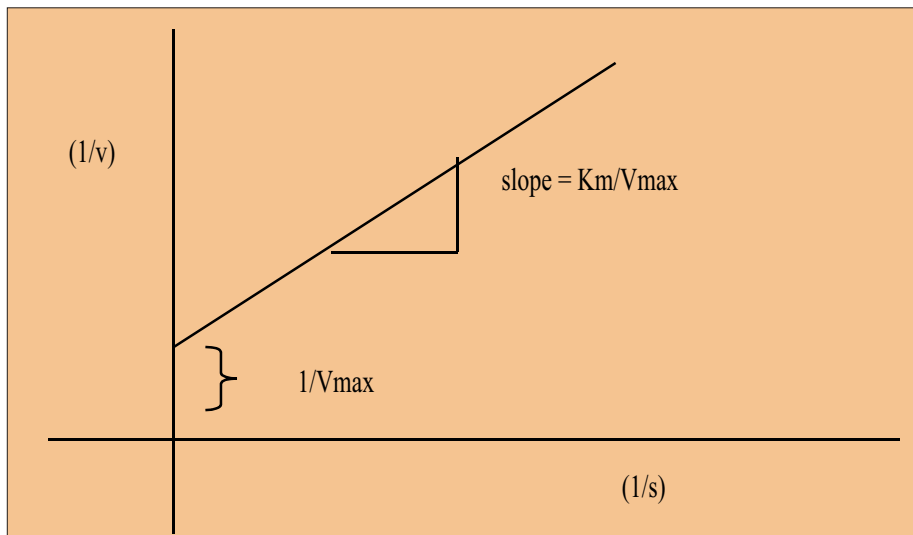


Fig: 3

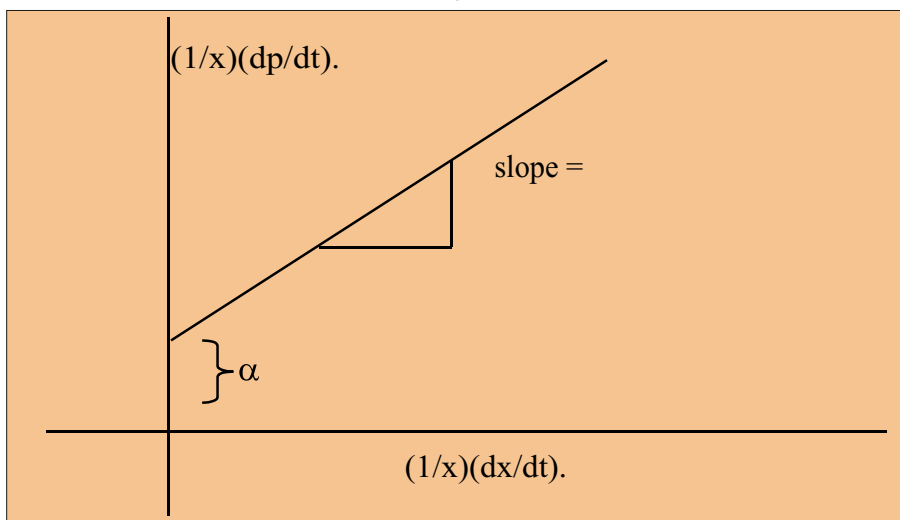


Fig: 4

The graph was plotted between $1/[v]$ and $1/[s]$. Fig. 3. The slope (K_m/V_{max}) and intercept ($1/V_{max}$) as found out. The production rate maximum reaction rate (V_{max}) was 4.678 and Michaelis Menten constant K_m was 1.075.

Leudeking piret model

$$dp/dt = (dx/dt) + x \dots\dots\dots (2)$$

The graph was plotted between the product concentration and time. The slope (dp/dt) at various points were found out. The values (dx/dt) were found out from the slopes at various points from the cell concentration curve. Now the graph was plotted between $((1/x)(dp/dt))$ and $(1/x)(dx/dt)$. Fig.4. The intercept $= 0.152$ and $= 0.046$ had been found out

Growth kinetics

The growth kinetics was done using Monod's kinetic model. The graph was plotted between time versus biomass concentration and spent growth rate was obtained by taking slopes to the curve at various points. Specific growth rate ($\mu = (1/x)(dp/dt)$). The graph was plotted between $(1/\mu)$ Vs $(1/s)$. Fig.5. From the graph the intercept ($1/\mu_{max}$) and slope (K_s/μ_{max}) was found the volume of specific growth rate was 0.523 hr⁻¹ and Monod's constant (K_s) was 1.285.

Conclusion

The results of batch process show the potential advantage of cellulase production process using saw dust. Further enhancement of enzyme production can be done by pH optimization, temperature optimization, inoculum size optimization, substrate optimization. Large scale production of cellulase is required in the food, paper, textile and for the production of cellulosic ethanol.

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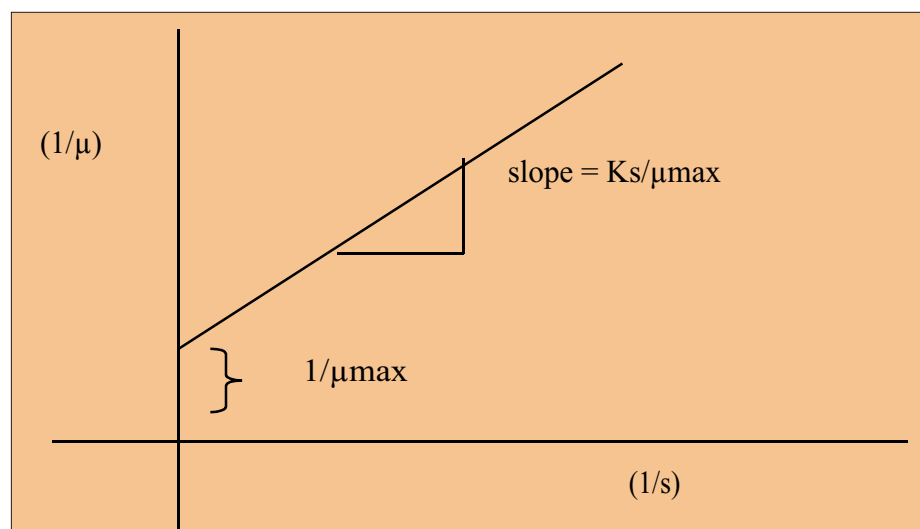


Fig: 5

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