



Optimization of Cellulose Production by *Curvularia pallescens* Isolated from Textile Effluent

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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Short Communication

ABSTRACT

Introduction: Celluloses are important industrial enzymes and find application in several industrial processes. Effects of pH, temperature, incubation time, source of carbon and nitrogen were tested in submerged fermentation process in the production of cellulose by *Curvularia pallescens* isolated from textile effluent.

Aims: The present study was attempted in a fungus; *Curvularia pallescens* isolated from textile effluent for maximizing its production under optimal conditions in submerged fermentation by using inexpensive substrate wheat bran.

Study Design: The production medium was prepared in distilled water, supplemented with 4.5% wheat bran, 0.05% KCl, 0.2% KH₂PO₄, (carbon source), yeast extract (nitrogen source), maintained with pH of 5.5 and incubated at 28°C for 120 h was found optimal for the production of cellulose.

Results: The test fungus achieved maximum FPA activity followed by cellobiohydrolase, endoglucanase and β -glucosidase activity at 46.76, 42.06, 26.94 and 3.56 U/ml respectively at pH 5.5 (Fig. 4). The temperature of 28°C produced maximum cellulase activity. Highest activity recorded was of FPA (38.94 U/ml), followed by cellobiohydrolase (30.29 U/ml), endoglucanase (22.41 U/ml), and β -glucosidase (3.98 U/ml). The effect of process parameters such as the effect of temperature, pH and inoculum size was investigated. Maximum cellulase and xylanase having an enzyme activity of 694.45 and 931.25 IU, respectively, were produced at 30°C incubation temperature.

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Conclusion: The effect of process parameters such as effect of temperature, pH and inoculum size was also investigated. The production of primary metabolites by microorganisms is highly influenced by their growth, which is determined by the availability of the nutrients in the substrates.

Keywords: Cellulase; *Curvularia pallescens*; textile effluent; submerged fermentation; wheat bran.

1. INTRODUCTION

Cellulases are important industrial enzymes and find application in several industrial processes [1]. Currently, the most important application is the bio-bleaching of pulp and, the production of dissolving pulp, the treatment of wastewater. The cost of production and low yields of these enzymes are the major problems for industrial application. Therefore, investigations on the ability of the cellulose hydrolyzing microbial strains to utilize inexpensive substrate have been done [1,2]. The enzyme is commercially used after extracting from many microorganisms especially fungal sources [3,1] of mostly terrestrial origin but less from marine sources.

Therefore, in the present study, the enzyme production was attempted in a fungus, *Curvularia pallescens* isolated from textile effluent for maximizing its production under optimal conditions in submerged fermentation by using inexpensive substrate wheat bran.

2. MATERIALS AND METHODS

2.1 Organism and Culture Condition

Curvularia pallescens was isolated from textile effluent using serial dilution and spread plate method [4].

All the enzyme production studies were carried out under submerged conditions in a medium contain wheat bran 4.5%, yeast extract 1.5%, glucose 1%, NH_4Cl 0.25%, Thiamine dichloride 0.05%, KH_2PO_4 0.2%, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05%, CaCl_2 0.01%, KCl 0.05%. Ten (10) agar plugs of 8 mm diameter of the fungus grown for 7 days on PDA culture plates were inoculated in 100 ml of the medium. The flasks were incubated at 28°C under shaker conditions at 120 rpm. Cultures were harvested on 5th day and assayed for cellulase activity.

2.2 Optimization of the Medium

Standardization of the optimum condition for the growth of the isolated organism as well as for

cellulase production was determined by varying temperature, and initial pH of the medium, carbon and nitrogen sources, inoculum size, incubation period, mechanical shaking with different speed during incubation.

2.3 Cellulase Assay

The test fungus was assayed for total cellulolytic activity by filter paper assay (FPA) [5]; endoglucanase (Cx) activity by carboxymethyl assay (CMC), cellobiohydrolase (C1) activity by cotton assay and β -glucosidase activity by using p-nitrophenyl- β -D-pyranoglucosidase (PNPG) method [6].

1 unit of FPA, CMCase and cotton activity was defined as the amount of enzyme that releases 1 micromole of glucose from the substrate per minute and 1 unit of β -glucosidase was defined as the amount of enzyme required to liberate 1 micromole of 4-nitrophenol per minute.

3. RESULTS

Glucose favoured cellobiohydrolase and endoglucanase activity in *C. pallescens* (30.35 and 21.24 U/ml respectively) whereas sucrose and fructose proved to be best for FPA (61.35 U/ml) activity and β -glucosidase (6.97 U/ml) activity respectively (Fig. 1).

Organic nitrogen sources used for optimization were peptone, malt and yeast. *C. pallescens* showed maximum FPA activity 38.59 U/ml, cellobiohydrolase activity 30.35 U/ml, β -glucosidase activity 3.08 U/ml in the presence of yeast whereas endoglucanase activity 33.71 U/ml reported higher with malt extract (Fig. 2).

$(\text{NH}_4)_2\text{SO}_4$ was reported as a best inorganic nitrogen source for cellobiohydrolase, endoglucanase and β -glucosidase activities at 57.18, 56.82 and 6.77 U/ml respectively. FPA activity was shown highest at 87.59 U/ml with NaNO_3 (Fig. 3).

The test fungus achieved maximum FPA activity followed by cellobiohydrolase, endoglucanase

and β -glucosidase activity at 46.76, 42.06, 26.94 and 3.56 U/ml respectively at pH 5.5 (Fig. 4). The temperature of 28°C produced maximum cellulase activity. Highest activity recorded was

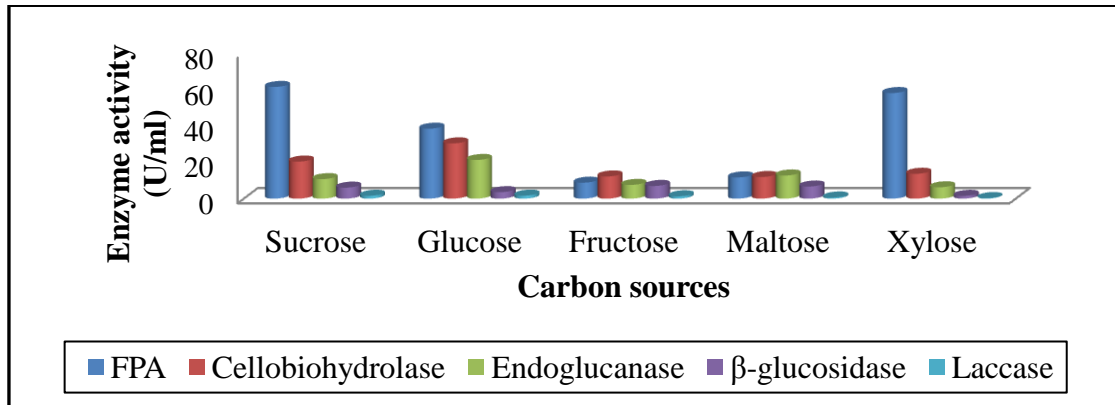


Fig. 1. Optimization of carbon source for lignocellulases production by *Curvularia pallescens*

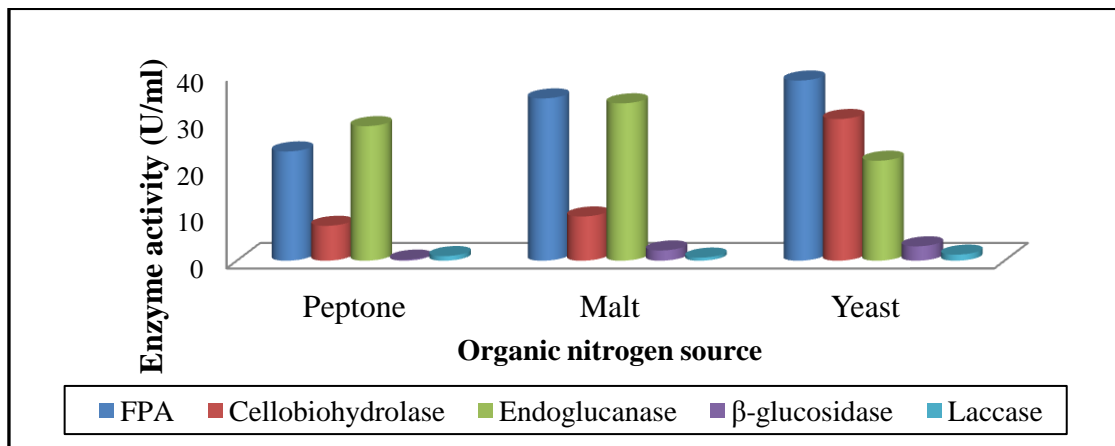


Fig. 2. Optimization of nitrogen source (organic) for lignocellulases production by *Curvularia pallescens*

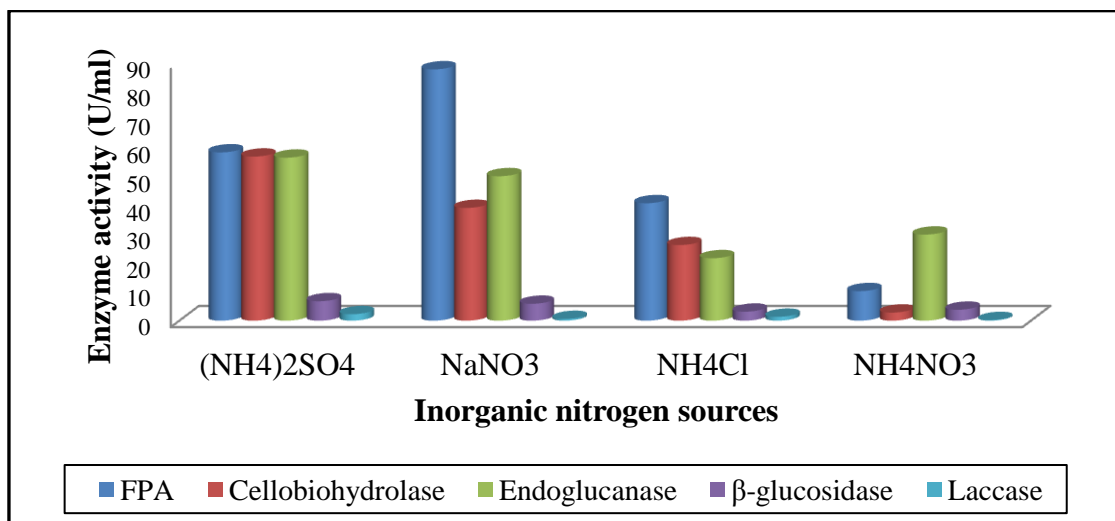


Fig. 3. Optimization of nitrogen source (inorganic) for lignocellulases production by *Curvularia pallescens*

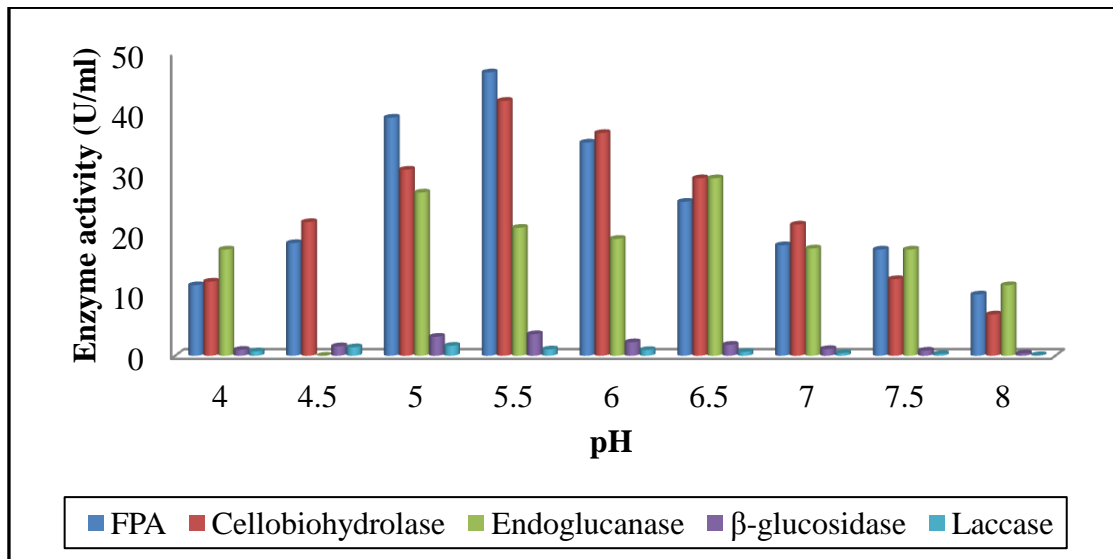


Fig. 4. Optimization of pH for lignocellulases production by *Curvularia pallescens*

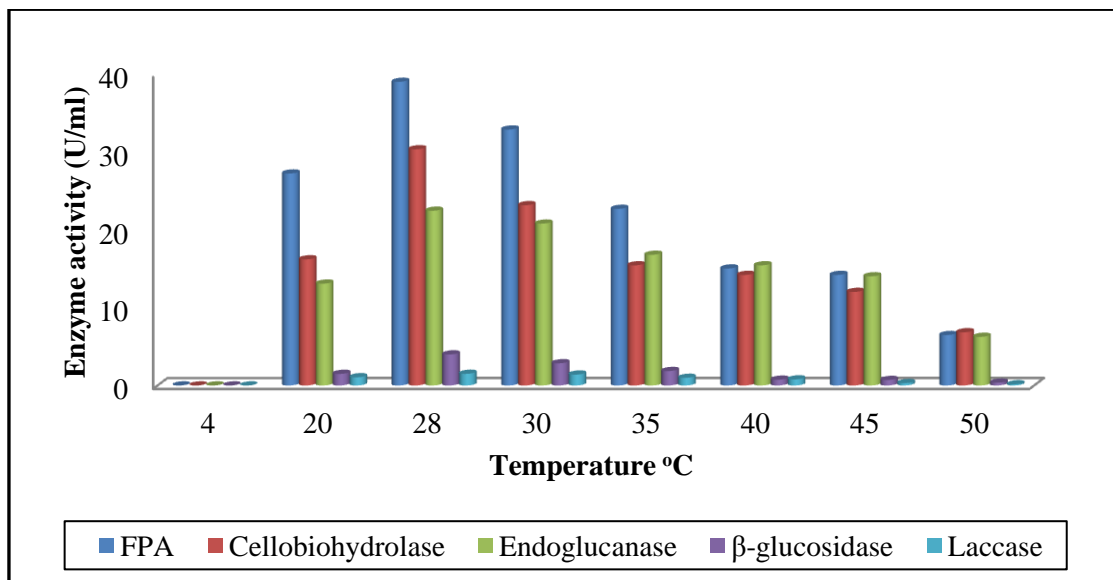


Fig. 5. Optimization of temperature for lignocellulases production by *Curvularia pallescens*

of FPA (38.94 U/ml), followed by cellobiohydrolase (30.29 U/ml), endoglucanase (22.41 U/ml), and β -glucosidase (3.98 U/ml) (Fig. 5). FPA activity 38.65 respectively was obtained maximum for *C. pallescens* after 168 hrs whereas cellobiohydrolase, endoglucanase and β -glucosidase activities 40.29, 57.41 and 2.98 U/ml respectively were recorded highest at 120 hrs of incubation (Fig. 6).

Media containing various amounts of inocula were used for studying the effect of inoculum size on lignocellulolytic activity. Results are shown in Fig. 7. Reported maximum FPA,

Cellobiohydrolase, endoglucanase and β -glucosidase activities 37.94, 30.01, 22.24 and 3.98 U/ml by inoculation 10 disc of 8 mm size in the production medium. *C. pallescens* also gave maximum cellulase production at 120 rpm. Endoglucanase activity was observed highest followed by FPA, cellobiohydrolase and β -glucosidase activities as 38.59, 30.35, 27.41 and 1.91 U/ml respectively (Fig. 8).

4. DISCUSSION

Medium optimization is an important aspect to be considered in the development of fermentation

technology. The production of primary metabolites by microorganisms is highly influenced by their growth, which is determined by the availability of the nutrients in the substrates. Garcia et al. [7] reported that submerged fermentation for aerobic microorganisms is a well known and widely used method for the production of cellulase and xylanase. Chellapandi and Jani [8] reported enhanced endoglucanase production by soil isolates of *Fusarium* sp. and *Aspergillus* sp. through the submerged fermentation process. Papinutti and Lechner [9] studied the influence of the carbon source on the growth and

lignocellulolytic enzyme production by *Morchella esculenta*. Arora and Sehgal [10] reported the production of cellulase and xylanase by *Scopulariopsis acremonium* through submerged fermentation using shake flask cultivation media. The effect of process parameters such as the effect of temperature, pH and inoculum size was investigated. Maximum cellulase and xylanase having an enzyme activity of 694.45 and 931.25 IU, respectively, were produced at 30°C incubation temperature. The pH optimum to achieve these enzyme activities was 5.5 with an inoculums size of 1×10^5 spores ml^{-1} of tween – 80.

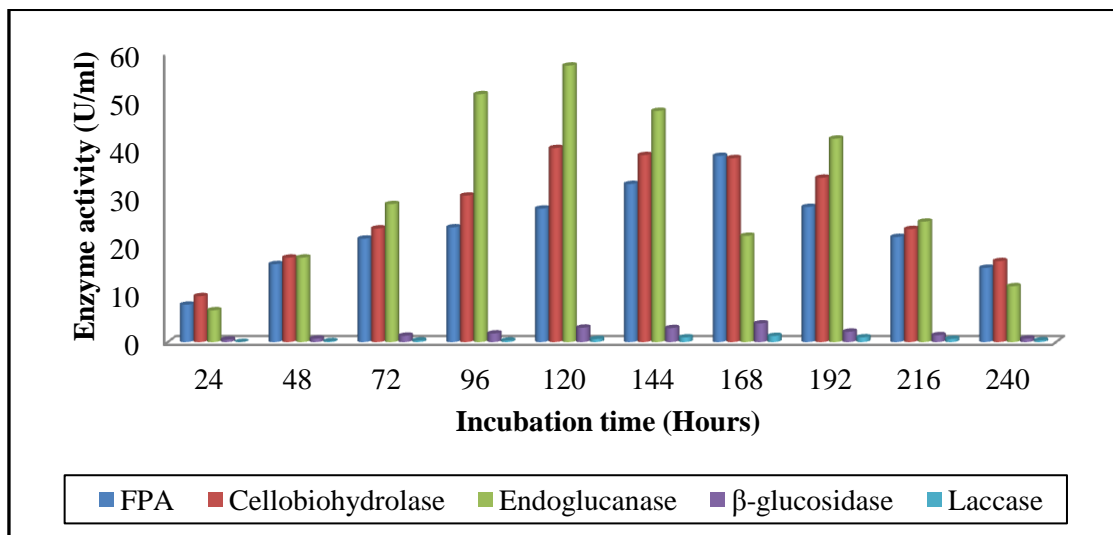


Fig. 6. Optimization of incubation time for lignocellulases production by *Curvularia pallescens*

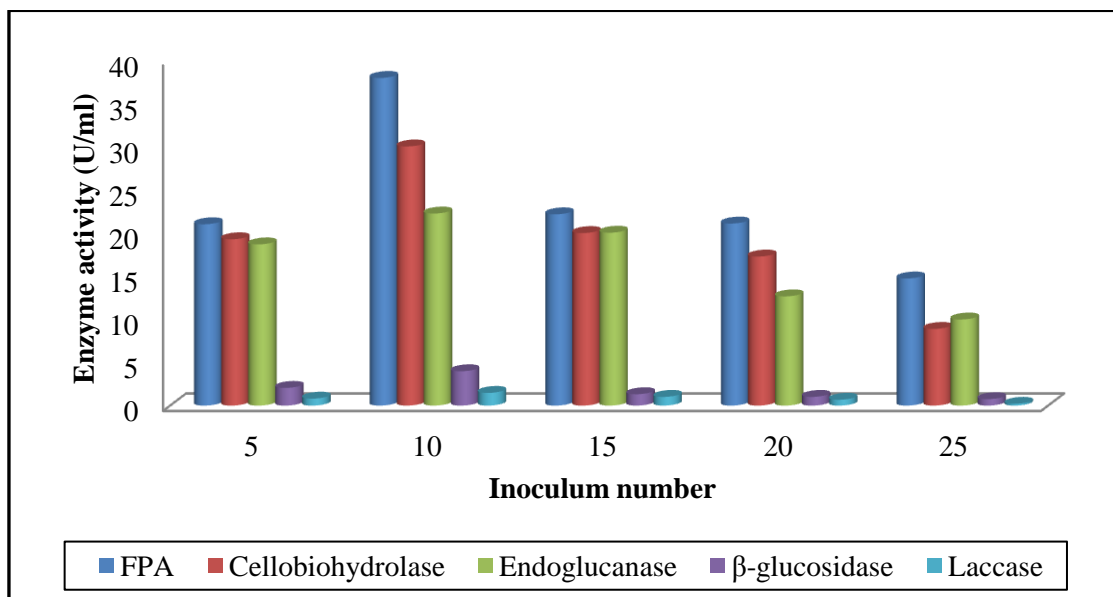


Fig. 7. Optimization of inoculum size for lignocellulases production by *Curvularia pallescens*

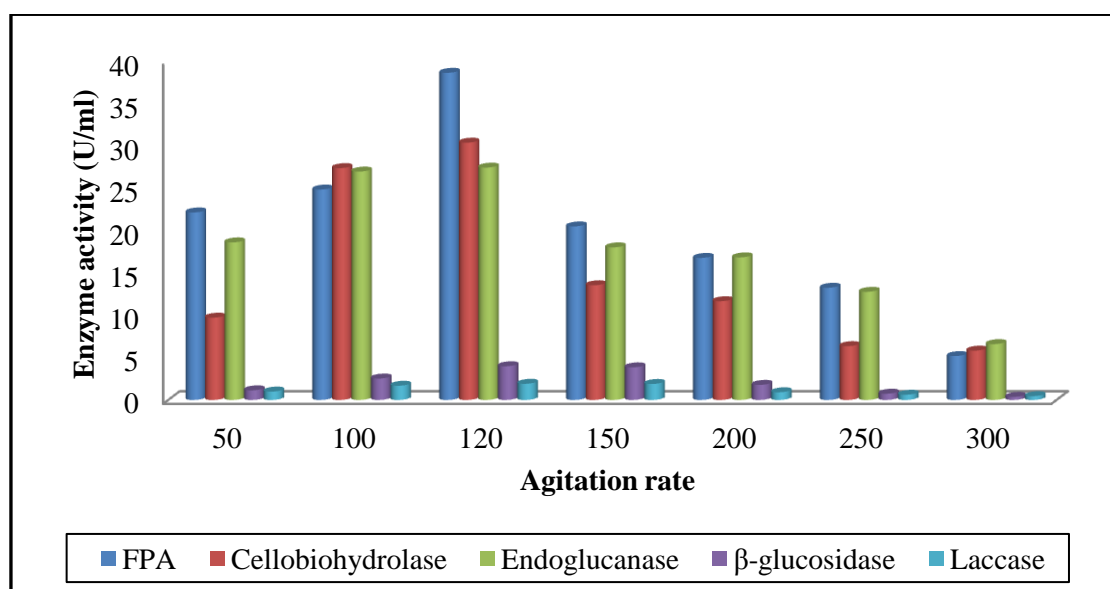


Fig. 8. Optimization of agitation rate for lignocellulases production by *Curvularia pallescens*

Gupta et al. [11] studied microbial proteins and cellulase production from cellulosic materials by *Coprinus atramentarius* and reported the optimum pH for protein production and extracellular enzymes (cellulase and xylanase) by *C. atramentarius*, utilizing cellulose to be 6 and optimum temperature 30°C. The resulting enzyme activities were endoxylanase as 7.2 IU ml⁻¹, exoglucanase as 1.0 IU ml⁻¹ and xylanase as 5 IU ml⁻¹. Li et al. [12] reported pH of 4.14 was reported to be optimum for the production of endoxylanase production by *Aspergillus awamori* under submerged fermentation which gave an enzyme activity of 28.25 U ml⁻¹.

Shear stress within the medium, which is directly related to the stirrer speed, has also been shown to have a marked influence on enzyme production by *Thermomyces lanuginosus* SSBP [13,14]. Acharya et al. [15] reported maximum cellulase production by *Aspergillus niger* in submerged fermentation at 120 rpm. However, Ojumu et al. [16] observed maximum cellulase production by *Aspergillus flavus* Linn isolate NSPR 101 at the agitation of rate 200 rpm.

5. CONCLUSION

Effects of pH, temperature, incubation time, source of carbon and nitrogen were investigated in the submerged fermentation process in the production of cellulose by *Curvularia pallescens* isolated from textile effluent. The effect of process parameters such as the effect of temperature, pH and inoculum size was also

investigated. The production of primary metabolites by microorganisms is highly influenced by their growth, which is determined by the availability of the nutrients in the substrates.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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