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Effect of Different Inducer Sources on Cellulase Enzyme Production by White-Rot Basidiomycetes *Pleurotus ostreatus* and *Phanerochaete chrysosporium* under Submerged Fermentation

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Abstract: Cellulase enzymes attract a lot of research due to their industrial application. Diverse cellulase-producing organisms and substances that induce cellulase are highly sought after. This study aimed to evaluate the effect of different inducer sources on cellulase production by white rot fungi *P. ostreatus* CGMCC 3.7292 and *P. chrysosporium* CGMCC 3.7212 under submerged fermentation employing a completely randomized experimental design. The different inducer sources tested were nitrogen (yeast, potassium nitrate, sodium nitrate, ammonium sulphate, aqueous ammonia and urea), carbon (malt extract, glucose, fructose, carboxymethylcellulose, starch and xylose) and agro-biomass (stevia straw, wheat straw, oat straw, alfalfa straw, corn cobs and corn stover). These inducer sources strongly impacted enzyme activities by *P. ostreatus* CGMCC 3.7292 and *P. chrysosporium* CGMCC 3.7212. The suitable nitrogen and carbon inducer sources for cellulase activity by *P. ostreatus* and *P. chrysosporium* were yeast (1.354 U/mL and 1.154 U/mL) and carboxymethylcellulose (0.976 U/mL and 0.776 U/mL) while the suitable agro-biomass were wheat straw (6.880 U/mL) and corn stover (6.525 U/mL), respectively. The least inducer sources in terms of nitrogen, carbon and agro-biomass for cellulase activity by *P. ostreatus* and *P. chrysosporium* were urea (0.213 U/mL and 0.081 U/mL), glucose (0.042 U/mL and 0.035), xylose (0.042 U/mL and 0.035 U/mL) and stevia straw (1.555 U/mL and 0.960 U/mL). In submerged fermentation, the cellulase enzyme activity of *P.* ostreatus in response to various inducer sources was relatively higher than *P. chrysosporium*.

Keywords: submerged fermentation; *P. ostreatus*; *P. chrysosporium*; inducer sources; agro-biomass; cellulase enzyme; basidiomycetes

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1. Introduction

Cellulase of microbial origin has become a crucial biocatalyst due to its intricate nature and extensive industrial uses. Cellulases refer to a group of glycosyl hydrolases that includes endoglucanase, exoglucanase and β-glucosidase, acting in a synergistic way to promote the hydrolysis of cellulose into glucose [1]. Cellulases are inducible enzymes synthesized by many microorganisms, including filamentous fungi and bacteria [2,3]. Among microbes, the genera of *Clostridium*, *Cellulomonas*, *Thermomonospora*, *Trichoderma* and *Aspergillus* are the widely considered cellulase producers [4]. White rot basidiomycetes are a group of wood-decaying fungi that can be exploited as an alternative and safe source for extracellular cellulolytic enzymes. Production of cellulase by white rot basidiomycetes is known to be influenced by several factors, such as species [5], the composition of the growth medium (carbon, nitrogen) [6], type of cultivation (solid state cultivation or submerged cultivation) [7], culturing condition (pH, temperature, aeration, humidity) [8] and the nature of the substrate (purified, lignified or non lignified) [9], among others.

Pleurotus spp. and *Phanerochaete* spp. are two of the most widely studied basidiomycetes species for cellulolytic enzyme assessment under different fermentation processes. Submerged fermentation (SmF) is the fancied method of cultivation because it is currently the most conveniently used in industry for the commercial production of cellulase enzymes [10]. Moreover, Elisashvili et al. [11] reported that the production of hydrolytic enzymes is favored by submerged fermentation compared to solid-state fermentation processes. Several species of *P. ostreatus* and *P. chrysosporium* are distributed across different geographical regions. Various independent studies have also been conducted to ascertain and verify the factors that affect cellulolytic enzymes production involving several species of these fungi [12–15]. In China, numerous *Pleurotus* and *Phanerochaete* species have been isolated, characterized and exploited for their potential to produce cellulase, among other uses. However, a detailed investigation of the existing literature revealed a paucity of information regarding the cellulase production from *P. ostreatus* CGMCC 3.7292 and *P. chrysosporium* CGMCC 3.7212 in response to different inducer sources.

The purpose of this investigation was thus to study the effect of different inducer sources, i.e., nitrogen (yeast, potassium nitrate, sodium nitrate, ammonium sulphate, aqueous ammonia and urea), carbon (malt extract, glucose, fructose, carboxymethyl cellulose, starch and xylose) and agro-biomass (stevia straw, wheat straw, oat straw, alfalfa straw, corn cobs and corn stover) under conditions of submerged fermentation (SmF) on cellulase production by *P. ostreatus* CGMCC 3.7292 and *P. chrysosporium* CGMCC 3.7212. This study sought to fill the existing knowledge gap, and the results obtained would help to identify suitable inducer sources that favor cellulase production by these fungi species. This would be the first study on cellulase production by *P. ostreatus* CGMCC 3.7292 and *P. chrysosporium* CGMCC 3.7212 under submerged fermentation in response to different inducer sources.

2. Materials and Methods

2.1. Organisms and Inoculum Preparation

Basidiomycete white-rot fungi, *P. ostreatus* CGMCC 3.7292 and *P. chrysosporium* CGMCC 3.7212 were acquired from the China Agricultural University in Beijing, China. Fungi culture were maintained on malt extract agar slants at 4 °C. Fungal inoculants were prepared by growing fungi at 24 ± 1 °C in 200 mL flasks containing 100 mL of the following nutrient medium: glucose,10.0 g/L; NH_4NO_3, 1.0 g/L; KH_2PO_4, 0.8 g/L; Na_2HPO_4, 0.2 g/L; $MgSO_4 \cdot 7H_2O$, 0.5 g/L; yeast extract, 2.0 g/L. The pH of the medium was altered to 6.0 using 2 M NaOH before sterilization for 20 min at 121 °C. After culturing fungi for 6 days, mycelial pellets were harvested and homogenized with a Waring laboratory blender.

2.2. Cultivation Conditions

2.2.1. Enzyme Activities with Carbon Inducer Sources

Submerged fermentation of the fungi was performed in a static condition at 24 ± 1 °C in 200 mL flasks containing 50 mL of standard medium: carbon source 10.0 g/L; NH_4NO_3, 1.0 g/L; KH_2PO_4, 0.8 g/L; Na_2HPO_4, 0.2 g/L; $MgSO_4 \cdot 7H_2O$, 0.5 g/L. The medium was adjusted to pH 6.0 with 2 M NaOH before sterilization at 121 °C for 20 min. To elucidate the effect of different carbon inducers on enzyme activities, equal amounts (10 g/L) of malt extract, glucose, fructose, carboxymethylcellulose, starch and xylose procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) were used as substitute carbon sources. About 3 mL of homogenized fungus were inoculated into 200 mL flasks containing 50 mL of standard medium and incubated at 24 ± 1 °C for 10 days. Controls without carbon were run in parallel. All experiments had four replications. After 10 days of incubation, when the cultures had completed the beginning, middle and end of the logarithmic phase and at the stationary phase of growth, solid fungal mass was separated by filtration using Whatman filter paper, followed by centrifugation (14,000× *g*; 15 min) at 4 °C. The supernatants obtained after fungi mass separations were assayed to determine cellulase enzyme activity.

2.2.2. Enzyme Activities with Nitrogen Inducer Sources

Nitrogen inducers were appraised similarly by substituting ammonium nitrate (NH_4NO_3) with other nitrogen inducers such as yeast, potassium nitrate (KNO_3), sodium nitrate ($NaNO_3$), ammonium sulphate ($(NH_4)_2SO_4$), ammonium hydroxide (NH_4OH) and urea (CH_4N_2O) procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The medium was supplemented with all nitrogen-containing inorganic and organic substances at a final concentration of 20 mM. The pH of the medium was altered to 6.0 using 2 M NaOH before sterilization for 20 min at 121 °C. About 3 mL of homogenized fungus mycelium were transferred into 200 mL flasks containing standard medium and incubated at 24 ±1 °C for 10 days. There were parallel runs of controls without nitrogen. All experiments had four replications. After 10 days of incubation, solid fungal mass was separated by filtration using Whatman filter paper, followed by centrifugation (14,000× *g*; 15 min) at 4 °C. The supernatants obtained after biomass separations were assayed to determine cellulase enzyme activity.

2.2.3. Enzyme Activities with Agro-Biomass Inducer Sources

The agro-biomass substrates stevia straw, wheat straw, oat straw, alfalfa, corn cobs and corn stover were procured from the cereal crop test field of Yangzhou University, Jiangsu, China. They were firstly dried at 60 °C in a drying oven (Shanghai Jinghong Co., Ltd., Shanghai, China) for 24 h and chopped into miniature pieces using a chopper (Zhengzhou Yike Heavy Machinery Manufacturing Co., Ltd., Henan, China). The chopped agro-biomasses were ground into lesser particles in a hammer mill (Shanghai Jinghong Co., Ltd., Shanghai, China) and separated by a 0.45 mm (40 meshes) sieve. The portion that made it through the sieve was used for the medium preparation in the submerged fermentation (SmF). Submerged fermentation of agro-biomass was carried out in a static condition at 24 °C in 200 mL flasks containing 50 mL of the above-mentioned standard medium with 40 g/L of agro-biomass as carbon substituents. The pH of the medium was altered to 6.0 using 2 M NaOH before sterilization for 20 min at 121 °C. Three mL of mycelial homogenate was transferred into the flasks containing the media with agro-biomass. All experiments had four replications. After 10 days of incubation, fungal solid mass and agro-biomass were separated by filtration through Whatman filter paper. This was followed by centrifugation (14,000× *g*; 15 min) at 4 °C. The supernatants obtained after biomass separations were assayed to determine cellulolytic enzyme activity.

2.3. Enzyme Assay

Endoglucanase activity was employed to assess the cellulase enzyme activities. Endoglucanase activity (carboxymethyl cellulase; CMCase) was measured as described previously [16] using a reaction mixture having 1 mL of 1% carboxymethyl cellulose (CMC) in 0.05 M citrate acetate buffer (pH 5.0) and aliquots of appropriately diluted filtrate. The reaction mixture was incubated at 50 °C for 30 min. The reducing sugar produced was determined by the dinitrosalicylic acid (DNS) method and measuring the absorbance at 540 nm using a Multiskan Go UV-Vis spectrophotometer (Thermo Fisher Scientific Oy, Ratastie 2, 01620 Vantaa, Finland). Reducing sugar content was determined via a glucose standard curve. One unit (IU) of endoglucanase activity was defined as the amount of enzyme releasing one µmole of reducing sugar per min. Enzyme yield was expressed as U/mL.

2.4. Statistical Analysis

The results of the enzyme activities are the mean ± SD (standard deviation) values of the four replicates. The means were compared by analysis of variance (ANOVA). One-way ANOVA followed by the Duncan test was employed to assess the effect of each inducer source on the enzyme production by each white rot fungi. Differences were considered significant at $p < 0.05$. Data analysis was done using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA, USA). Error bars in all figures referred to the SD of four

replicates values of each treatment. Prior to conducting the ANOVA, the assumption of homogeneity of variances was tested and satisfied based on Levene's test ($p > 0.05$).

3. Results

3.1. Cellulase Activity of White Rot Fungus P. ostreatus and P. chrysosporium under Submerged Fermentation on Different Nitrogen Inducer Sources

Significant differences were recorded in the cellulase activity of *P. ostreatus* on the different nitrogen inducers observed in the present study, as shown in Figure 1A. Yeast recorded significantly the highest cellulase activity (1.354 U/mL), while CH_4N_2O recorded the least cellulase activity (0.213 U/mL). Even though there was no significant difference between KNO_3 and $NaNO_3$, they did record comparatively a higher cellulase activity (0.756 U/mL and 0.757 U/mL), respectively, compared to the $(NH_4)_2SO_4$ (0.571 U/mL) and NH_4OH (0.444 U/mL). The cellulase activity of NH_4OH (0.444 U/mL) was comparatively lower compared to that of $(NH_4)_2SO_4$ (0.571 U/mL). Significant differences were recorded in the cellulase activity of *P. chrysosporium* on the different nitrogen inducers observed in the study, as shown in Figure 1B. Yeast recorded significantly the highest cellulase activity (1.154 U/mL), while CH_4N_2O, on the other hand, recorded the least cellulase activity (0.081 U/mL) in the current study. Much as there was no significant difference between the KNO_3 and the $NaNO_3$, they did record comparatively a higher cellulase activity (0.557 U/mL and 0.556 U/mL, respectively) compared to the $(NH_4)_2SO_4$ (0.371 U/mL) and NH_4OH (0.144 U/mL). The cellulase activity of NH_4OH (0.144 U/mL) was comparatively lower compared to that of $(NH_4)_2SO_4$ (0.371 U/mL).

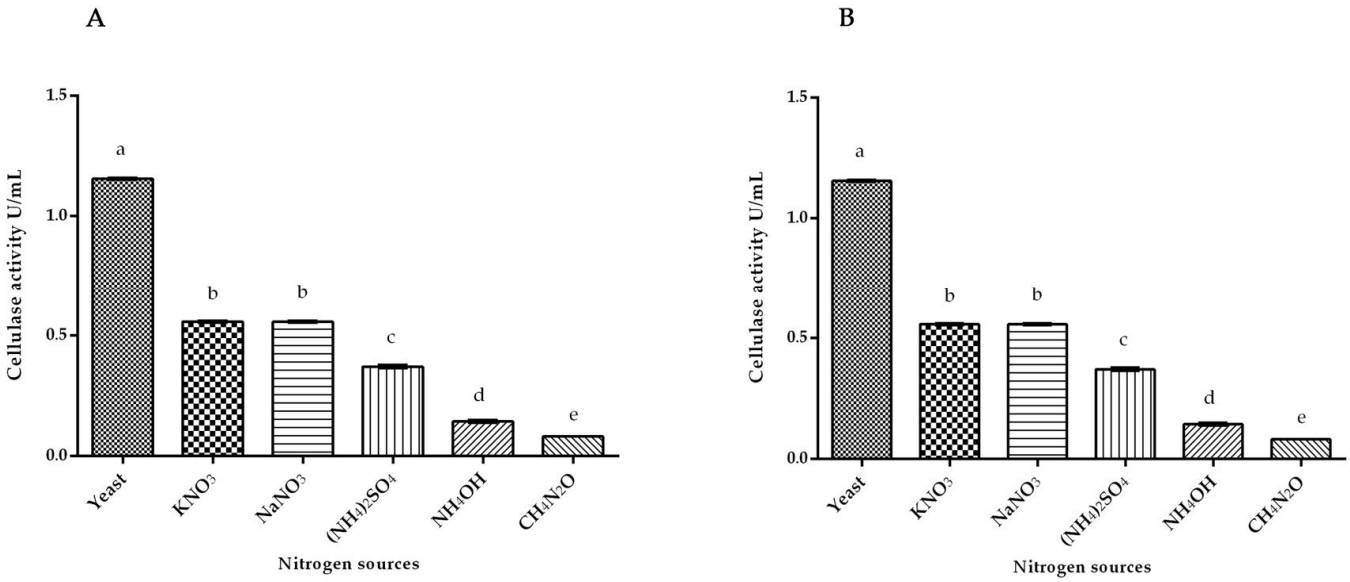

Figure 1. (**A**) Cellulase activity of white rot fungus *P. ostreatus* under submerged fermentation on different nitrogen inducers. Bars with different letters (a–e) are significantly different at $p < 0.05$. (**B**) Cellulase activity of white rot fungus *P. chrysosporium* under submerged fermentation on different nitrogen inducers. Bars with different letters (a–e) are significantly different at $p < 0.05$.

3.2. Cellulase Activity of White Rot Fungus P. ostreatus and P. chrysosporium under Submerged Fermentation on Different Carbon Inducer Sources

As shown in Figure 2A, with the exception of glucose and xylose, which did not record any significant differences in the activity of cellulase (0.042 U/mL and 0.042 U/mL), all the other carbon inducers (fructose, 0.211 U/mL; malt extract, 0.694 U/mL; starch, 0.824 U/mL and carboxymethylcellulose, 0.976 U/mL) considered in the study recorded significant differences in cellulase activity of *P. ostreatus* with the carboxymethylcellulose recording the highest cellulase activity. The cellulase activity of *P. ostreatus* on starch (0.824 U/mL) was significantly higher compared to malt extract (0.694 U/mL) and fructose (0.211 U/mL). Fruc-

tose, however, recorded significantly lower cellulase activity (0.211 U/mL) compared to the malt extract (0.694 U/mL). With the exception of glucose and xylose, which did not record any significant differences and the least cellulase activity (0.035 U/mL and 0.035 U/mL), all the other carbon sources (fructose, 0.181 U/mL; malt extract, 0.536 U/mL; starch, 0.624 U/mL and carboxymethylcellulose, 0.776 U/mL) considered in the study recorded significant differences in cellulase activity of *P. chrysosporium*, with the carboxymethylcellulose recording the highest cellulase activity, as shown in Figure 2B. The cellulase activity of *P. chrysosporium* on the fructose was significantly lower (0.181 U/mL) compared to the starch (0.6204 U/mL) and malt extract (0.536 U/mL). On the other hand, starch recorded a significantly higher cellulase activity (0.624 U/mL) than malt extract (0.536 U/mL).

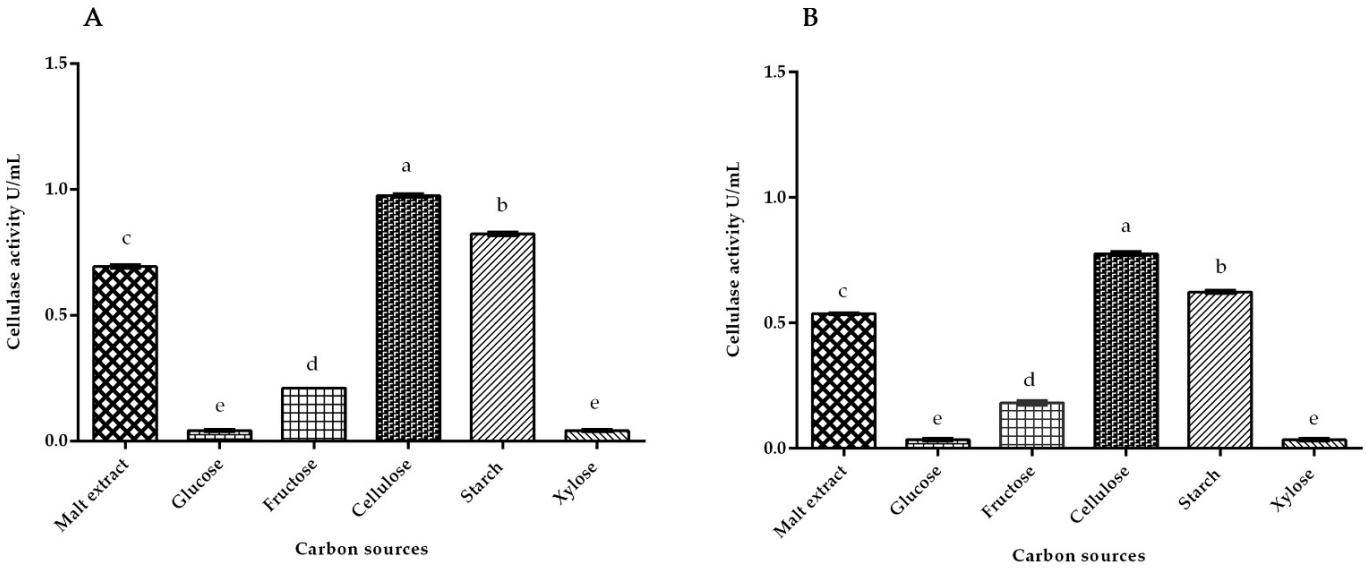

Figure 2. (**A**) Cellulase activity of white rot fungus *P. ostreatus* under submerged fermentation on different carbon inducers. Bars with different letters (a–e) are significantly different at $p < 0.05$. (**B**) Cellulase activity of white rot fungus *P. chrysosporium* under submerged fermentation on different carbon inducers. Bars with different letters (a–e) are significantly different at $p < 0.05$.

3.3. Cellulase Activity of White Rot Fungus P. ostreatus and P. chrysosporium under Submerged Fermentation on Different Agro-Biomass

Significant differences were recorded in the cellulase activity of *P. ostreatus* on all the agro-biomass observed in the study under submerged fermentation, as shown in Figure 3A. Wheat straw recorded significantly the highest cellulase activity (6.880 U/mL), while stevia straw recorded the least cellulase activity (1.555 U/mL) in the current study. Corn stover did record comparatively a higher cellulase activity (6.342 U/mL) compared to the oat straw (5.247 U/mL) and alfalfa straw (3.172 U/mL). Although the cellulase activity of the alfalfa straw (3.172 U/mL) was significantly higher than that of the corn cob (2.037 U/mL), it was not comparable to that of the oat straw (5.247 U/mL). Significant differences were recorded in the cellulase activity of *P. chrysosporium* on all the agro-biomass observed in the study under submerged fermentation, as shown in Figure 3B. Corn stover recorded significantly the highest cellulase activity (6.525 U/mL), while stevia straw recorded the least cellulase activity (0.960 U/mL). Wheat straw recorded comparatively a higher cellulase activity (5.550 U/mL) compared to the oat straw (4.240 U/mL), alfalfa straw (2.025 U/mL), and corn cob (1.617 U/mL). Although the cellulase activity of the alfalfa straw (2.02 U/mL) was significantly higher than that of the corn cob (1.617 U/mL), it was not comparable to that of the oat straw (4.240 U/mL).

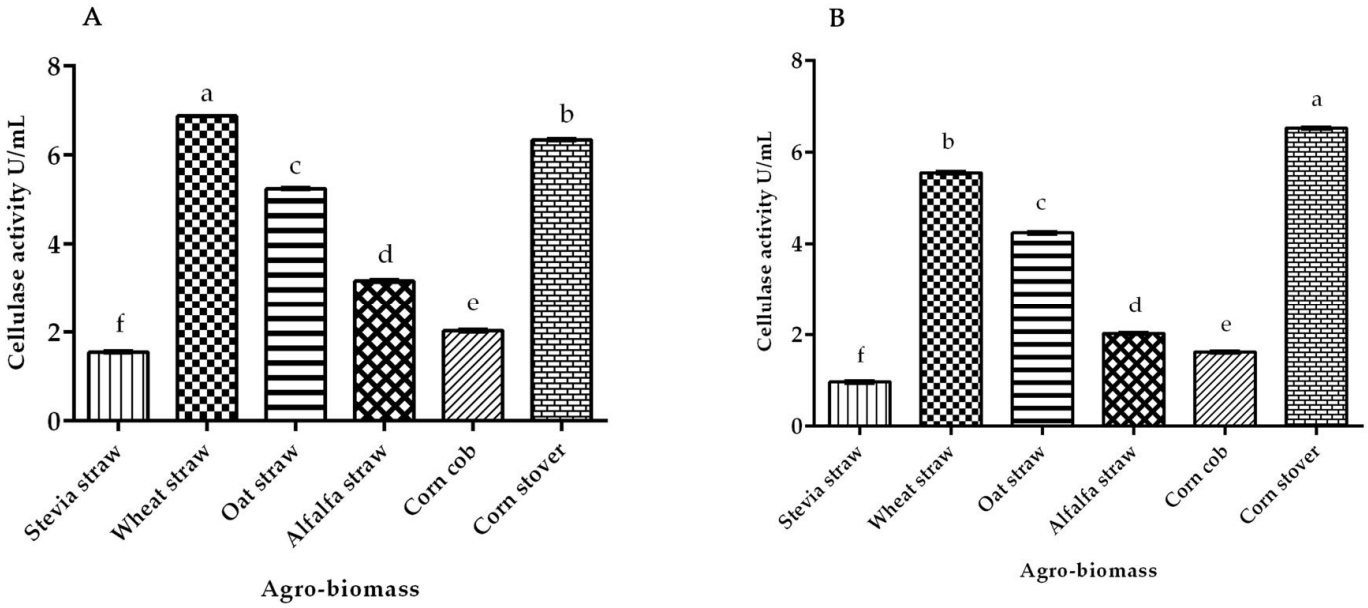

Figure 3. (**A**) Cellulase activity of white rot fungus *P. ostreatus* under submerged fermentation on different agro-biomass. Bars with different letters (a–f) are significantly different at $p < 0.05$. (**B**) Cellulase activity of white rot fungus *P. chrysosporium* under submerged fermentation on different agro-biomass inducers. Bars with different letters (a–f) are significantly different at $p < 0.05$.

4. Discussion

Naturally, nitrogen stimulates fungal cell growth, enhancing biomass formation and cellulase enzyme expression [11]. *P. ostreatus* and *P. chrysosporium* fungi used in the current study showed a similar response to different nitrogen sources with respect to cellulase activity. The highest enzyme activity from the nitrogen sources considered in the study was recorded in the yeast extract. Yeast extract is the sole complex source of nitrogen among those tested. This observation is coherent with the findings of Chuwech et al. [17] and Jonathan and Adeoyo [18]. The enhanced enzyme production by yeast extract, according to Jonathan and Fasidi [19], could be attributed to its chemical composition: high amino acid and vitamin. That is, nitrogen sources: nitrate-based vis-à-vis ammonium-based equally has influence on the cellulase enzyme activity. This gives a basis for the observed higher cellulase activity of the nitrate-based compound (potassium nitrate and sodium nitrate) compared with the ammonium-based compounds (ammonium sulphate, ammonium hydroxide, and urea). Similarly, Rajmane and Korekar [20] indicated that nitrogen sources like sodium nitrate stimulated higher cellulase activity while phosphate and sulphate ammonium-based nitrogen were proven to hamper cellulase activities. This may be due to the tendency of the mycelium to absorb the ammonium ions, which resulted in the lowering of the pH, which subsequently reduced the production of cellulase enzymes [21]. Potassium nitrate and sodium nitrate enhancing cellulase activity could be due to the simplicity of the nitrogen sources utilized for the biosynthesis of cellulase enzymes. Though adding yeast as the sole organic nitrogen source resulted in increased cellulase enzyme activity in this study, the authors share the same view with Hammad et al. [22], who explained that organic nitrogen sources are not effective substituents for inorganic nitrogen sources because they are costly. The cellulase activity of both fungi on urea which recorded the least cellulase activity is similar to the reports of both Elsebaay et al. [23] and Sethi and Gupta [24].

Much as carbon is known to have a positive effect on cellulase production [25], the selection of carbon-based materials/substrates from plant polymers and their degraded products/derivatives in the assessment of enzymatic activity was attributed to their abundance or availability and cost-effectiveness compared to other sources. Therefore, carbon sources such as malt extract, glucose, fructose, carboxymethylcellulose, starch and xy-

lose used in the current study were intentional. Among the carbon sources tested in the study, carboxymethylcellulose produced the highest cellulase activity. This observation agrees with the study of Niranjane et al. [26], in which carboxymethylcellulose as a carbon substrate proved to be the strongest inducer of cellulase activity. Similarly, crystalline cellulose was noted to be the best carbon source and to have the highest cellulase activity among the three substrates evaluated among all filamentous fungi [27]. The observed high performance of fungi on carboxymethylcellulose, according to Kobakhidze et al. [28], may be due to the presence of several inducers capable of initiating cellulase production and catalyzing the hydrolysis of cellulose to simple assimilable sugars.

Moreover, Yoav et al. [29] and Suzuki et al. [30], respectively, stated that the cellulases of *P. ostreatus* and *P. chrysosporium* are inducible enzymes. According to Elisashvili et al. [31], these filamentous white rot basidiomycetes secrete cellulases with high efficiency upon cultivation in the presence of carboxymethylcelluloses. Most studies also have appreciated cellobiose as a primary product of cellulose degradation that considerably induces cellulase production in most filamentous fungi [6]. On a transcriptional level, cellobiose is known to induce transcriptional factor Clr-Clr1which subsequently induces the expression of the transcriptional factor Clr-2 responsible for the expression of significant cellulase genes [32].

Cellulase enzyme activity of the starch was higher than fructose, glucose, and xylose. Filamentous fungi possess starch-active enzymes [33] whose expression is known to be induced by starch as well as its intermediary metabolite maltose [34,35]. This, therefore, might explain the enhanced cellulase activity observed with starch. Cellulase from filamentous basidiomycetes fungi is induced by cellulose/polysaccharide-containing materials [28,36]. Thus, since malt extracts are heterogeneous polysaccharides [37], their ability to elicit increased cellulase activity compared to fructose¸ glucose, and xylose may originate from several cellulase-inducing metabolites generated during its degradation. In various metabolic processes, glucose serves as a significant energy. However, the efficiency of filamentous white rot fungi to secrete cellulase is informed by the presence of a cellulase intermediary metabolite like cellobiose. In this study, glucose and xylose demonstrated the least cellulase activity. Similarly, Elisashvili, Khardziani, Tsiklauri and Kachlishvili [31], Kobakhidze et al. [38], and Cai et al. [39] reported the least cellulase activity when white rot fungi were cultured on glucose as the sole carbon source. Similarly, Salmon, et al. [40] analyzed the effect of different carbon sources, including xylose, as inducers of cellulase activity by the filamentous basidiomycete fungi *Ganoderma applanatum* mr-56 and reported that xylose has the least cellulase activity, which is coherent with the observation made in this study. According to Lynd et al. [41] and Suzuki et al. [42] readily, metabolizable carbon sources turn to lower/suppress cellulase synthesis. Thus, the comparatively reduced cellulase activity in the presence of glucose and xylose in the current study could be attributed to the repression of the filamentous white rot fungi due to the glucose concentration in the extracellular media [6,43] since the readily available glucose is utilized by the fungi to meet its energy requirement and not an intermediary metabolite.

From a genetic perspective, glucose, in the presence of filamentous fungi, usually serves as a repressor of cellulolytic genes [44]. That is, the cellulase regulatory mechanism is influenced by the presence of glucose as it acts as a negative feedback mechanism which suppresses cellulase induction [32]. Aslam et al. [45] monitored cellulase activity in the culture filtrate, which had only glucose as a carbon source in the medium and witnessed very low levels to almost zero activity. Muthuvelayudham and Viruthagiri [46] explained that glucose is the end product of carbohydrate hydrolysis by cellulases, and glucose in the medium shows the end product inhibition. In other words, although glucose may promote mycelia growth, it is not a good inducer of cellulase enzyme. Cellulase enzyme activity on fructose by both fungi was thus higher than glucose and xylose. Similarly, Mehboob et al. [47] observed an increased cellulase enzyme activity when filamentous fungi were grown in a wheat straw-based medium with additional fructose than glucose. Although glucose, xylose, and fructose are easily metabolizable sugar, the cellulase activity

of fructose being higher than glucose and xylose suggests the absence or low levels of repressors during fructose metabolism.

The suitability of common agricultural biomass wastes as carbon sources for cellulase production by *P. ostreatus* and *P. chrysosporium* revealed that cellulase activity levels varied among the substrates, suggesting that the composition of these substrates induces different quantities (levels) of enzymes. In accordance with the general observations [7,48–50], *P. ostreatus* and *P. chrysosporium* produced cellulases when grown on different lignocellulose agro-biomass. The capacity of these basidiomycetes filamentous fungi to produce these enzymes is vital in furnishing the growing cultures with a carbon source essential for their biosynthetic activity. The substrate not only provides a supply of carbon but also generates the required inducing compounds for the filamentous fungi [51].

In the present study, cellulase activity by *P. ostreatus* and *P. chrysosporium* on wheat straw, oat straw, and corn stover were generally high. The corn stover used in this study referred to the above-ground structure; the stalk with its leaves after harvesting of the main crop. Similarly, among the several agro-biomass tested, wheat straw, corn stover, and oat straw exhibited enhanced cellulase activity by filamentous fungi *Morchella spongiola* [52], *Aspergillus terreus* [25], and *T. reeesi* [53], respectively. The variation in terms of the level of cellulase activity observed in the present study may be due to the variation in agro-biomass compositions. These findings were in agreement with Elisashvili et al. [54], who mentioned that the nature of the lignocellulosic biomass substrate had a positive impact on enzyme secretion. The level of cellulase activity of the corn cob was lower compared to the alfalfa, oat straw, wheat straw, and corn stover. Similarly, among several agro-biomasses, Reddy et al. [55], Ganash et al. [56], and Govumoni et al. [57], respectively, reported the least cellulase activity when *Aspergillus niger*, *P. ostreatus*, and *Phanerochaete chrysosporeum* (MTCC 787) were grown on corn cobs. Corn cob's inability to produce higher cellulase compared to the other agro-biomass is due to its structural nature. Corn cob has a dense or compact micro-structural carbohydrate configuration [58]. As such, compared to the other agro-biomass, the fungi mycelium could not easily penetrate and access the hydrolyzable fibers, which are essential for cellulase enzyme biosynthesis. Furthermore, Reyes et al. [59] stated that better contact with the cellulosic material could initiate improved enzyme production associated with cellulose hydrolysis.

Among the agro-biomasses considered in the study, the cellulase activity of stevia was the least. The logical explanation is that stevia biomass mainly consists of soluble sugars [60,61]. According to Amore et al. [62], genes encoding hydrolytic enzymes, including cellulase implicated in plant cell wall breakdown in filamentous fungi, can be repressed during the growth period in the presence of easily utilizable carbon substrate sources but are induced/stimulated in the presence of cellulosic substrates (or product thereof) [63]. Authors, therefore, concur that besides stevia having a poly-carbohydrate composition, it still has more easily assimilable sugars compared to other agro-biomass that would serve as a carbon source and possibly repress cellulase activity. In general, the cellulase enzyme activity of *P. ostreatus* in response to various inducers was relatively higher than *P. chrysosporium* in submerged fermentation. The genomes of *P. ostreatus* are known to encode more glycoside hydrolase family cellulase genes compared to *P. chrysosporium* [64].

5. Conclusions

It is clear from the present study that yeast and carboxymethylcellulose are suitable nitrogen and carbon inducer sources of cellulase activity by *P. ostreatus* and the reference culture *P. chrysosporium* under submerged fermentation, while the most suitable agro-biomasses are wheat straw and corn stover, respectively. The least inducer sources in terms of nitrogen, carbon, and agro-biomass for cellulase activity by both *P. ostreatus* and *P. chrysosporium* were urea, glucose, xylose, and stevia straw, respectively. Further research could focus on optimizing the various inducers aiming at higher enzyme activities by *P. ostreatus* and *P. chrysosporium*.

Author Contributions: Conceptualization, O.D.; Data curation, O.D.; Formal analysis, O.D.; Methodology, O.D. and Q.Y.; Supervision, G.Z. and L.M.; Validation, O.D., Q.Y. and L.M.; Writing—original draft, O.D.; Writing—review and editing, O.D. and L.O.-M. All authors have read and agreed to the published version of the manuscript.

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