CYANOBACTERIAL BIOMASS AS N-SUPPLEMENT TO OIL PALM EMPTY FRUIT BUNCH (OPEFB) FIBRE FOR IMPROVEMENT OF CELLULASE PRODUCTION BY *ASPERGILLUS TERREUS* IN SUBMERGED FERMENTATION

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The possibility of using dry biomass of a cyanobacterium, *Anacystis nidulans,* as nitrogen source supplement for improvement of cellulase production by *Aspergillus terreus* was studied in submerged fermentation using oil palm empty fruit bunch (OPEFB) fibre as a carbon source. For comparison, four other nitrogen sources (ammonium sulphate, urea, peptone, and yeast extract) were also tested. Growth and cellulase production were greatly enhanced in fermentation using biomass of cyanobacterium as the nitrogen source. The use of cyanobacterial biomass as a nitrogen source also reduced the inhibitory effect of high concentrations of $CaCl_2$ to growth of *A. terreus* and cellulase production. The addition of 0.3 g L^{-1} $CaCl_2$ to the medium containing OPEFB fibre and cyanobacterial biomass further enhanced the cellulase production, though growth remained unchanged. The final FPase, CMCase, and β-glucosidase obtained in fermentation using 10 g L^{-1} OPEFB fibre and 6 g/L cynaobacterial biomass with the addition of 3 mM $CaCl_2$ was 0.97 U mL^{-1}, 14.1 U mL^{-1}, and 10.4 U mL^{-1}, respectively.

Keywords: Cyanobacterial biomass; Anacystis nidulans; Aspergillus terreus; Cellulase; Fermentation

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INTRODUCTION

Cellulase enzymes, which hydrolyze cellulose to form glucose and other commodity chemicals, can be divided into three main components: endoglucanase (endo-1,4-β-D-glucanase, EG; EC 3.2.1.4); cellobiohydrolase (exo-1,4-β-D-glucanase, CBH; EC 3.2.1.91), and β-glucosidase (1,4-β-D-glucosidase, BG; EC 3.2.1.21) (Hong et al. 2001; Li et al. 2006). Scientific communities have a keen interest in cellulases for their various applications in industries such as starch processing, animal food production, grain alcohol fermentation, malting and brewing, extraction of fruit and vegetable juices, the pulp and paper industry, and the textile industry (Adsul et al. 2007; Kaur et al. 2007).

Agricultural waste, especially lignocellulosic biomass, is known to be an excellent carbon source for industrial microbial fermentation. The utilization of hemicellulose and cellulose components of plant tissue for the production of liquid fuel would be economically attractive (Pushalkar and Rao 1998). However, many microorganisms that produce extracellular cellulase when grown in medium containing cellulose or cellulase

inducers cannot ferment sugar to ethanol. Most microorganisms capable of fermenting sugars to ethanol lack the hydrolytic enzymes needed to break down cellulose (Bayer et al. 2007). Only a few filamentous fungi such as *Monilia* sp., *Fusarium oxysporum* VTT-D-80134, *Fusarium oxysporum* F-3, and *Neurospora crassa* have been reported to ferment sugars to ethanol (Christakopoulos et al. 1989; Deshpande et al. 1986; Gong et al. 1981; Suihko 1983). Cellulase production has been found to be a feature of many *Aspergillus* spp. (Lockington et al. 2002; Ong et al. 2004; Wang et al. 2006), but only a few studies are available on the production of cellulase from *Aspergillus terreus* (Emtiazi et al. 2001; Gao et al. 2008; Workman and Day 1982). *A. terreus* has ability to produce extracellular endo-β-1,4-glucanase and exo- β-1,4-glucanase with high levels of β-glucosidase and has the ability to ferment glucose to ethanol (Pushalkar et al. 1995).

The major impediment to the comprehensive application of cellulase in industry is the high cost of raw materials for its production. Considerable cost reduction may be feasible if cellulose conversion can be enhanced by using microorganisms that produce cellulolytic enzymes. Thus, it is necessary to seek microorganisms that have a high rate of cellulase production (Kotchoni and Shonukan 2002). In addition to cellulose-producing characteristics of the microorganisms, research has shown that the choice of carbon and nitrogen sources to be used in cultivation of such microorganisms is one of the most important factors affecting the cost and yield of cellulase production. Consequently, careful choice of a cheap and easily available substrate seems to be essential to reduce the cost of enzyme production (Beg et al. 2000; Senthilkumar et al. 2005).

Cellulase production, by means of an inducible extracellular enzyme, is greatly influenced by the medium composition. The effect of various commercial nitrogen sources on cellulase production by white rot fungi have been reported by many reserachers. The diazotrophic cyanobacteria are microbes, found worldwide, that contribute significantly to the nitrogen economy of the biosphere (Borowitzka 1988). Cyanobacteria that cause water bloom often release toxic compounds, leading to purification and an objectionable odour. These bacteria are nevertheless not only a rich source of nitrogen, but also a rich source of the sugars, lipids, and proteins needed for the production of useful compounds (Dwi et al. 2001).

The objective of this study was to examine the potential application of dry biomass of a diazotrophic cyanobacterium (*Anacystis nidulans*) as nitrogen source for the enhancement of cellulase production by *Aspergillus terreus* in submerged fermentation using oil palm empty fruit bunch (OPEFB) fibres as carbon source. The effect of different concentrations of $CaCl_2$ on growth of *A. terreus* and cellulase production during fermentations using cyanobacterium and OPEFB fibres was also evaluated.

MATERIALS AND METHODS

Microorganism

The mold *Aspergillus terreus*, isolated from the compost of oil palm empty fruit bunch (OPEFB) waste at a local oil palm processing factory (Sri Ulu Langat Palm, Dengkil, Selangor, Malaysia) was used in this study as cellulase producer. Details of the method of isolation and identification of this fungus have been described in our previous

paper (Shahriarinour et al. 2011a). The fungus was grown on potato dextrose agar (PDA) at 30°C for 7 days to allow development of spores, which were then stored at 4°C for subsequent use in inoculum preparation. The cyanobacterium *Anacystis nidulans* IU625 (ATCC 27144), as non-viable biomass, was used as nitrogen supplement for lipase fermentation. This cyanobacterium was grown in broth medium BG-11 for blue green algae at 26°C for 12 days. The cell mass was collected by filtration with filter paper (Whatman Grade No. 1) and then dried in an oven at 80°C for 24 h to obtain dry cell mass as nitrogen supplement.

Media Formulation and Preparation

The basal medium described by Mandels and Weber (1969) was used in this study for cellulase fermentation. The medium was added with Tween 80 (2 mL L^{-1}), trace element solution (1 mL L^{-1}), nitrogen sources and OPEFB fibres as a carbon source. The original mandel medium contained 1 mL L^{-1}, and from the preliminary study a higher Tween 80 concentration (2 mL L^{-1}) was required to enhance cellulase production (data not shown).

The OPEFB fibres obtained from an oil palm processing factory (Sri Ulu Langat Palm, Dengkil, Selangor, Malaysia) were first washed with water. The dried fibres were shredded by grinding in a hammer mill (Mill Powder Tech Solutions, Taiwan) to obtain fibres with an average length of 1 mm. The fibres were delignified by physico-chemical pretreatment by soaking in phosphoric acid and then exposed to hydrothermal treatment at 160°C for 10 min, followed by biological treatment with effective microorganisms (Shahriarinour et al. 2011b). The pretreated OPEFB fibers were filtered and washed with distilled water until no traces of acid could be detected and then dried in an oven at 95°C for 2 days. In all experiments, 10 g/L of dry pretreated OPEFB fibre were used as a carbon source.

Fermentations

All fermentations were carried out in 500-mL flasks containing 100 mL of medium (initial pH 5.5), and the flasks were sterilized in an autoclave at 121°C, 15 psi for 15 min. The flasks were inoculated with 5 mL of spore suspension containing approximately 6×10^6 spores mL^{-1}. The flasks were then incubated at 30 °C for 12 days on a rotary orbital shaker, agitated at 200 rpm. All experiments were carried out in triplicate. During the fermentation, samples were withdrawn at regular time intervals (0, 24, 48, 72, 96, 120, 144, 168, and 192 h) for analysis.

In the initial stage, the effect of the addition of different nitrogen sources [$(NH_4)_2SO_4$, urea, yeast extract, peptone and dry cyanobacterial biomass] at different concentrations (3, 6, 9, and 12 g L^{-1}) to the basal medium containing 10 g L^{-1} of delignified OPEFB as a carbon source on cellulase production by *A. terreus* was evaluated.

The effect of the supplementation of different concentrations of $CaCl_2$ (0, 0.1, 0.2, 0.3, 0.4, and 0.5 g L^{-1}) on cellulase production by *A. terreus* was also evaluated. In this set of experiment, the $CaCl_2$ component of the basal medium was omitted prior to the addition of different concentrations of $CaCl_2$.

Analytical Procedure

The culture sample withdrawn during fermentation was centrifuged at 15,000 rpm for 15 min at 4°C. The clear supernatant was analyzed for cellulase activities. Since the mechanical separation of mycelium from OPEFB fibres is not possible, the amount of mycelium was estimated indirectly by measuring the amount of glucosamine according to the method reported in our previous study (Shahriarinour et al. 2011b).

Carboxymethylcellulase (CMCase) activity was determined by measuring the reducing sugars produced from 2% (w/v) carboxymethylcellulose, while filter-paper-hydrolysing (FPase) activity was determined by estimating the reducing sugars liberated from Whatman filter-paper no. 1 (Wood and Bhat 1988). Both reactions were carried out in 0.05 M sodium acetate buffer at pH 5 and incubated at 50 °C for 30 min for CMCase assay and for 1 h for FPase assay. One unit of CMCase and FPase activity was defined as 1 μmol reducing sugar released per volume of enzyme sample (mL) per incubation time (min). β-Glucosidase was determined by the method described by Wood and Bhat (1988). The *p*-nitrophenol released from *p*-nitrophenyl-β-D-glucopyranoside (Fluka, Buchs, Switzerland) was measured using a spectrophotometer (Thermo Fisher Scientific, Genesys 20). One unit of β-glucosidase activity was defined as 1 μmol *p*-nitrophenol liberated per volume of enzyme sample (mL) per incubation time (min). Specific activity was defined as units/mg protein. The concentration of protein was estimated by the dye binding method of Bradford (1976), using the dye reagent Bio-Rad determination kit (Bio-Rad-500-0.006) in micro plates. A standard curve was generated using 1 μg μL^{-1} bovine serum albumin (BSA). All measurements were performed in triplicate.

Statistical Analysis

The results were compared by one-way analysis of variance (one-way ANOVA) and multiple range tests to find the differences between measurement means at the 5% (0.05) significance level. Data were analyzed using Minitab 14 statistical software (Minitab, PA, USA).

RESULTS AND DISCUSSION

Chemical Composition of OPEFB

The chemical composition of untreated and delignified OPEFB fibres is shown in Table 1. Delignification reduced the lignin content in OPEFB fibres from 10% to 4.2%, while hemicellulose was reduced from 21.7% to 4.3 %. On the other hand, cellulose content increased from 50.3% to 63.4%.

Table 1. Chemical Composition of Untreated and Delignified Oil Palm Empty-Fruit-Bunch (OPEFB) Fibres

Treatment	* Chemical Composition (%)			
	Cellulose	Hemicellulose	Lignin	Ash
Untreated	50.3 ± 1.5	21.7 ± 0.3	10.0 ± 1.4	0.5 ± 0.05
Treated with phosphoric acid, followed by hydrothermal treatment and biological treatment	63.4 ± 1.9	4.30 ± 0.6	4.20 ± 0.2	0.6 ± 0.03

* Result are based on the mass of dry matter.

Effects of Nitrogen Source on Cellulase Production by *A. terreus*

The effects of different nitrogen sources on three main components of cellulase (CMCase, FPase, and β-glucosidase) production by *A. terreus* using delignified OPEFB fibres as carbon source are summarised in Table 2. Maximum growth of *A. terreus* was achieved when either yeast extract or dry cyanobacterial biomass were used as a nitrogen source, and inhibition of growth at high concentrations of these nitrogen sources was observed. The protein content of the cyanobacterium *A. nidulans* ranged from 60 to 70% of its dry weight (Singh et al. 2002). High growth of *A. terreus* and cellulase production in fermentation using cyanobacterial biomass may be due to its high nutritional value, especially protein. Growth was very poor when $(NH_4)_2SO_4$ or urea was used as the nitrogen source. Inhibition of cellulase production with increasing concentrations of $(NH_4)_2SO_4$ and urea was also observed.

Among the nitrogen sources investigated, dry cyanobacterial biomass provided the highest production of all three main components of the cellulase complex by *A. terreus*, followed by yeast extract, peptone, urea, and $(NH_4)_2SO_4$. β-Glucosidase activity was not detected when $(NH_4)_2SO_4$ was used. Cyanobacterial biomass and yeast extract concentrations of above 9 g L^{-1} inhibited cellulase production. Cellulase production was greatly inhibited at urea concentrations of more than 3 g L^{-1}. The optimum initial concentrations of $(NH_4)_2SO_4$, yeast extract, peptone, urea, and dry cyanobacterial biomass for the highest cellulase production by *A. terreus* with 10 g L^{-1} treated OPEFB fiber as carbon source were 3, 6, 6, 3, and 6 g L^{-1}, respectively. The maximum activities of FPase, CMCase, and β-glucosidase obtained with 6 g L^{-1} dry cyanobacterial biomass were 0.82, 10.1, and 8.53 U mL^{-1}, respectively. A comparison of the profiles for all three main components of cellulase (CMCase, FPase, and β-glucosidase) production by *A. terreus* using yeast extract and cynaobacterial biomass is given in Fig. 1.

Enhanced cellulase production in fermentation using organic nitrogen-supplemented medium may be attributed to the presence of some nutrients and activators naturally present in organic nitrogen sources. Carbon- and nitrogen-rich raw materials, such as cyanobacterial biomass, in appropriate concentrations can serve as a complete nutrient pool for the fermentation process for the production of enzymes. Lignocellulosic substrate with a high concentration of organic nitrogen stimulates the production of cellulase enzymes (Ravankar and Lele 2006). Nitrogen content greatly influenced fungal growth and enzyme production (Vikineswary et al. 2006).

Effect of Different Concentrations of Calcium on Cellulase Production

The effect of calcium concentration in the medium on cellulase production by *A. terreus* using OPEFB fibre as a carbon source and yeast extract as a nitrogen source is shown in Tables 3. On the other hand, Table 4 shows the results for the fermentation using cynobacterial biomass as a nitrogen source. For fermentations using both types of nitrogen source, $CaCl_2$ greatly influenced growth of *A. terreus* and the production of all cellulase components. For fermentation using yeast extract, increased cellulase activity was observed with increasing $CaCl_2$ concentration, and the highest production of cellulase components: FPase (0.79 U mL^{-1}), CMCase (9.23 U mL^{-1}) β-glucosidase (7.58 U mL^{-1}) and cell concentration (5.21 g L^{-1}), were obtained at 0.3 g L^{-1} $CaCl_2$. At $CaCl_2$ concentration of above 0.3 g L^{-1}, significant decrease in cell growth and cellulase activity were observed (Table 3).

Table 2. Effect Different Nitrogen Sources on Growth of *A. terreus* and Cellulase Production in Fermentation using OPEFB Fibre as Carbon Source

Nitro-gen source	Concentration* (g L^{-1})	Cell Growth (g L^{-1})	FPase* (U mL^{-1})	CMCase* (U mL^{-1})	B-glucosidase* (U mL^{-1})	Yield of FPase# (U g^{-1} cellulose)	Protein (mg mL^{-1})
$(NH_4)_2SO_4$	3	2.3±0.5	0.05±0.01	2.85±0.1	0	7.88	0.75±0.03
	6	2.2±0.1	0.05±0.03	1.74±0.2	0	7.88	0.77±0.01
	9	2.1±0.3	0.04±0.02	0.82±0.1	0	6.30	0.78±0.01
	12	2.0±0.2	0.03±0.03	0.61±0.3	0	4.73	0.79±0.02
Yeast extract	3	4.6±0.1	0.61±0.04	6.57±0.1	5.40±0.04	96.21	1.05±0.04
	6	5.1±0.2	0.76±0.01	8.64±0.3	6.81±0.01	119.87	1.16±0.09
	9	5.1±0.4	0.64±0.02	7.59±0.2	4.95±0.02	100.94	1.17±0.05
	12	5.0±0.3	0.58±0.01	5.51±0.3	4.48±0.07	91.48	1.20±0.08
Peptone	3	4.5±0.2	0.53±0.03	6.16±0.1	4.93±0.03	83.59	1.06±0.01
	6	5.0±0.4	0.69±0.05	7.41±0.2	6.37±0.02	108.83	1.09±0.02
	9	5.0±0.1	0.56±0.01	7.36±0.5	4.86±0.02	88.32	1.11±0.02
	12	4.9±0.2	0.51±0.03	5.14±0.2	4.15±0.01	80.44	1.12±0.03
Urea	3	3.7±0.1	0.31±0.02	4.08±0.1	2.46±0.03	48.89	0.85±0.06
	6	3.3±0.3	0.28±0.01	3.11±0.3	0.84±0.02	44.16	0.88±0.03
	9	3.3±0.1	0.21±0.01	1.23±0.1	0.65±0.04	33.12	0.91±0.02
	12	3.2±0.2	0.10±0.03	0.93±0.2	0.13±0.01	15.77	0.92±0.01
Dry cyanobacterial biomass	3	4.8±0.1	0.68± 0.03	7.23±0.4	6.35±0.11	107.10	1.08±0.02
	6	5.6±0.5	0.82± 0.11	10.1±0.1	8.53±0.21	129.15	1.21±0.05
	9	5.6±0.2	0.71± 0.14	7.11±0.1	5.98±0.13	111.82	1.23±0.01
	12	5.4±0.3	0.62± 0.05	6.05±0.3	5.21±0.09	97.65	1.25±0.04

Values are means of triplicate; ± standard deviation; * Maximum concentration obtained during fermentation; # Yield of FPase calculated based cellulose consumed during the fermentation

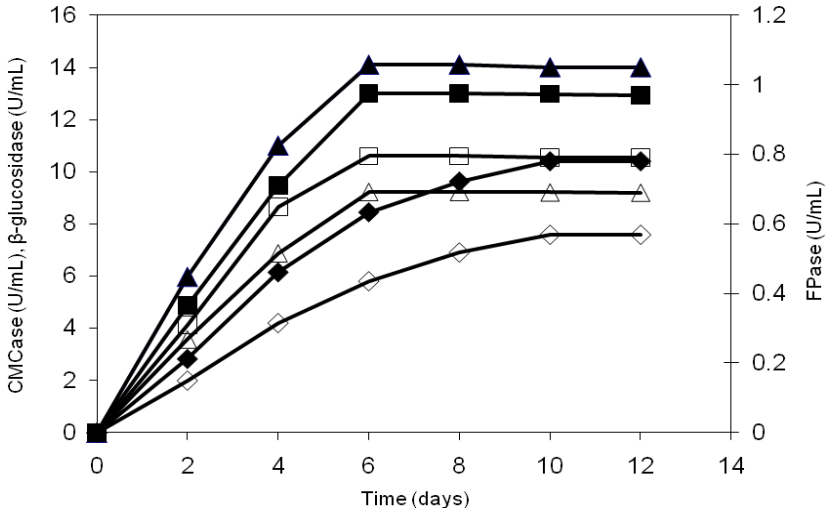

Fig. 1. Comparison of yeast extract and cyanobacterial biomass as N source for cellulase production by *A. terreus*. Values are means of 3 replicates with ± SD of 2% of measure values. Cellulase activities when using cyanobacterial biomass were significantly higher ($p<0.05$) than cultures using yeast extract as N sources. Symbols represent: (▲) CMCase activity; (■) FPase activity; (◆) β-glucosidase. Open symbols: yeast extract; Closed: dry cyanobacterial biomass

Table 3. Effect of Calcium Chloride Concentration on Growth of *A. terreus* and Cellulase Production in Fermentation using OPEFB fiber as a Carbon Source and Yeast Extract as a Nitrogen Source

$CaCl_2$ (g L^{-1})	Cell Growth* (g L^{-1})	FPase Activity* (U mL^{-1})	CMCase Activity * (U mL^{-1})	β-glucosidase Activity* (U mL^{-1})
0	2.65 ± 0.23 [a]	0.24 ± 0.23[a]	2.18 ± 0.38 [a]	3.48 ± 0.16 [a]
0.1	4.98 ± 0.31 [b]	0.69 ± 0.04 [b]	8.64 ± 0.41 [b]	7.04 ± 0.85 [b]
0.2	5.10 ± 0.25 [c]	0.72 ± 0.11 [b]	8.91 ± 0.26 [c]	7.21 ± 0.31 [b]
0.3	5.21 ± 0.32 [c]	0.79 ± 0.03 [c]	9.23 ± 0.54 [c]	7.58 ± 0.18 [c]
0.4	5.19 ± 0.27 [c]	0.70 ± 0.08 [b]	8.77 ± 0.23 [b]	7.15 ± 0.25 [b]
0.5	4.97 ± 0.28 [d]	0.59 ± 0.05 [d]	7.72 ± 0.38 [d]	6.72 ± 0.29 [d]

Values are means of triplicate ± standard deviation
[a-d] Means values in same column with different superscripts are significantly different (P<0.05).
* Maximum cell concentration and enzyme activity obtained during the fermentation

For fermentation using cyanobacterial biomass as N source, the highest activity of FPase (0.97 U mL^{-1}), CMCase (14.1 U mL^{-1}), and β-glucosidase (10.4 U mL^{-1}), as well as cell concentration (6.08 g L^{-1}) were observed at 0.3 g L^{-1} $CaCl_2$ (Table 4). Reduced activity of all component of cellulase was observed at $CaCl_2$ higher than 0.3 g L^{-1}, though growth of *A. terreus* was not significantly changed. This inhibitory effect may be attributed to calcium's interference with the utilization of other essential microelements by fungal cells. In addition, the change in pH toward greater acidity in the presence of chloride ions may be considered as a substantial factor in decreasing the growth and cellulolytic activity of *A. terreus*. Some changes in the permeability of the cell wall may occur with the presence of $CaCl_2$, resulting in greater excretion of the enzyme, which in turn enhances cellulase synthesis (Chen and Wayman 1992).

Table 4. Effect of Calcium Chloride Concentration on Growth of *A. terreus* and Cellulase Production using OPEFB fiber as a Carbon Source and Dry Cyanobacterial Biomass as a Nitrogen Source

$CaCl_2$ (g L^{-1})	Cell Growth (g L^{-1})	FPase* (U mL^{-1})	CMCase* (U mL^{-1})	β-glucosidase* (U mL^{-1})
0	3.06 ± 0.16 [a]	0.39 ± 0.18 [a]	3.87 ± 0.29 [a]	4.73 ± 0.21[a]
0.1	5.29 ± 0.16 [b]	0.71 ± 0.08 [b]	8.23 ± 0.23 [b]	7.84 ± 0.36 [b]
0.2	5.58 ± 0.27 [c]	0.85 ± 0.11 [c]	11.6 ± 0.16 [c]	8.72 ± 0.19 [c]
0.3	6.08 ± 0.12 [d]	0.97 ± 0.05 [c]	14.1 ± 0.31 [d]	10.4 ± 0.11 [d]
0.4	6.17 ± 0.09 [d]	0.81 ± 0.15 [c]	9.36 ± 0.38 [e]	8.31 ± 0.09 [e]
0.5	6.21 ± 0.21 [d]	0.69 ± 0.02 [d]	8.57 ± 0.19 [e]	6.60 ± 0.24 [e]

Values are means of triplicate ± standard deviation.
[a-e] Mean values in same column with different superscripts are significantly different (P<0.05).
* Maximum cell concentration and enzyme activity obtained during the fermentation

Among the nitrogen sources investigated, dry cyanobacterial biomass provided the maximum production for all three main components of the cellulase complex by *A. terreus*, followed by yeast extract, peptone, urea, and $(NH_4)_2SO_4$. The highest activities of FPase, CMCase, and β-glucosidase obtained in fermentation using 6 g L^{-1} dry

cyanobacterial biomass as nitrogen source were 0.82, 10.1, and 8.53 U mL^{-1}, respectively. It is also important to note that the production of β-glucosidase acitivity was significantly enhanced in fermentation using dry cyanobacterial biomass as compared to other nitrogen sources tested in this study. However, β-glucosidase production was greatly reduced at high dry cyanobacterial biomass concentration (8 g L^{-1}), though growth was not inhibited.

Results from this study have demonstrated that a substantial amount of the complete set of cellulase components can be produced by *A. terreus* grown in delignified OPEFB fibres and dry cyanobacterial biomass as the carbon and nitrogen sources, respectively. However, one of the problems related to the economic viability of the enzymatic hydrolysis of cellulose is the low β-glucosidase levels in the culture filtrates containing the cellulase enzymes that have been found to be produced by many fungi (Ghani and Rickard 1990; Woodward and Wiseman 1982). For example, the production of β-glucosidase by *A. terreus* in shake flask with an initial medium pH in the range of 4.0-5.5 reached maximum activity (2.18 U mL^{-1}) after 7 days fermentation (Pushalkar et al. 1995). During enzymatic hydrolysis of cellulose by cellulase, production of cellobiose becomes inhibitory to FPase and CMCase. Hence, high β-glucosidase activity is required to hydrolyze the cellobiose to relieve the inhibition (Ghani and Rickard 1990).

It is also important to note that *A. terreus* was able to grow and produce substantial amounts of cellulase using delignified OPEFB fibres as carbon source, an inexpensive and an abundant carbon source, in a simple batch fermentation process. The yield and productivity achieved by this fungus may be further improved by using other modes of fermentation, such as fed-batch or batch cultures with controlled culture conditions. Further treatments of OPEFB fibres, such as further reduction of the size and optimization of the medium with the addition of cellulase inducers, may also be found to be necessary to further improve the production of cellulase by *A. terreus*. Results from this study showed that water bloom cyanobacteria could be used as C and N supplement for the production of a variety of industrially essential metabolites. This represents the first report on the potential application of cyanobacterial biomass as an additive for the production of cellulase by *A. terreus* in submerged fermentation. A large quantity of this bacterial biomass is generated as waste from various bioprocesses. The use of such wastes not only reduced the projected cost of raw materials for the production but also enhanced enzyme yield. Further research along the lines of this study will open the way for new efforts to utilize this nutritionally essential prokaryote as a cost-effective and eco-friendly tool in fermentation technology to improve the production of industrially important fungal metabolites.

CONCLUSIONS

1. Growth of *A. terreus* and production of all the three main components of cellulase were greatly enhanced in fermentation using biomass of the cyanobacterium *Anacystis nidulans* as nitrogen source as compared to other commercial nitrogen sources such as ammonium sulphate, urea, peptone, and yeast extract.
2. The inhibitory effect of high concentrations of $CaCl_2$ to growth of *A. terreus* and cellulase production was also greatly reduced when cyanobacterial biomass was used as the nitrogen source.

3. The addition of 0.3 g L^{-1} $CaCl_2$ to the medium containing OPEFB fibre and cyanobacterial biomass enhanced further cellulase production, though growth remained unchanged.
4. In fermentation using 10 g L^{-1} OPEFB fibre and 6 g L^{-1} cynaobacterial biomass with the addition of 0.3 g L^{-1} $CaCl_2$, the final activity of FPase, CMCase, and β-glucosidase was 0.97 U mL^{-1}, 14.1 U mL^{-1}, and 10.4 U mL^{-1}, respectively.

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