KINETICS OF CELLULASE PRODUCTION BY *ASPERGILLUS TERREUS* AT VARIOUS LEVELS OF DISSOLVED OXYGEN TENSION IN A STIRRED TANK BIOREACTOR

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In this study the effect of different levels of dissolved oxygen tension (d.o.t) on the production of three main components of extracellular cellulases (FPase, CMCase and β-glucosidase) at a fixed agitation speed by *Aspergillus terreus* was investigated. Growth of *A. terreus* and cellulase production were modeled based on logistic and Luedeking-Piret equations. The results from the model fit well with the experimental data, confirming that the models were appropriate for describing and representing growth and cellulase production at various d.o.t levels. The models showed that the production of FPase and CMCase were growth-associated processes. Cell growth and cellulase production were approximately two-fold higher in a stirred tank bioreactor compared with a shake-flask culture. At a d.o.t of 55% air saturation, cell growth and cellulase production were higher than at low d.o.t (40% air saturation) or high d.o.t (80% air saturation). The highest activities of FPase (2.33 U ml^{-1}), CMCase (51.10 U ml^{-1}), and β-glucosidase (16.18 U ml^{-1}) were obtained at a d.o.t of 55% air saturation, yielding overall productivities of 19.40, 425.00, and 67.40 U $l.h^{-1}$, respectively.

Keywords: Kinetics; Cellulase; Aspergillus terreus; Oil palm empty fruit bunch; Stirred tank bioreactor

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INTRODUCTION

Because of their ability to decompose cellulosic biomass into glucose, cellulase enzymes constitute some of the most extensively investigated multi-component enzyme systems (Hong et al. 2001; Li et al. 2006). Cellulase production is a major factor in the hydrolysis of cellulosic material, and it is essential to unlocking the economic potential of biomass resources. The cellulolytic fungi *Trichoderma* and *Aspergillus* have been widely studied relative to their high abilities of secreting cellulose-hydrolysis enzymes (Zhou et al. 2008).

Aspergillus species play a key role in decay and decomposition due to their ability to produce a wide range of enzymes. Although cellulase production has been reported for several *Aspergillus* species (Lockington et al. 2002; Ong et al. 2004; Yang et al. 2006),

only a few studies have reported on the production of cellulase from *Aspergillus terreus* (Emtiazi et al. 2001; Hui et al. 2010). The amount of cellulase produced is affected by the composition of the nutrient medium and environmental conditions, which can be inducers or repressors of cell density and growth rate (Umikalsom et al. 1998). In addition, cellulase production is influenced by the hydrodynamic conditions in the bioreactor (Garia-Soto Mariano et al. 2006). The influence of the shear rate and agitation intensity on the cellulase production activity in fungal microorganisms has been investigated by many researchers (Ahamed and Vermette 2010; Patel et al. 2009, 2010; Umikalsom et al. 1998). The effect of d.o.t in the culture during fermentation (at a fixed agitation speed) on the synthesis of cellulase has not been adequately studied. In most cases, a high d.o.t was maintained throughout the cellulase fermentation for maximum production.

Oil palm empty fruit bunch (OPEFB) fiber is an appropriate renewable raw material for bioconversion into value added products because it is easily accessible, plentiful, and rich in lignocellulose. Improved cellulase production by *A. terreus* has been previously reported, and the optimized conditions in shake-flask and a stirred tank bioreactor were achieved through response surface methodology (Shahriarinour et al. 2010, 2011c). No significant interaction was observed between d.o.t (31% to 88% air saturation) and initial pH (4.7 to 6.2) on the cellulase production. Despite all these studies, no report have been published which deals with the kinetics and modeling of cellulase production particularly at large scale. A mathematical model based on some equations can be used to describe the relationship of the experimental variables and to explain the behavior of the process itself. The information gathered may be useful in optimization and designing of the fermentation process for improvement of the cellulase production. The kinetic equation is a basic element in the scale-up of fermentation process, and the validity of such general model equations must then be tested for each particular fermentation process from the experimental data. The kinetic study is useful in obtaining kinetic parameter values that describes the kinetic behavior. Hence, the main objective of this study was to investigate the kinetics of *A. terreus* growth and cellulase production, which are FPase, CMCase, and β-glucosidase, using a simplified model in a stirred tank bioreactor using oil palm empty fruit bunch (OPEFB) fiber. Experimental data from batch fermentation of cellulase at various d.o.t levels on the growth and the production of the three major components of cellulase (Shahriarinour et al. 2011c) were analyzed in order to form the basis for a kinetic model of the process. The model may allow better understanding and control of cellulase production by *A. terreus*. The distribution of the three major components of cellulase of *A. terreus* was also identified in fermentation, using soluble CMC as carbon source, and all the three main components of cellulase activities in both the extracellular and cell associated (intracellular) fractions were analyzed.

MATERIALS AND METHODS

Microorganism

The mould *Aspergillus terreus* used in this study for cellulase production was isolated from a sample collected from the oil palm empty fruit bunch (OPEFB) compost (Shahriarinour et al. 2011a). It was grown on potato dextrose agar (PDA) at 30 °C for 7

days to allow the development of spores and then stored at 4 °C until use in inoculum preparation.

Medium and Inoculum Preparation

The basal medium as proposed by Mandels and Weber (1969) was used for cellulase production. In all experiments, yeast extract (8 g L^{-1}) was added as the only nitrogen source and delignified OPEFB fibre (13.90 g L^{-1}) as a carbon source to the basal medium for cellulase production (Shahriarinour et al. 2010). 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. For inoculums preparation, spores were harvested from the PDA slants using a sterile 0.01 % (v/v) Tween 80 solution with the aid of wire loop. The spore suspension containing, on average 6×10^7 spores mL^{-1}, was used as an inoculums in all fermentation.

Crude Enzyme Extraction

After an appropriate time of incubation, the cultures were harvested at 24 h intervals by centrifugation at 15,000 rpm at 4 °C for 15 min using a refrigerated ultracentrifuge (SORVALL RT7 PLUS). The supernatant was then analyzed for soluble protein and extracellular enzyme activities.

Fermentations in Bioreactor

The fermentations were carried out in 2 L stirred tank bioreactor (Biostat B, Melsungen, Germany); the vessel was made of 5 mm thick borosilicate glass. The vessel had an internal diameter of 0.13 m and liquid height of 0.12 m. Two six-bladed Rushton turbine impellers with a diameter (D) of 52 mm were mounted on the impeller shaft used for agitation. The bioreactor was equipped with temperature and dissolved oxygen controllers. Air was supplied into the culture via a single air sparger (0.1 mm internal diameter). During the fermentation, agitation speed (N) was fixed at 225 rev/min (impeller tip speed=πND =0.613 m/s), and d.o.t in the culture broth was controlled using a sequential cascade control of airflow rate. The maximum and minimum set points of permitted airflow rates were 1.5 L/min and 0.1 L/min, respectively. A polarographic dissolved oxygen probe (Ingold, Urdorf, Switzerland) was used to measure d.o.t in the culture. The output of the oxygen master controller functions directly on the set point input value of the airflow controller. In all cases, d.o.t was effectively controlled to within ± 2 % of the required set points (40, 55, 60, and 80 % saturation). The initial pH (5.5) of the culture were adjusted to appropriate values either by addition of 1 N HCl or 1N NaOH. The temperature within the bioreactor was controlled at 29 °C. Spore suspension (15 mL) was inoculated into the bioreactor containing 1.5 L medium. During the fermentation, samples were withdrawn at regular time intervals for analysis.

Analytical Procedure

To estimate mycelium concentration, the chemical method based on the measurement of glucosamine was selected, while the physical separation of mycelium from the OPEFB fibres for measurement was not possible (Khan and Strange 1975). This method required the production of chitosan from fungal chitin and liberation of glucosamine from chitosan using a chemical reaction. Chitin is an insoluble linear polymer of α-1, 4-linked-*N*-acetylglucosamine units produced by most fungi but not found in plant tissues. Absorbance due to the resulting of the reaction was measured with a spectrophotometer (Model Shimadzu, UV-1601 PC) at 650 nm. The glucosamine concentration in the mycelia of *A. terreus* was found to be proportional to the mycelial weight and remained constant throughout the growth phases.

Carboxymethylcellulase (CMCase) activity was determined by measuring spectrophotometrically the reducing sugar produced from 2% (w/v) carboxymethyl-cellulose, while filter-paper-hydrolysing (FPase) activity was determined by estimating the reducing sugar liberated from filter paper (Wood and Bhat 1988). Both reactions were carried out in 0.05 M sodium acetate buffered at pH 5 and incubated at 50°C. The reaction time was 30 min and 60 min for CMCase and FPase, respectively. One unit of CMCase or FPase activity was defined as 1 µmol reducing sugar released/mL enzyme/min. Meanwhile, β-glucosidase was determined using the method described by Wood and Bhat (1988). In this method, *p*-nitrophenol released from *p*-nitrophenyl-β-D-glucopyranoside (Fluka) was measured using a spectrophotometer (Shimadzu, UV-1601 PC). One unit of β-glucosidase activity is defined as 1 µmol *p*-nitrophenol liberated/mL of enzyme/min, while the specific activity is defined as units/mg protein. Protein content was determined by the method of Bradford using bovine serum albumin as a standard (Bradford 1976).

Mathematical Approach

The following simplified batch fermentation kinetic models for cell growth and product formation based on logistic and Luedeking-Piret equations were used to evaluate the kinetics of production of the three major components of cellulase (FPase, CMCase, and β-glucosidase) by *A. terreus*,

Cell growth
$$dx/dt = \left[\mu_{max}\left(1 - x/x_{max}\right)\right]x \quad (1)$$

Product formation

CMCase
$$dA/dt = p\left(dx/dt\right) + qx \quad (2)$$

FPase
$$dB/dt = a\left(dx/dt\right) + bx \quad (3)$$

β-glucosidase
$$dC/dt = e\left(dx/dt\right) + fx \quad (4)$$

where x is the cell concentration (g L^{-1}); x_{max} is the maximum cell concentration (g L^{-1}); μ_{max} is the maximum specific growth rate (h^{-1}); A is the CMCase activity (U mL^{-1}); B is the FPase activity (U mL^{-1}); C is β-glucosidase activity (U mL^{-1}); p is the growth

associated coefficient for CMCase formation (U g-cell^{-1}); q is the non-growth associated coefficient for CMCase formation (U g-cell.h^{-1}); a is the growth associated coefficient for FPase formation (U g-cell^{-1}); b is the non-growth associated coefficient for FPase formation (U g cell.h^{-1}); e is the growth associated coefficient for β-glucosidase formation (U g-cell^{-1}); and f is the non-growth associated coefficient for β-glucosidase formation (U g-cell.h^{-1})

The kinetic models (Eq. 1-4) were fitted to the experimental data using a non-linear regression with a Marquadt algorithm using MATLAB computer software (MATLAB 7.2, The MathWorks. 2006 U.S.). The model parameter values were first evaluated by solving Equations (1-4), and then the computer program was used as an exploritory method to minimize the sum of squares of the differences between the predicted and measured values. The predicted values were afterward used to simulate the profiles of cell, substrate, and enzymes concentrations through the fermentation. In order to find out whether the deviations between the experimental and calculated data are significant or not-significant, data were analysed statistically using the unpaired *t*-test of the SAS Institute Inc. (Allison 2010) with a significant probability of 5%.

RESULTS AND DISCUSSION

Distribution of Cellulase in *A. terreus*

The distribution of the three major components of cellulase (FPase, CMCase, and β-glucosidase) of *A. terreus* was determined in the course of fermentation using soluble CMC as carbon source; all three main components of cellulase activities in both the extracellular and cell associated (intracellular) fractions were analyzed. Table 1 shows the maximum production and location of FPase, CMCase, and β-glucosidase in *A. terreus* using soluble CMC as the carbon source. The FPase and CMCase of *A. terreus* were fully extracellular, while β-glucosidase was both an extracellular (cell-free) and a cell-bound (surface bound and intracellular) enzyme.

Table 1. Localization of Cellulase in *Aspergillus terreus* Grown in Shake-Flask Culture

Enzyme	Intracellular or cell bound (U ml^{-1})	Extracellular or free (U ml^{-1})
FPase	0	0.71 ±0.03
CMCase	0	8.16 ±0.71
B-glucosidase	10.23±0.74	5.85 ±0.53

Values are ± standard deviation (based on three replicates)

Generally, in fungi, β-glucosidase is mainly intracellular and this is true in the case of *Trichoderma reesei (Hypocrea jecorina*), for which about 50 to 100% of β-glucosidase can be cell bound during cultivation (Saloheimo et al. 2002). The amount of extracellular β-glucosidase of *T. reesei* is less than in the mycelium at all stages of growth. Unlike *T. reesei,* the distribution of β-glucosidase in *A. terreus* was dependent on the age of the mycelium (Ahamed and Vermette 2010). Most β-glucosidase was intracellular when the culture was in the active stage of growth (Umikalsom et al. 1998).

It is possible that this enzyme is released by autolysis, i.e., when the culture is in the stationary phase. However, it is often difficult to show whether an enzyme found in medium is actively secreted by growing cells or is passively secreted because of cellulolysis (Linger et al. 2010).

Production of Cellulase in a Shake-Flask

Typical time course results are shown in Fig. 1 for free cellulase production by *A. terreus* in a shake-flask cultures. The basal medium as proposed by Mandels and Weber (1969) was used with an addition of yeast extract (8 g L^{-1}) as the only nitrogen source and treated OPEFB fibres (13.90 g L^{-1}) as a carbon source. The CMCase and FPase activities were increased throughout the growth cycle and reached their maximum during the stationary phase. The rate of free β-glucosidase production was low during the initial stages of the fermentation and very rapid towards the end of the fermentation. In contrast to CMCase and FPase production, free β-glucosidase was produced during the stationary phase. Figure 1 shows that the data calculated from the kinetic models (Eq. 1-4) fit the experimental data with more than 90% confidence. From *t*-test analysis, the deviations between the calculated and experimental data were not significant at a significance probability of 5%. This result suggests that the proposed models based on logistic and Luedeking-Piret equations are sufficient to simulate growth of *A. terreus* and the production of the cellulase complex.

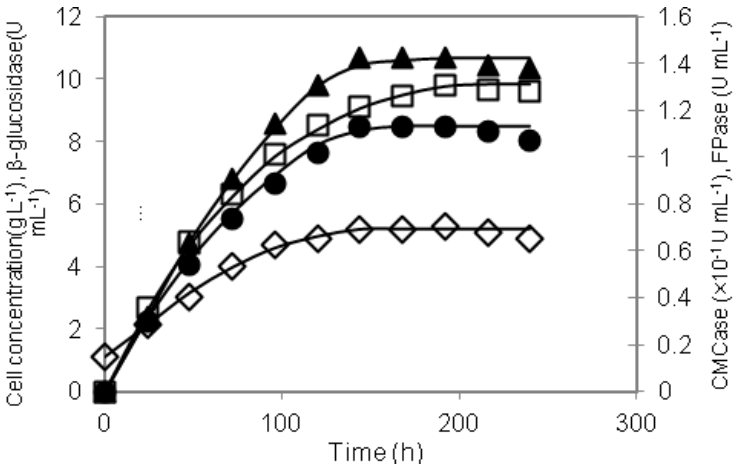

Fig. 1. Comparison between the calculated and experimental data for shake-flask fermentation of cellulase by *A. terreus.* ◇, Cell concentration; ●, FPase activity; ▲, CMCase activity; □, β-glucosidase activity; (—) data calculated according to Eq. 1-4

The kinetic parameter values calculated by the models for fermentation in a shake-flask can be used to describe the kinetics of the production of all three main components of cellulase by *A. terreus* (Table 2). The non-growth associated rate constants for FPase (*b*) and CMCase (*q*) were zero, suggesting that production of these enzymes was growth associated processes. Instead, the production of free β-glucosidase was a mixed process indicated by non-zero values of the growth associated (*e*) and non- growth associated rate constants (*f*).

Table 2. The Kinetics Parameter Values of Fermentation in Shake-Flask and Bioreactor for Production of All Three Main Components of Cellulase Production by *A. terreus*

Kinetic Parameter	Shake-flask	Bioreactor at different d.o.t levels (% saturation)			
		40	55	60	80
X_{max}(g/L)	5.20 ± 0.02[a]	11.54 ± 0.01[b]	13.07 ± 0.03[c]	12.68 ± 0.02[d]	7.15 ± 0.01[e]
μ_{max}(h^{-1})	0.0041± 0.0003[a]	0.0069 ± 0.0002[b]	0.0071± 0.0002[b]	0.0070 ± 0.0001[b]	0.0054 ± 0.0003[c]
FPase B_{max}(U/m L)	1.13 ± 0.01[a]	1.95±0.01[b]	2.33 ± 0.04[c]	2.24 ± 0.02[d]	1.36 ± 0.03[e]
a (U/g cell)	0.238 ± 0.05[a]	0.207 ± 0.03[b]	0.287 ± 0.06[c]	0.273± 0.07[d]	0.161 ± 0.02[e]
b (U/g cell.h)	0	0	0	0	0
CMCase A_{max}(U/m L)	14.25 ± 0.04[a]	45.6 ± 0.07[b]	51.10 ± 0.12[c]	49.2 ± 0.09[d]	22.4 ± 0.06[e]
p (U/g cell)	2.76 ± 0.05[a]	3.55 ± 0.02[b]	4.28 ± 0.05[c]	3.83 ± 0.02[d]	2.96 ± 0.03[e]
q (U/g cell.h)	0	0	0	0	0
β- glucosidase C_{max}(U/m L)	9.86 ± 0.03[a]	14.66 ± 0.05[b]	16.18 ± 0.06[c]	15.83 ± 0.04[d]	11.5 ± 0.09[e]
e (U/g cell)	0.819 ± 0.06[a]	0.515 ± 0.01[b]	1.039 ± 0.08[c]	0.856 ± 0.02[d]	0.267 ± 0.04[e]
f (U/g cell.h)	0.118 ± 0.009[a]	0.0049 ± 0.0004[b]	0.0053 ± 0.0002[b]	0.0051± 0.0006[b]	0.0048 ± 0.0003[b]
B_{max}/X_{max} (×1000 U/g)	0.217 ± 0.01[a]	0.169 ± 0.02[b]	0.178 ± 0.01[b]	0.176 ± 0.03[b]	0.19 ± 0.02[c]
A_{max}/X_{max} (×1000 U/g)	2.74 ± 0.04[a]	3.96 ± 0.02[b]	3.90 ±0.03[c]	3.88 ± 0.02[c]	3.13 ±0.05[d]
C_{max}/X_{max} (×1000 U/g)	1.89 ± 0.03[a]	1.27 ± 0.01[b]	1.23 ± 0.02[c]	1.24 ±0.01[c]	1.60 ±0.04[d]
Yield (U FPase / g cellulose	178.2 ± 1.5[a]	307.5 ± 1.9[b]	367.5 ± 2.3[c]	353.3 ± 3.2[d]	214.5 ± 2.1[e]
Productivity (U FPase/ l.h)	9.41± 0.16[a]	15.25 ± 0.22[b]	19.41± 0.25[c]	18.66 ± 0.23[d]	11.33 ± 0.19[e]

[a-e] Values in the same row with different superscripts are significantly different (P<0.05)
Values are the means of triplicate ± standard deviation

Cellulase Production in the Bioreactor at Various Dissolved Oxygen Tension Levels

Growth and production of three major components of cellulase in the fermenter at different d.o.t levels by *A. terreus* are shown in Fig. 2. The models of growth and cellulase production in the stirred tank bioreactor were comparable to that achieved in a

shake-flask. However, growth and cellulase production by *A. terreus* were significantly affected by the d.o.t levels during the fermentation. Growth and cellulase production were high at a d.o.t of 55% air saturation, while the growth was inhibited at high d.o.t (80% air saturation). The effect of d.o.t on cellulase production was similar to the effect on growth, indicating that cellulase production was directly associated with the rate of growth and cell concentration in the culture. Figure 2 also shows how the calculated points from the proposed models (Eqs. 1 to 4) fit the experimental data that determine fermentation at various d.o.t levels. In all cases, the calculated data fit well with the experimental data with greater than 85 % confidence.

Table 2 shows the evaluation of the function and the kinetic factor values of cellulase production in the stirred tank fermenter at different d.o.t levels. In all cases, the values of b and q were zero, while the value of f was not zero. These results explain a previous outcome of the experiment using a shake-flask: the FPase and CMCase production were growth-associated processes, while the production of free β-glucosidase was a mixed-growth-associated process. The highest x_{max}, A_{max}, B_{max}, and C_{max} values were obtained at d.o.t levels of 55 % air saturation. In addition, the μ_{max} was also higher at d.o.t levels of 55% air saturation. However, the values of a, p, e and f were significantly different at various d.o.t levels.

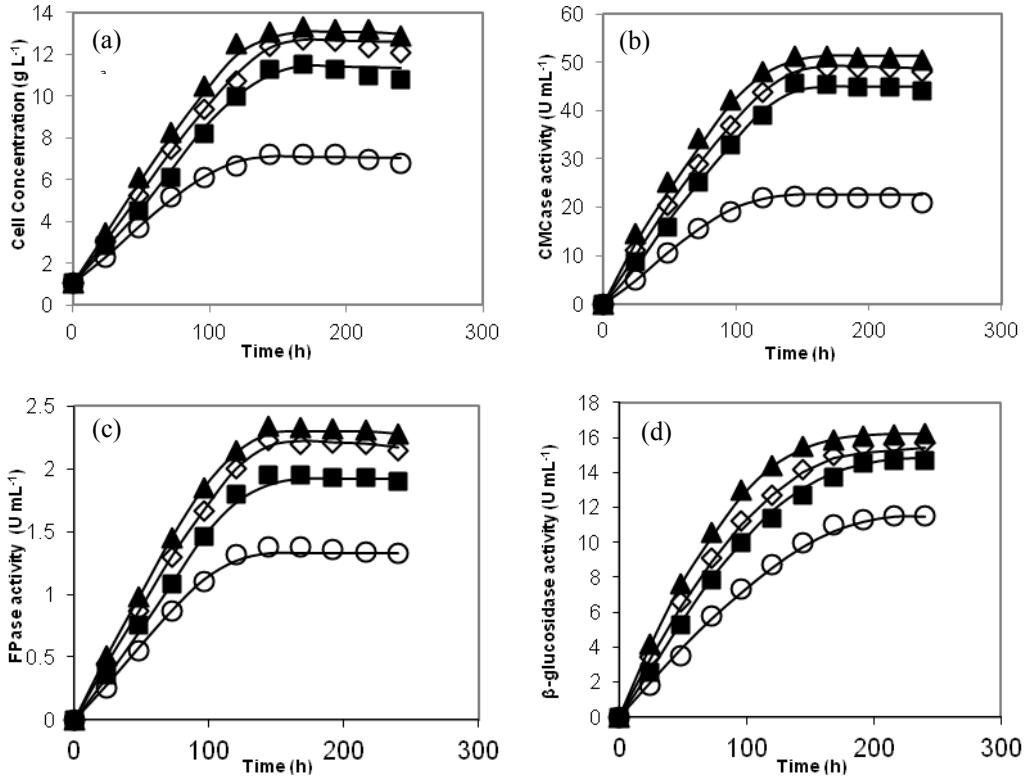

Fig. 2. Comparison between the calculated data and the experimental data for batch fermentation of cellulase by *A. terreus* in the bioreactor at different d.o.t levels. (a) Cell concentration; (b) FPase activity; (c) CMCase activity; (d) β-glucosidase activity. ■ 40%, ▲ 55%, ◇ 60%, ○ 80%; (—) data calculated according to Eqs.1 through 4

During the fermentation, x_{max} was different with various d.o.t levels in the culture. Therefore, the maximum amount of cellulase components should be determined per unit cell weight (B_{max}/x_{max}, A_{max}/x_{max}, and C_{max}/x_{max}) to signify whether the fungi produced the enzyme efficiently under certain culture conditions or if the production was simply proportional to cell mass. The values of B_{max}/x_{max} and A_{max}/x_{max} were not significantly different at various d.o.t levels (Table 2). This result suggests that CMCase and FPase production corresponded to cell weight. However, the value of C_{max}/x_{max} (1.60 × 1000 U/g) was the highest at 80% air saturation, while the value was not significantly different at 40%, 55% and 60% air saturation, signifying that the efficiency of cell to produce β-glucosidase was improved at very high d.o.t. The highest yields of FPase (367.50 U/g-cellulose and 19.41 U/l.h, respectively) were obtained at a d.o.t of 55% air saturation. Higher shear rates in the bioreactor disrupt the fungal mycelium and reduce cellulase production (Lejeune and Baron 1995). At a fixed agitation speed (shear rate) and different d.o.t levels, which were controlled via the air flow rate, the production of all three main components of cellulase by *A. terreus* was affected.

During excess oxygen conditions (80% air saturation), the production of CMCase and FPase were significantly reduced. Additionally, improved cellulase production at a d.o.t level that was higher than the critical value of dissolved oxygen (C_{crit}) was detected in *T. reesei* (Lantz et al. 2010). The ability of *A. terreus* to produce β-glucosidase (as measured by C_{max}/x_{max}) was increased at high d.o.t (80 % air saturation); however, growth was significantly inhibited, which resulted in a reduction in the maximum activity of free β-glucosidase. Typically, higher d.o.t and agitation intensity were required to enhance β-glucosidase production. The production of all three main components of cellulase in the stirred tank bioreactor, where the d.o.t was controlled at 55 % air saturation, was approx.-imately two-fold higher than the production in a shake-flask. The high shear rate created by the agitation using an impeller may have caused excessive breakdown of the OPEFB fibers, which reduced the fiber size and increased the degree of attack by microbes and enzymes.

In a shake-flask culture, the fungus grew in the form of a pellet with an atypical diameter between 0.5 and 1 mm, whereas in the bioreactor, the fungus grew in the form of mycelia. The low shear rate in a shake-flask culture facilitated growth into the form of a pellet, and this had been detected in other cellulase producers, such as *T. reesei* (Lejeune and Baron 1995). The pelletization of hyphae in the shake-flask fermentation led to severe mass transfer limitation, which was responsible for lower μ_{max} compared with the μ_{max} for growth in the bioreactor at 55% air saturation. However, the pelletization enhanced the leakage of enzyme from inside the pellet. Therefore, the reduction in cellulase production in the shake-flask culture was attributed to the partial lack of dissolved oxygen and to the leakage of enzymes from the internal parts of the pellet.

In the bioreactor, where a high d.o.t level (80% air saturation) was maintained, growth of *A. terreus* was inhibited. The influence of high d.o.t levels in the inhibition of microbial growth in the bioreactor has been accounted for by noting that the surplus oxygen is, at some level, toxic to the growth of some microorganisms (Lee et al. 2003). Superoxide radicals (O_2^-), which are critical to cell metabolism, are major inhibitors of cell growth (Lund et al. 2000). A simple model using logistic and Luedeking-Piret equations was found sufficient to describe growth of *A. terreus* and the production of all

three main components of cellulase. From the model, it was recognized that the production of FPase and CMCase were growth-associated, while free β-glucosidase production was mixed. In order to attain a high amount of β-glucosidase in the culture filtrate, the fermentation should be extended after growth has reached stationary phase.

The cellulase produced by *A. terreus* exhibited a high level of β-glucosidase activity. The ratio of β-glucosidase to FPase for cellulase obtained in fermentation at 55% d.o.t was about 8:1, and this was significantly higher than the ratios for *T. reesei* (Doppelbauer et al. 1987), *T. lignorum* (Baig 2005), *Fusarium oxyporum* (Ramanathan et al. 2010), *Gliocladium virens* (Gomes et al. 1989), and *Chaetomium globosum* (Umikalsom et al. 1998), which were in the range 0.44 to 7. The importance of a high proportion of β-glucosidase, because of the cellulose hydrolysis, was increased by the addition of β-glucosidase since it reduces inhibition caused by the end product of cellobiose hydrolysis (Berlin et al. 2005).

CONCLUSIONS

1. A considerable agreement among the calculated data and the experimental data for both cell growth and the production of all the three main components of cellulase was observed, suggesting that the recommended model based on logistic and Luedeking-Piret equations was sufficient to describe the growth of *A. terreus* and the three main components of cellulase production.
2. FPase and CMCase production were growth-associated processes (fully extracellular), while the production of free β-glucosidase was a mixed-growth-associated process (both an extracellular and a cell bound).
3. Cell growth and cellulase production were higher at 55% saturation of d.o.t as compared to the production at low condition oxygen (40% saturation) and at very high d.o.t (80% saturation).
4. The production cellulase in stirred tank bioreactor with d.o.t maintained at 55% air saturation was improved approximately two-fold compared to the production of the shake-flask culture.

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