Optimization of Cellulase Production by *Chaetomium globosum* 17BDSM Using Solid Phase Fermentation Method

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Abstract. Cellulose is still present in agricultural byproducts. Fungi can use cellulose as a source of carbon and energy as well as a substrate for cellulase production. *Chaetomium globosum* 17BDSM is a fungus with cellulolytic properties. Cellulase activity is influenced by the following factors: pH, temperature, substrate thickness, and substrate type. This study aims to find the optimal substrate thickness, pH, and temperature for *C. globosum* 17BDSM growth in order to produce high-activity cellulase. A factorial Randomised Block Design experimental design was used in this study to treat substrate type, temperature, pH, and substrate thickness. According to the findings, thickness affects cellulase activity; at 1.5 cm of thickness, cellulase activity is 0.154 U/mL. The substrate type affects cellulase activity, notably sugarcane bagasse and corncob, which had cellulase activity values of 0.321 U/mL and 0.040 U/mL, respectively. Both temperature and pH treatments showed no influence on cellulase activity.

Keywords: Cellulose, Cellulase, *Chaetomium globosum*, Optimization.

1 Introduction

Cellulose is a glucose polymer joined by 1-4 glycosidic linkages and is one of the most abundant biopolymers in nature [1]. Cellulose is an important biopolymer in plants that can only be destroyed by cellulase enzymes produced by diverse microbes. The cellulase enzyme slowly converts cellulose into energy and glucose, which bacteria and other creatures can use. Bacteria, actinomycetes, and fungi are examples of microbes that can produce cellulase enzymes [2].

Fungi that degrade cellulose are known as cellulolytic fungi. Many fungi rely on complex carbon sources like cellulose to develop. Fungi can digest these nutrients by producing degrading enzymes and absorbing them as a source of carbon and energy for growth [2, 3]. Agricultural byproducts can be used as a growth substrate for fungi that produce cellulase. There are two techniques for producing enzymes: solid-state fermentation (solid media fermentation) and submerged fermentation (liquid media fermentation). Solid substrates such as rice straw, sugar cane bagasse, and maize cobs are used in solid-state fermentation. Compared to submerged

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fermentation (liquid fermentation), solid-state fermentation has more advantages: the level of productivity is higher, the amount of water wasted is lower, the product is better, the cost is lower, and the substrate is easier to obtain [4, 5].

The fungus *C. globosum* 17BDSM was isolated from forest soil. Previous studies revealed that this fungus strain grew on pure substrate and had cellulolytic properties [6]. Environmental parameters like as pH and temperature have a considerable influence on *C. globosum* 17BDSM cellulase production. Enzymes have an optimal pH that allows them to catalyse a process with maximum activity [7]. At temperatures ranging from 30 to 37°C, enzyme activity increases because the kinetic energy increases, increasing the intensity of the reaction between the substrate and the enzyme and producing more products. The thickness of the substrate will influence fungal growth during cellulase production [8, 9]. Optimisation is a technique to identify the best circumstances for fungus to grow to produce cellulase. Researchers were prompted to investigate the optimisation and production of cellulase by *C. globosum* from sugarcane bagasse, corn cobs and rice straw using solid fermentation due to limited research and the usage of agricultural by-products as substrates in the production of cellulase enzymes.

2. Methodology

2.1. Preparation of biomass by-products of agricultural products

Corn cobs, rice straws, and sugar cane bagasse are dried and crushed into 60 mesh powder. Corn cobs, rice straw, and sugarcane bagasse were prepared in 5% NaOH (w/v) at 90ºC for 90 minutes or room temperature for 12 hours. Sugarcane bagasse preparation was continued in a 5% peracetic acid (PAA) solution at 70ºC for 150 minutes. The prepared biomass was washed with distilled water until the pH was neutral (pH = 7) and dried in an oven at 60ºC. The cellulose content is analyzed first before being used as a substrate for fungal growth [10].

2.2. Experimental design

The research used a factorial Randomised Block Design with substrate type (sugarcane bagasse, rice straw and corn cobs), temperature (room temperature, 37°C), pH (5, 6, 7) and substrate thickness (1 cm, 1.5 cm). There were two repeats of this Randomised Block Design. A single repetition requires 36 samples. The fermentation process uses Mandels & Weber's medium.

2.3. Optimization of production conditions through solid-phase fermentation

Fungal spores (10^6 propagules/g substrate), which had been grown on slanted PDA media for 14 days, were inoculated in Mandels & Weber's medium. Fermentation was carried out for 14 days using the solid surface method [11].

2.4. Crude enzyme extract

Extraction of enzymes from fermentation results was carried out by adding 50 mL of cold, sterile distilled water (4ºC) to each treatment, incubating in a shaker incubator at 150 rpm for 30 minutes at room temperature. The supernatant obtained was used as a source of crude enzymes for analysis [12].

2.5. Cellulolytic activity using the Filter Paper Assay method

Cellulase activity was determined using the DNS method with Whatman paper no. 1. as a substrate measuring 1 x 6 cm. 1 mL of 0.05M Na-citrate pH 4.8 buffer was mixed with 0.5 mL of enzyme crude extract dissolved in the same buffer. Whatman paper no. 1 was placed in a

mixture of crude enzyme extract and buffer, then incubated at 50°C for 60 minutes. The reaction was stopped by adding 3 mL of DNS solution, boiling at 100°C for 5 minutes, and then cooling on ice. Absorbance was measured at λ=540 nm. The absorbance results obtained were compared with the standard absorbance results using a standard curve. Glucose was used as a standard. One unit (U) of cellulase is defined as the amount of enzyme that can liberate 1 µmol glucose per minute at 50 °C [13].

2.6. Data analysis

The cellulolytic activity data was then statistically analysed using the Analysis of Variance (ANOVA) method at a significance level of 5%. If the results show a significant difference, a further test using the Duncan Multiple Range Test (DMRT) is carried out.

3. Results and Discussion

3.1. Cellulose Content in Sugarcane Bagasse, Rice Straw, and Corn Cobs

Based on the results of the analysis of cellulose content using the Van Soest method, the results shown in Table 3.1. were obtained as follows:

Table 3.1. Cellulose content on various substrate

No	Substrate	Cellulose (%)
1	Corn cobs	2.044
2	Sugarcane bagasse	1.473
3	Rice straw	3.068

According to Table 3.1, all substrates tested in the present research contain cellulose in different quantities. Fungi will use this substrate content as a source of carbon and energy [3]. Substrates derived from agricultural by-products can be used naturally or pre-treated [14]. In this research, a substrate prepared using acid was used. Pretreatment of substrates is one way to remove persistent structures in lignocellulosic complexes and facilitate fungal access to cellulose [15]. However, acid preparation causes the cellulose content to be less and produces toxic compounds with the potential to inhibit fungi. Chemical pretreatment is widely practiced because it is effective and can produce the desired product [16].

Research comparing the use of natural substrates with those prepared shows that the cellulase enzyme activity on natural substrates is more significant. Saini et al. [14] obtained high fungal cellulase activity using non-pretreated plant biomass substrate. *Trichoderma koningii*, on the other hand, developed the most cellulases from natural sugarcane bagasse (8.2 IU/g substrate), followed by *Penicillum* sp (1.7 IU/g substrate) [17].

3.2. The Activity Cellulase Crude Enzyme Extract

Statistical results of analysis of variance of all treatments independently and their interactions showed insignificant results, except for substrate thickness and substrate type independently ($P<0.05$). The ANOVA test findings of the relationship between cellulase activity - thickness and cellulase activity - type of substrate were significant. Hence, a further 5% DNMRT test was performed. There are two thickness treatments (1 cm and 1.5 cm), and three substrate treatments (sugar cane bagasse, corn cobs and rice straw). The results obtained based on the DNMRT 5%

follow-up test are reported in Table 3.2.

Based on Table 3.2, the 5% DNMRT test results show that substrate thickness is one of the factors that can influence cellulase enzyme activity. The analysis results on substrate thickness showed a p-level value of 0.025 < 0.05, making it significant for cellulase activity. This proves that the substrate thickness is high, which means there is more substrate and supports the growth of fungi in producing cellulase [8, 9].

Table 3.2 DNMRT 5% test result on temperature, pH, substrate, and substrate thickness treatments

Variation	DB	JK	KT	F	P
Intercept	1	1.225	1.225	166.678	0.000
Treatment	35	1.610	0.046	6.260	0.000
Temperature	1	0.004	0.004	0.579	0.452
pH	2	0.005	0.003	0.363	0.698
Substrate	2	1.319	0.660	89.735	0.000
Substrate thickness	1	0.040	0.040	5.428	0.026
Temperature*pH	2	0.018	0.009	1.219	0.308
Temperature*Substrate	2	0.028	0.014	1.875	0.168
pH*Substrate	4	0.020	0.005	0.677	0.612
Temperature*Substrate thickness	1	0.001	0.001	0.044	0.852
pH*Substrate thickness	2	0.022	0.011	1.507	0.236
Substrate*Substrate thickness	2	0.007	0.003	0.447	0.643
Temperature*pH*Substrate	4	0.034	0.008	1.150	0.349
Temperature*Substrate*Substrate thickness	2	0.002	0.001	0.128	0.881
Temperature*pH*Substrate thickness	2	0.044	0.022	3.027	0.061
pH*Substrate*Substrate thickness	4	0.046	0.012	1.573	0.203
Temperature*pH*Substrate*Substrate thickness	4	0.021	0.005	0.711	0.590
Repeats	1	0.007	0.007	0.886	0.353
Error	35	0.257	0.007		
Total	71	1.874			

The cellulase produced by *C. globosum* 17BDSM on a substrate thickness of 1.5 cm is more supportive of fungal growth; this is thought to be because the fungal hyphae can spread to the bottom and there are sufficient nutrients. This study showed that the thicker of the substrate, the more the cellulase activity increased. Substrate thickness of 2 cm and the addition of starter glucose will stimulate the growth of the *Aspergillus niger* fungus in the solid inoculation process. This is proven by the resulting cellulase activity value, 0.064 U/mL [18]. Thinner substrates can keep their water content during fermentation if opposed to thinner substrates. Haryati et al. [9] research shows that a substrate thickness of 3 cm yields more enzyme activity than 1.5 cm. Still, according to Ab Rashid et al. [19], the maximum enzyme activity was found at low substrate thickness (0.5 cm) — both of these studies measured mannase activity.

Table 3.3. Cellulase activity of *Chaetomium globosum* 17BDSM at substrate type and substrate thickness

No	Treatments	Value	Cellulase activity (U/mL)
1.	Substrate thick-ness	1 cm	0.107 [a]
		1.5 cm	0.154[b]
2.	Type substrate	Paddy straw	0.030 [a]
		Corn cobs	0.040 [b]
		Sugarcane bagasse	0.322 [b]

Table 3.3. shows that the type of substrate has a significant effect on the cellulase activity value. Fungi have different abilities in degrading cellulose on various substrates. Therefore, each substrate has different cellulase activity. The research about cellulase enzyme activity produced by *Trichoderma* in various agricultural biomass showed that five agricultural byproducts (corn cob, banana peel, groundnut shell, sugarcane bagasse, as well as, pigeon pea stalk) were utilised as substrates growing *Trichoderma harzianum* and T. *viride* for the production of cellulase under solid-state fermentation conditions. The findings revealed that the evaluated agricultural by-products can be used as a low-cost feedstock for cellulase synthesis. The high or low activity of the cellulase enzyme using the same substrate will be different if different isolates are used [20].

The difference in the growth of the fungus mycelium *C. globosum* 17BDSM on the sugarcane bagasse, rice straw, and corncob substrates is thought to be due to the chemical substances contained in these substrates, such as lignin and cellulose. Sugar cane bagasse, as the chemical content of plants in general, they also contain lignin hemicellulose besides cellulose. The high lignin content causes the rate of degradation of lignocellulosic materials by fungi to be slower and can inhibit the growth of the fungi themselves [21]. The structure of sugar cane bagasse is finer than the structure of rice straw and corn cob substrates, making it possible for fungi to break down cellulose into cellulase easily.

C. globosum 17BDSM was isolated from soil containing litter [6] at a low temperature so that the cellulase that *C. globosum* 17BDSM fungus will produce enzyme at the same temperature as the place where the fungus grows. *C. globosum* has an optimum growth temperature, namely at room temperature, which causes the decomposition of cellulose into glucose at high speed, which cells can then use as a carbon source, and cell division will be more optimal. Thus, enzyme production will be optimal. According to Subowo [22], the *Aspergillus niger* PA2 fungus has an optimum temperature of 50 C; at this temperature, the cell growth of the *Aspergillus niger* PA2 fungus can grow well by producing the highest biomass so that the fungus has the highest cellulase enzyme activity.

Chaetomium is a cellulolytic fungus that is commonly used for cellulose waste degradation and the production of single-cell proteins (SCP) [23]. *C. globosum* 17BDSM can be used to degrade cellulose substrates in agricultural byproducts, which can then be used to make other products like as biofuels. The moisture content in the substrate must be maintained throughout solid substrate fermentation so that substrate moisture may be evaluated. Furthermore, to ensure optimum cellulase activity specifically for *C. globosum* 17BDSM, it is necessary to ferment with a thicker substrate than the results reported in this work, which is 1.5cm.

4. Conclusion

According to the results, sugarcane bagasse is the best substrate with an average cellulase activity of 0.32 and a substrate thickness of 1.5 cm as the best substrate thickness with a cellulase activity of 0.15, which can increase the growth of the fungus *C. globosum* 17BDSM in producing cellulase with high activity. pH and temperature have no significant effect on the fungus *C. globosum* 17BDSM growth in agricultural by-product substrates for producing cellulase with high activity.

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