Optimization and Production of Industrial Important Cellulase Enzyme from *Penicillium citrinum* in Western Ghats of Sathuragiri Hills Soil Sample Isolate

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Abstract In optimization of cellulase production by different pH, substrates, nitrogen, carbon sources were detected from *penicillium citrinum* micro-fungal species. The maximum cellulase activity was recorded in the range of pH- 5 to 6.5 and wheat bran (91 U/ml) and rice bran (81 U/ml) is the best raw substrate for the production of cellulase. Estimation of extracellular protein was recorded in different production medium of *Penicillium citrinum* (1.63 mg/ml) for maltose followed by sucrose (1.46 mg/ml), fructose (1.13 mg/ml), wheat bran (1.12 mg/ml), peptone (0.88 mg/ml) and yeast extract (0.82 mg/ml). The highest extracellular protein was recorded in pH 5.5 to 6.5 followed by substrate for wheat bran (1.12 mg/ml), rice bran (1.04 mg/ml), paddy straw (1.03 mg/ml). In nitrogen sources, ammonium sulphate (141 U/ml) and ammonium nitrate (121 U/ml) is the best nitrogen source for the production of cellulase enzyme. The maximum cellulase activity was recorded in glucose (134 U/ml) and maltose (159 U/ml) was best carbon source for the production of cellulase.

Keywords Cellulase, *Penicillium citrinum*, Optimization, Micro-fungal Enzyme

suited for production of higher level of extracellular cellulases. Among microorganisms, fungi are of great interest for enzyme production because they excrete their enzymes extracellularly [2]. Today, the new potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated renewed interest in the exploration of extracellular enzymatic activity in several microorganisms [3]. Selection of the right organism plays a key role in high yield of desirable enzymes [4]. Fungi are microorganisms which are well known for their wide range of novelty of enzymes they produce and enzymes of fungal origin are used in the industrial process for which, amount to billions of dollars of revenue annually [5].

The cellulolytic system of aerobic fungi and bacteria consists of three types of cellulases such as Endoglucanases, Exoglucanases and B- Glucosidases. Cellulase is a consortium of hydrolytic enzyme capable of hydrolysing cellulose in to simple glucose units. Several studies were carried out for the isolation and identification of the cellulolytic fungi possessing activity [6, 7]. The ability of cellulolytic microorganisms to degrade cellulose varies greatly with the physico-chemical characteristics of the substrate, such as: (a) the size and permeability of cellulolytic enzymes and other reagent molecules, which are involved in relation to the size and surface properties of the grown fibrils and the space between microfibrils and cellulose molecules from amorphous region; (b) the degree of crystallinity of cellulose; (c) the unit cell dimensions of cellulose; (d) the stereoscopic conformation and rigidity of the anhydroglucose units; (e) the degree of polymerization of cellulose molecules; (f) the nature of components with which cellulose is associated; (g) the nature, concentration and distribution of substituted groups [8]. The crystallinity degree of cellulose is one of the most important structural parameters which affect the rate of enzymatic degradation by hydrolysis. Therefore, the rate of degradation should be a function of the surface properties of cellulose which makes

1. Introduction

Microorganisms play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi. Fungi play an important role in bioconversion and composting of organic waste and can be an important contributor to optimal agricultural and forest waste bioconversion [1]. In biological processes, aerobic microorganism biodegrade complex cellulose solid waste into simple compound such as C, N, H and mineral salts. The ability to secrete large amount of extracellular protein is a characteristics of certain fungi and such strains are most

possible the access of enzymes to polymeric molecules [9].

Cellulolytic enzymes play an important role in natural biodegradation processes in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi, bacteria, actinomycetes and protozoa. In industry, these enzymes have found novel applications in the production of fermentable sugars and ethanol [10, 11, and 12], organic acids [13] detergents and other chemicals [14]. They have been used in the pulp and paper industry, e. g., in deinking of fiber surfaces and in improving pulp drainage [15, 16] in the textile industry [17, 18] animal feed and even in the food industry [19, 20] for the processing of paper and cellophane, as well as for biotransformation of waste cellulose to fermentable sugars [21]. As lytic enzymes, they are of prime importance and are the protoplast production [22]. The demand for more thermostable, highly active and specific cellulases is on the increase. Fungi are well-known agents of decomposition of organic matter in general and cellulose substrates in particular [23]. Fungal cellulases are inducible enzymes that are usually excreted into the environment and depend on cellulose type (amorphous or crystalline) acting on the organism [24]. The objects of the present work is to identify the fungal strains *Penicillium citrinum* have potential for cellulose production, and compare enzyme production during varying incubation time and different substrate, carbon and nitrogen source by submerge fermentation technology.

2. Materials and Methods

Sampling Site

The leaf litter soil samples were collected out in Sathuragiri Hills (Western Ghats, Tamil Nadu) which is about 15 Kms from Srivilliputhur. The town is situated on latitude 9.7273° North and longitude 77.6186° East. The town is about 4500 feet above the sea level.

Sample Collection

Soil and litter samples were collected in Sathuragiri Hills in 12 different locations (Western Ghats, Tamil Nadu). Using a sterilized spatula, 50g of soil sample was collected from each location using sterile zip cover. The samples from the 12 different sites were conveyed to the Microbiology Laboratory within same day, where they were analyzed.

Isolation from Serial Dilution Technique

Soil and litter sample was mixed thoroughly in 99 ml of sterile water in a glass tube and shaken thoroughly. From this initial suspension, serial dilutions were prepared. One ml of the required dilution (1/1000) was pipette into five replicate plates containing potato dextrose agar medium with antibiotic. The plates were incubated at room temperature in

glass chambers under aseptic conditions for 4 days and then examined for fungal growth.

Morphological Identification of Fungi

Lactophenol and Lactophenol cotton blue stain (Hi Media laboratories private limited) were used as the staining solution. Slides prepared were sealed with DPX mountant. Identification of the fungal species was done using “The Genera of Hyphomycetes from soil” by [25] Compendium of soil fungi by [26].

Screening of Cellulase Enzymes

The fungal isolates were screened for cellulase activity was carried out on agar media in petri plates containing the respective substrates. The following substrate was used, for cellulase production 1% carboxy methyl cellulose (CMC) and supplemented with 1% yeast extract and malt extract, agar-2g. The cultures were spot inoculated at the center of the plate and incubated at 28° C for a period of 5 days to get good growth of the cultures. For cellulase, the plates were flooded with an aqueous solution of 0.1% Congo red. After 30 minutes, the plates were repeatedly washed with 1 M sodium chloride and zone of clearance around their growing margin indicated cellulase activity [27].

Production Medium of Cellulase Enzyme

To prepare the production medium of cellulase using CMC (Carboxy methyl cellulase)- 1g, yeast extract- 1g, glucose- 1g and autoclaved to add basal mineral salt medium. Then 5-7 days fungal culture was inoculated into the all flask. The flasks were incubated at 28°c in a shaker for 7 days [28].

Quantitative Assay of Cellulase

Cellulase activity was measured by the DNS (3, 5-dinitrosalicylic acid) method [29] through the determination of the amount of reducing sugars liberated. Glucose was used as a standard; 0.5 ml of extract was reacted with phosphate buffer (pH 5). Reaction was stopped by the addition of DNS solution. The reaction mixture was boiled for 10 min, cooled in water for color stabilization, and the optical density was measured at 540 nm.

Estimation of Protein by Lowry's Method

One ml of partially purified protein solution was taken in a test tubes and 2 ml of alkaline copper sulphate reagent (analytical reagent) was added, mixed and incubate at room temperature for 10 mins. Then 0.2 ml of reagent FolinCiocalteaus solution (reagent solutions) was added to each tube and incubated for 30 min. Zero the UV -spectrometer with blank and take the optical density (measure the absorbance) at 660nm. The absorbance plotted

against protein concentration to get a standard calibration curve. By using the absorbance of unknown sample, the concentrations of the unknown sample were determined using Bovine serum albumin (BSA) standard curve [30].

Optimization of Cellulase Production by Different Carbon Sources

The production medium of cellulase using various carbon sources namely, fructose, glucose, sucrose, lactose and maltose used for optimized the production rate. The broth contained 1.0% of each carbon sources, 1% CMC (carboxy methyl cellulose) and was autoclaved to add basal mineral salt medium. The fungal cultures were inoculated into all flasks. The flasks were incubated at 7 days for 28°C in a shaker [32].

Optimization of Cellulase Enzyme Production by Different Nitrogen Sources

The production medium of cellulase using various nitrogen sources namely, ammonium nitrate, ammonium sulphate, peptone, yeast extract and sodium nitrate used for optimized the production rate. The broth contained 1.0% of each nitrogen sources, 1% CMC (carboxy methyl cellulose) and was autoclaved to add basal mineral salt medium. The fungal cultures were inoculated into all flasks. The flasks were incubated at 7 days for 28°C in a shaker at 200 rpm [32].

Optimization of Cellulase Enzyme Production by Different pH

The production medium of cellulase using substrate 1% CMC (carboxy methyl cellulose) with carbon source and nitrogen sources were added to optimized the production rate. Each flask was adjusted to different pH such as 5.0, 5.5, 6.0, 6.5, 7.0 using 0.1 N NaOH and 0.1 N HCL. Then add autoclaved basal mineral salt medium. The fungal cultures were inoculated into all flasks. The flasks were incubated up to 7 days for 28°C in a shaker at 200 rpm [32].

Optimization of Cellulase Enzyme Production by Different Substrate

The production medium of cellulase using different substrate namely, wheat bran, rice bran, paddy straw, paddy husk and crud paper used for optimized the production rate. The broth contained 1.0% of each substrate, and suitable carbon and nitrogen sources were added. Then add autoclaved basal mineral salt medium. Fungal cultures were inoculated into all flasks. The flasks were incubated for 7 days at 28°C in a shaker at 200 rpm [32].

3. Results

Micro-fungal Species Isolate and Cellulase Screening

The micro-fungal species *Fusarium oxysporum*, *Curvularia lunata*, *Alternaria alternata*, *Aspergillus niger*, *Cladosporium cladosporioides*, *Penicillium citrinum*, *Aspergillus nidulans* were recorded in Potato dextrose Agar medium but screening of cellulase production in carboxymethyl cellulose plate assay medium in highest zone of clearance, were recorded in after floated with Congo red solution in 4th day of micro-fungal culture plate of *Penicillium citrinum* (fig-1, 2). Further cellulase assays were carried out by selected micro-fungal species *Penicillium citrinum*.

Figure 1. PDA Plate isolation of *Penicillium citrinum*

Figure 2. *Penicillium citrinum* Microscopic observation

Cellulase Activity of *Penicillium citrinum* in Different Carbon Sources

The fungal cellulase activity was recorded in 3rd, 5th and 7th day of fungal culture broth and the highest fungal cellulase activity was recorded in 5th day. The maximum cellulase activity was recorded in 5th day maltose (159 U/ml) followed by glucose (134 U/ml), sucrose (123 U/ml), fructose (95 U/ml) and lactose (83 U/ml). Similarly maximum cellulase activity was recorded in 3rd day glucose (78 U/ml) followed by maltose (70 U/ml), sucrose (65 U/ml), fructose (52 U/ml) and lactose (49 U/ml). Minimum cellulase activity was recorded in 7th day glucose (54 U/ml) followed by sucrose (52 U/ml), maltose (51 U/ml), fructose (46 U/ml) and lactose (37 U/ml) (Fig -3).

Extra Cellular Protein Estimation of *Penicillium citrinum* for Different Carbon Sources

The estimation of extra cellular protein were recorded in 3rd, 5th and 7th day of fungal culture broth and the highest extra cellular protein were recorded in 5th day. The maximum amount of extra cellular protein were recorded in 5th day maltose (1.63 mg/ml) followed by glucose (1.58 mg/ml), sucrose (1.46 mg/ml), fructose (1.13 mg/ml) and lactose (1.03 mg/ml). Similarly maximum amount of extra cellular protein were recorded in 3rd day glucose (0.97 mg/ml) followed by maltose (0.92 mg/ml), sucrose (0.89 mg/ml), fructose (0.71 mg/ml) and lactose (0.65 mg/ml). The minimum amount of extra cellular protein were recorded in 7th day glucose (0.78 mg/ml) followed maltose (0.72 mg/ml), sucrose (0.63 mg/ml), fructose (0.62 mg/ml) and lactose (0.45 mg/ml) (Fig -4).

Figure 3. Cellulase activity of *Penicillium citrinum* in different carbon sources

Figure 4. Extra cellular protein in different carbon sources for *Penicillium citrinum*

Cellulase Activity of *Penicillium citrinum* in Different Nitrogen Sources

The fungal cellulase activity was recorded in 3rd, 5th and 7th day of fungal culture broth and the highest fungal cellulase activity was recorded in 5th day. The maximum cellulase activity was recorded in 5th day ammonium sulphate (141 U/ml) followed by ammonium nitrate (121 U/ml), peptone (112 U/ml), yeast extract (98 U/ml) and sodium nitrate (72 U/ml). Similarly maximum cellulase activity was recorded in 3rd day peptone (78 U/ml) followed by ammonium sulphate (71 U/ml), yeast extract (69 U/ml), ammonium nitrate (68 U/ml) and sodium nitrate (54 U/ml). Minimum cellulase activity was recorded in 7th day ammonium nitrate (64 U/ml) followed by ammonium sulphate (59 U/ml), peptone (58 U/ml), yeast extract (49 U/ml) and sodium nitrate (41 U/ml) (Fig -5).

Extra Cellular Protein Estimation of *Penicillium citrinum* for Different Nitrogen Sources

The estimation of extra cellular protein were recorded in 3rd, 5th and 7th day of fungal culture broth and the highest extra cellular protein were recorded in 5th day. The maximum amount of extra cellular protein were recorded in 5th day peptone (0.88 mg/ml), yeast extract (0.82 mg/ml), ammonium sulphate (0.78 mg/ml), ammonium nitrate (0.64 mg/ml) and sodium nitrate (0.64 mg/ml). Similarly the maximum amount of protein was recorded in 3rd day ammonium sulphate (0.61 mg/ml) followed by peptone (0.59 mg/ml), yeast extract (0.56 mg/ml), ammonium nitrate (0.55 mg/ml) and sodium nitrate (0.42 mg/ml). The minimum amount of extra cellular protein was recorded in 7th day peptone (0.64 mg/ml) followed by yeast extract (0.55 mg/ml), ammonium sulphate (0.50 mg/ml), sodium nitrate (0.49 mg/ml) and ammonium nitrate (0.37 mg/ml) (Fig -6).

Figure 5. Cellulase activity of *Penicillium citrinum* in different nitrogen sources

Figure 6. Extra cellular protein in different nitrogen sources for *Penicillium citrinum*

Cellulase Activity of *Penicillium citrinum* in Different pH

The fungal cellulase activity was recorded in 3rd, 5th and 7th day of fungal culture broth and the highest fungal cellulase activity was recorded in 5th day. The maximum cellulase activity was recorded in 5th day pH – 6.0 (86 U/ml) followed by pH – 5.5 (71 U/ml), pH – 5.0 (64 U/ml) and pH – 6.5 (62 U/ml) pH – 7.0 (24 U/ml). Similarly maximum cellulase activity was recorded in 3rd day pH – 5.5 (39 U/ml) followed by pH – 6.0 (35 U/ml), pH – 6.5 (35 U/ml), pH – 5.0 (32 U/ml) and pH – 7.0 (24 U/ml). Minimum cellulase activity was recorded in 7th day pH – 6.5 (37 U/ml) followed by pH – 5.5 (36 U/ml), pH – 6.5 (32 U/ml), pH – 5.0 (31 U/ml) and pH – 7.0 (12 U/ml) (Fig -7).

Extra Cellular Protein Estimation of *Penicillium citrinum* for Different pH

The estimation of extra cellular protein were recorded in 3rd, 5th and 7th day of fungal culture broth and the highest extra cellular protein were recorded in 5th day. The maximum amount of extra cellular protein were recorded in 5th day pH – 6.0 (0.88 mg/ml) followed by pH – 6.5 (0.82 mg/ml), pH – 5.5 (0.78 mg/ml), pH – 5.0 (0.64 mg/ml) and pH – 7.0 (0.64 mg/ml). Similarly maximum amount of extra cellular protein were recorded in 3rd day pH – 5.5 (0.61 mg/ml) followed by pH – 6.0 (0.59 mg/ml), pH – 6.5 (0.56 mg/ml), pH – 5.5 (0.55 mg/ml) and pH – 7.0 (0.42 mg/ml). The minimum amount of extra cellular protein were recorded in 7th day pH – 6.0 (0.64 mg/ml) followed by pH – 6.5 (0.55 mg/ml), pH – 5.5 (0.50 mg/ml), pH – 7.0 (0.49 mg/ml) and pH – 5.0 (0.37 mg/ml) (Fig -8).

Figure 7. Cellulase activity of *Penicillium citrinum* in different pH

Figure 8. Extra cellular protein in different pH for *Penicillium citrinum*

Cellulase Activity of *Penicillium citrinum* in Different Substrate

The fungal cellulase activity was recorded in 3rd, 5th and 7th day of fungal culture broth and the highest fungal cellulase activity was recorded in 5th day. The maximum cellulase activity was recorded in 5th day wheat bran (91 U/ml) followed by rice bran (88 U/ml), paddy straw (86 U/ml), paddy husk (71 U/ml), and crude paper (68 U/ml). Similarly maximum result was recorded in 3rd day wheat bran (52 U/ml) followed by rice bran (49 U/ml), paddy straw (48 U/ml), paddy husk (41 U/ml) and crude paper (39 U/ml). Minimum cellulase activity was recorded in 7th day wheat bran (42 U/ml) followed by rice bran (40 U/ml), paddy husk (38 U/ml), paddy straw (37 U/ml) crude paper (36 U/ml) (Fig -9).

Extra Cellular Protein Estimation of *Penicillium citrinum* for different Substrates

The estimation of extra cellular protein were recorded in 3rd, 5th and 7th day of fungal culture broth and the highest extra cellular protein were recorded in 5th day. The maximum amount of extra cellular protein were recorded in 5th day wheat bran (1.12 mg/ml) followed by rice bran (1.04 mg/ml), paddy straw (1.03 mg/ml), paddy husk (0.93 mg/ml) and crude paper (0.89 mg/ml). Similarly maximum amount of extra cellular protein were recorded in 3rd day wheat bran (0.71 mg/ml) followed by rice bran (0.68 mg/ml), paddy straw (0.62 mg/ml), crude paper (0.57 mg/ml) and paddy husk (0.54 mg/ml). The minimum amount of extra cellular protein were recorded in 7th day rice bran (0.62 mg/ml) followed by wheat bran (0.59 mg/ml) and paddy husk (0.59 mg/ml), paddy straw (0.53 mg/ml) and crude paper (0.52 mg/ml) (Fig -10).

Figure 9. Cellulase activity of *Penicillium citrinum* in different substrate

Figure 10. Extra cellular protein in different substrate for *Penicillium citrinum*

4. Discussion

Fungi are not only beautiful but also play a significant role in the daily life of human beings besides their utilization in industry, agriculture, medicine, food industry, textiles, bioremediation, natural cycling, as biofertilizers and many other ways. Fungal biotechnology has become an integral part of the human welfare. Microorganism performs their metabolic processes rapidly and with remarkable specificity under ambient conditions, catalyzed by their diverse enzyme-mediated reactions. There is a wide spectrum of microorganisms which can produce the variety of enzymes like cellulase under appropriate conditions [31].

Emoghene [33] reported that cellulolytic fungal isolates *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium citrinum*, *Penicillium chrysogenum* isolated from decayed wood samples. In present study of fungal isolates *Penicillium citrinum*, recorded from Sathuragiri Hills samples. Sonia Sethi and Saksham Gupta [32] were reported that maximum enzyme activity was observed at pH 5.0 for *Penicillium chrysogenum* (0.95 U/ml) and *Aspergillus niger* (0.85 U/ml). Gautam [31] reported that optimum pH for cellulase activities were found at pH 6.5. The enzyme activity was influenced by pH variation and depends on fungal species. Fungal enzyme also noted that the enzyme activity was stable at pH range of 5.0–8.0. Beldman [34] also reported that *Aspergillus* species grow and metabolize well in acidic pH medium between pH 3.0–5.0. Latifian [35] and Wen [36] was reported in the production of most fungal cellulases in the pH range of 4.5–6.0. In present study maximum yield of cellulase enzyme recorded in the range of pH- 5 to 6.5 in *Penicillium citrinum* (86 U/ ml).

Muthuvelayudham and Viruthagiri [37] were reported that Cellulose (78 U/ml), glucose (24 U/ml), xylose (28 U/ml) and lactose (28 U/ml) is a best carbon source for the production of cellulase enzyme by *Trichoderma reesei*. Sonia Sethi and Saksham Gupta [32] were reported that glucose is a best carbon source for the production of cellulase enzyme by *Penicillium* sps (0.60 U/ml) and *Aspergillus niger* (0.52 U/ml). Gautam [31] reported that sucrose is a best carbon sources for the production of cellulose enzyme (exoglucanase (2.68 U/mL), endoglucanase (2.17 U/mL) and β -glucosidase (2.06 U/mL) in *Aspergillus niger* and *Trichoderma* sp. In present study maltose (159 U/ml) and glucose (134 U/ml) are the best carbon sources for the production of cellulase enzyme by *Penicillium citrinum*.

Sonia Sethi and Saksham Gupta [32] were reported that ammonium sulphate is a best nitrogen source for the production of cellulase enzyme by *Aspergillus niger* and *Penicillium citrinum*. Gautam [31] were reported that Peptone (2.40U/ml), Ammonium nitrate (2.28U/ml) and beef extract (1.99U/ml) is a best nitrogen source for the production of cellulase enzyme by *Aspergillus niger*. Sun [38] were reported that beef extract and peptone resulted in increased growth and enzyme production. In present study ammonium sulphate (141 U/ml) and ammonium nitrate (121 U/ml) are

the best nitrogen source for the production of cellulase enzyme by *Penicillium citrinum*.

Muthuvelayudham and Viruthagiri [37] were reported that rice straw (44 U/ml) and sugar cane bagasse (68 U/ml) is a best substrate for the production cellulase enzyme by *Trichoderma reesei*. Padmavathi [39] reported that wheat bran (20 U/ml) and rice bran (36 U/ml) are the best substrate for the production cellulase enzyme by *M. plumbeus*. Sonia Sethi and Saksham Gupta [32] reported that coconut cake, ground nut cake, wheat bran and soy cake is the best substrates for the production of cellulase enzyme by *Aspergillus niger* and *Penicillium chrysogenum*. Ojumu [40] reported that saw dust, rice straw and sugarcane bagasse is a best substrates for the production of cellulase enzyme by *Aspergillus flavus*. Lian Xiong [41] reported that rice straw was produced highest cellulase activity was obtained. Baiget [42] reported that banana waste based medium for production of cellulolytic enzymes by *T. lignorum*. Ananda Muniswaran *et al.*, [43] used banana stalk and coconut coir for production of cellulases by *Aspergillus niger* and *Penicillium* sp. In present study wheat bran (91 U/ml) and rice bran (81 U/ml) is a best substrate for the production of cellulase enzyme by *Penicillium citrinum*.

Mohammed InuwaJa'afaru, [44] reported that *Aspergillus ustus*, *Trichoderma* sp. *Trichoderma* sp. *Aspergillus ustus*, and *Trichoderma viride* activity of cellulose enzyme level were increased simultaneously extra cellular protein level also increased. Muthuvelayudham and Viruthagiri [37] reported that production of extra cellular protein in different carbon source such as Cellulose (9.74 mg/ml), glucose (1.78 mg/ml), xylose (8.02 mg/ml) and lactose (7.98 mg/ml) were recorded in micro-fungal species *Trichoderma reesei*, alteration of carbone sources influence in alteration of extra cellular protein. Nwodo-Chinedu, *et al.*, [45] reported that for extra cellular protein from fungal isolates for *A. flavus* (0.7 mg/ml) *A. niger I* (1.5 mg/ml) *A. niger II* (1.0 mg/ml) *B. cinerea* (1.4 mg/ml) *Penicillium* species (1.0 mg/ml). Similarly in present study extracellular protein is also recorded in *Penicillium citrinum* (1.63 mg/ml) for maltose followed by ammonium sulphate (1.62 mg/ml), yeast extract (1.12 mg/ml) at pH – 6, wheat bran (1.12 mg/ml).

5. Conclusions

Micro-fungal cellulase production rate depends on three important parameters such as pH, carbon, Nitrogen substances, which was used to alter the cellulase production in micro-fungal species. The present study focus on cheapest substrate used to improve the cellulase activity and is very important in industrial scale up of cellulase production rate.

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