

Culture Conditions for the Production of Cellulase by *Trichoderma reesei* RUT

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Effects of pH values and peptone concentrations on the production of cellulases by *Trichoderma reesei* RUT were investigated in this study. *Trichoderma* strains, particularly *Trichoderma reesei* RUT is the excellent sources of cellulase suitable for practical applications. Enzyme production was observed at pH 3.5, 4.0 and 4.5 in 0.05 and 0.1 % peptone containing mediums for 4 d. The activity of cellulase was measured by using carboxymethylcellulose (CMC) as the substrate. According to results the maximum specific activity was obtained in 0.5 % peptone containing medium in 72 h at pH 4.0, 0.05 % peptone containing medium at 96 h at pH 4.5.

Key Words: Cellulase, Activity, *Trichoderma reesei* RUT.

INTRODUCTION

Endoglucanases, exo-cellobiohydrolases and β -glucosidases are three major types of cellulolytic enzymes¹. Cellulosic material is the most abundant renewable carbon source in the world. Cellulose may be hydrolyzed using enzymes to produce glucose, which can be used for production of ethanol, organic acids and other chemicals². Cellulase and xylanases are industrially important enzymes with application in *e.g.*, food, feed, textile and pulp and paper industries^{3,4}. These enzymes are produced mainly with filamentous fungi, particularly with different *Trichoderma species*⁵. *T. reesei* has strong cellulose degrading activity. Its cellulase system has been widely studied and is considered to be a rational choice for industrial use¹. Cellulases are important enzymes that are sold in large volumes for use in different industrial applications. Among the application areas bio-polishing of textile, addition to washing powder, addition to animal feed to enhance its digestibility, pulping, processing of fruit juices and beverage, baking and in bioethanol production can be mentioned⁶.

pH is an important parameter in the production of enzymes by *T. reesei* RUT. According to earlier studies, production of cellulases was favoured by a low pH (4.0)⁷. In this work, the enzyme production by *T. reesei* RUT at different pH values and peptone concentrations has been reported.

EXPERIMENTAL

T. reesei RUT was obtained from the culture collection at the Biology Department of Uludag University, Turkey.

Culture medium: *T. reesei* RUT was maintained on potato dextrose agar slants for 4 d at 28 °C using in enzyme production experiments. The basal medium for the growth of *T. reesei* RUT and production of cellulase is as follows: (NH₄)₂SO₄ 1.4 g/L, KH₂PO₄ 2.0 g/L, urea 0.3 g/L, MgSO₄·7H₂O 0.3 g/L, FeSO₄·7H₂O 5.0 mg/mL, MnSO₄·H₂O 1.6 mg/mL, ZnSO₄·7H₂O 1.4 mg/mL and CoCl₂ 2.0 mg/mL. In addition carboxymethylcellulose (CMC) (1 %), Difco peptone (0.1 %) and Tween 80 (polyoxyethylene sorbitan monooleate, 0.1 %) were added to the medium to induce cellulase production. pH was controlled using 2 N HCl and 2 N NaOH. Medium was autoclaved for 0.5 h and seeded with a suspension of *T. reesei* RUT spores⁸.

Enzyme production: *T. reesei* RUT was propagated at 28 °C for 4 d in 250 mL medium with shaking on a shaker (220 rpm/min). Samples were taken at 24 h intervals (24, 48, 72 and 96 h). The supernatant of the culture after centrifugation (6000 rpm, 20 min) at 4 °C was used to determine enzyme activity⁹⁻¹¹.

Determination of enzyme activity: Cellulase assay was investigated by incubating 0.5 mL sample suitably enzyme and 0.5 mL of 1 % (w/v) CMC solution in citrate buffer (50 mM, pH 4.6) at 50 °C for 0.5 h. Estimation of reducing sugars was carried out by 3,5-dinitrosalicylic acid (DNS) method¹².

Effects of pH on enzyme activity: The effect of pH on cellulase activity was investigated by measuring enzyme activity at different pH values (50 mmol/citrate buffers pH 3-6) under standard assay condition with CMC as substrate.

RESULTS AND DISCUSSION

Cellulase production: The shake flasks with the mediums and the inoculum were adjusted, controlled at different pH (3.5, 4.0 and 4.5) and incubated for 4 d. During incubation, the samples were withdrawn every 24 h and analyzed for the enzyme levels. Maximum enzyme activity was reached at pH 4.0 in both concentrations of peptone after 72 h (Figs. 1 and 2).

Effect of pH on enzyme activity: The activities of cellulase at various pH were measured by using carboxymethylcellulose as the substrate. The optimal activity was observed at pH 4.0 in 72 h. Experiments were repeated three times and the mean values were used.

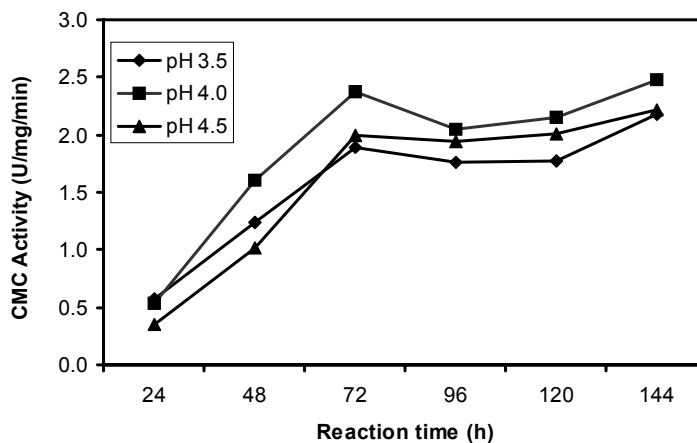


Fig. 1. Effect of 0.1 % pepton concentration on cellulase production

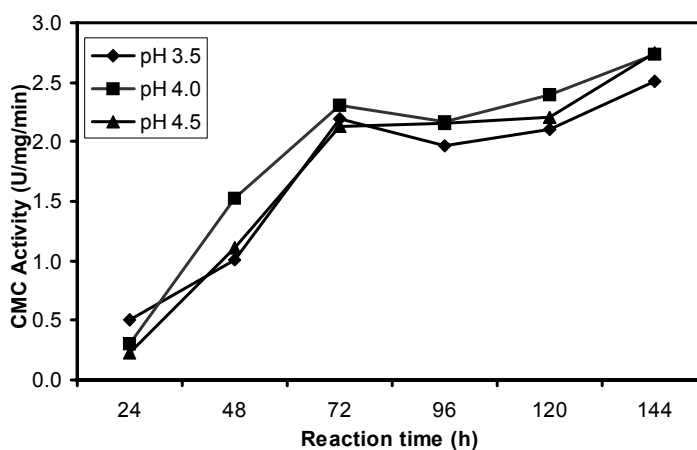


Fig. 2. Effect of 0.05 % peptone concentration on cellulase production

Effects of peptone concentrations on enzyme activity: To investigate the effects of various peptone concentrations (0.05-0.1 %), *Trichoderma reesei* RUT was incubated for 96 h and samples were analyzed at 24, 48, 72 and 96 h. Total activity and specific activity results are given in Tables 1 and 2.

Cellulase was isolated from *Trichoderma reesei* RUT in liquid basal medium containing peptone (0.05-0.1 % w/v). The peptone concentrations which gave maximum cellulase production were then studied at different periods in order to find its optimum level in the medium. The results showed

TABLE-1
EFFECT OF 0.05 % PEPTON CONTAINING MEDIUM ON CELLULASE
PRODUCTION FROM *Trichoderma reesei* RUT

Time (h)	Total activity (U)			Protein (mg/mL)			Total protein (mg)			Specific activity (U/mg)		
	pH 3.5	pH 4.0	pH 4.5	pH 3.5	pH 4.0	pH 4.5	pH 3.5	pH 4.0	pH 4.5	pH 3.5	pH 4.0	pH 4.5
24	125.10	74.43	56.50	1.95	1.90	1.95	477.64	465.36	477.21	0.26	0.16	0.12
48	250.62	382.27	278.38	2.02	1.88	1.91	485.31	450.05	459.18	0.52	0.85	0.61
72	549.35	577.67	533.27	2.19	1.92	1.80	513.78	452.05	424.02	1.07	1.28	1.26
96	491.71	542.52	539.25	2.39	1.95	1.98	548.96	449.59	456.34	0.90	1.21	1.18
120	526.44	599.30	552.62	2.46	2.00	2.07	553.75	450.31	466.25	0.95	1.27	1.19
144	627.63	683.56	682.26	2.86	2.26	2.53	628.52	497.72	555.89	1.00	1.37	1.23

TABLE-2
EFFECT OF 0.1 % PEPTONE CONTAINING MEDIUM ON CELLULASE
PRODUCTION FROM *Trichoderma reesei* RUT

Time (h)	Total activity (U)			Protein (mg/mL)			Total protein (mg)			Specific activity (U/mg)		
	pH 3.5	pH 4.0	pH 4.5	pH 3.5	pH 4.0	pH 4.5	pH 3.5	pH 4.0	pH 4.5	pH 3.5	pH 4.0	pH 4.5
24	142.46	134.63	89.66	2.46	2.69	2.64	601.70	659.29	646.16	0.24	0.20	0.14
48	309.12	401.48	255.60	2.36	2.68	2.63	567.02	643.35	630.49	0.55	0.62	0.41
72	472.64	593.33	492.83	2.43	2.57	2.48	570.24	603.54	582.42	0.83	0.98	0.85
96	441.05	511.92	485.59	2.19	2.71	2.80	504.44	623.30	643.57	0.87	0.82	0.75
120	444.32	539.39	500.96	2.42	2.61	2.70	544.03	587.19	608.58	0.82	0.92	0.82
144	544.37	618.80	553.34	2.67	2.81	3.03	587.45	617.49	665.78	0.93	1.00	0.83

that maximum total activity was observed in 0.1 % peptone containing medium at pH 4.0 (593.33 U) at 72 h. But maximum total activity was observed in 0.05 % peptone containing medium (577.67 U) at 72 h.

Thousands of microorganisms have the ability to grown on cellulose. Many of them grow quite rapidly, but only few produce extracellular cellulose that is capable of converting the native crystalline cellulose to sugar *in vitro*¹³. *Trichoderma* strains, particularly *Trichoderma reesei* RUT is the excellent sources of cellulase suitable for practical applications. Cellulase is an inducible enzyme in *Trichoderma*, with highest yields obtained when the fungus is grown on cellulose rich medium.

According to results, which were obtained from different peptone concentrations that were used for cellulase production in *Trichoderma reesei* RUT, maximum total activity was observed in 0.1 % peptone containing medium at pH 4.0 at 72 h. Krishna *et al.*⁸ found that the peptone was an

excellent additive an optimum concentration of 0.075-0.1 %. Maximum specific activity was observed as 0.92 U/mg 0.1 % peptone containing medium, at pH 4.0 at 72 h but it was 1.20 U/mg in 0.05 % peptone containing medium. Similarly, the optimum pH for cellulase production was found 4.0 by Kalogeris *et al.*¹⁴. These results are in agreement with these reported in the literature confirming that filamentous fungi prefer acidic environments with optima at pH 3.8-5¹⁵.

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