Malaysian Journal of Microbiology, Vol 9(3) 2013, pp. 227-236
http://dx.doi.org/10.21161/mjm.49712

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Investigation of newly developed solid state fermenter on carboxymethyl cellulase production

Lee Chee Keong[1*], Darah Ibrahim[1] and Ibrahim Che Omar[2]

[1]Industrial Biotechnology Research Laboratory, School of Biological Sciences, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia.
[2]Kampus Jeli, Universiti Malaysia Kelantan, Beg Berkunci No 100, 17600 Jeli, Kelantan, Malaysia.
Email: cklee1311@yahoo.co.uk

Received 11 December 2012; Received in revised form 13 March 2013; Accepted 17 March 2013

ABSTRACT

Aims: Enzyme (cellulase) contributes 10% to overall cost in bioethanol production from lignocellulosic biomass. This means that the cost for bioethanol production will be reduced if cellulase can be produced using cheaper method. Compared with submerged fermentation, it is recognized that the cost for cellulase production using solid state fermentation (SSF) process is much cheaper. The present study aimed to optimize cellulase production via SSF process using agro-industrial residual as substrate.
Methodology and result: Newly developed solid state bioreactor, FERMSOSTAT had been evaluated in cellulase production using local isolate *Aspergillus niger* USM AI 1 grown on sugarcane baggase and palm kernel cake as substrates at 1:1 (w/w) ratio. Under optimized SSF conditions of 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; aeration at 4 L/h.g fermented substrate for 5 min and mixing at 0.5 rpm for 5 min, about 62.6 U/g of CMCase activity obtained. At the same time, comparative studies of the enzyme production under the same SSF conditions indicated that CMCase produced by *Trichoderma reesei* was about 9% lower compared with *A. niger* USM AI 1.
Conclusion, significance and impact of study: It can be concluded that the performance of newly developed SSF fermenter is good since it can used to produce CMCase enzyme with reasonable good title (863% increased in CMCase production after optimization). Thus, this newly developed SSF bioreactor has highly potential be used as prototype for larger scale bioreactor design.

Keywords: solid state fermentation, cellulose, *Aspergillus niger*, sugarcane bagasse, palm kernel cake, FERMSOSTAT

INTRODUCTION

Malaysia spent about RM 80 million yearly in importing various types of enzymes for used in local industries and some for research purposes. Some of the reasons include high cost for development of facilities and equipment as well as poor support from the local industries in enzyme applications. Considering the fact that enzymes consumption in Malaysia will increase significantly in the near future, the production of enzyme within the country will definitely a cost saving approach for local industries. Compared with submerged fermentation process, SSF process possesses many advantages for enzyme production such as smaller working space and financial requirement. Based on this reasons, SSF technology has been given attention and have achieved to the commercial level in countries like India and Eastern Europe. Besides, the advantages associated with SSF are higher productivity, improved product recovery, reduced energy requirements, less effluent generated and simplicity of the equipment used (Kumar and Lonsane,1990; Wang and Yang, 2007; Pandey *et al.*, 2008). Besides that, the substrate itself contains most of the necessary nutrient required by the microbial to grow and this makes the fermentation media simpler (Cannel and Moo-Young, 1980; Steinkraus, 1984; Kumar and Lonsane, 1990; Raimbault, 1998; Perez-Guerra *et al.*, 2003). Furthermore, countries like Malaysia with abundant agro-industrial residual (> 5 million tons per year) will be a great advantage not only for the utilization of the agro-industrial residual for the production of enzymes, but also helps to lessen the undesirable impact to the environment.

This study focus on the production of cellulase (CMCase activity) via SSF process using agro-industrial residual such as palm kernel cake and sugarcane baggase as substrate. Local isolate *Aspergillus niger* USM AI 1 was used for the enzyme production in a newly developed solid substrate fermenter. In addition, factors that affect CMCase production such as amount of substrate, incubation temperature, moisture content, aeration rate and aeration time as well as mixing rate and mixing intensity were investigated and optimized.

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

MATERIALS AND METHODS

Microorganism

In this study *A. niger* USM AI 1 was used for the production of cellulase (CMCase) enzyme. It's a local isolate, which was isolated from decayed wood obtained from the Northern Region of Peninsular Malaysia in year 2002 (Lee *et al.*, 2011).

CMCase production via solid state fermentation process

Production of spore and preparation of SSF inoculum

Fungal spores were obtained by growing the culture on Potato Dextrose Agar (Amresco, Solon, Ohio, USA) at a room temperature (28 °C ± 2 °C) in a 250 mL medium bottle. The spores were harvested after 4-5 days of cultivation with sterile distilled water containing 0.1% (w/v) Tween-80 (Smits *et al.*, 1996). The spore suspension was passed through a 0.5 mm sieve to eliminate mycelia and the spore concentration was estimated by the method described by Raimbault and Alazard (1980). Twenty percent (v/w) of spore suspension at $1x10^8$ spore/mL was mixed with the previously autoclaved growth medium. This medium was used as inoculum for SSF process (Lee *et al.*, 2011).

Substrates preparation and sterilization

Sugarcane baggase (≤ 2 mm diameter) and palm kernel cake (PKC) (≤ 0.5 mm diameter) at 1:1 ratio was used as substrates for CMCase production. The substrates were mixed by hand and transfer into the fermenter (FERMSOSTAT) through the substrate port located on the top of the fermenter vessel. The substrate was pre-treated cum sterilized *in situ* with hot air sterilization at 130 °C for 3 h (Lonsane *et al.*, 1992). After the sterilization process, the fermenter and substrates were allowed to cool down to ambient temperature before inoculation of the substrates were carried out. PKC (0.5 kg) and sugarcane baggase (0.5 kg) were put into the fermenter for single run of SSF process (Lee *et al.*, 2011).

Medium preparation and inoculation process

The growth medium used in SSF process consisted of mineral salts and trace elements (Pang *et al.*, 2006). The pH was adjusted to 7.0 prior to autoclave but the pH of the growth medium was not controlled during the course of fermentation. For inoculation, spraying nozzles was used to spray the spore suspension and growth medium over the solid substrate. Mixing of the substrate by impeller continued during inoculation. The air supplied was discontinued after all the spore suspension was sprayed on the substrate. However, mixing was continued for another 15 min in order to allow the absorption of the growth medium by the solid substrate (Lee *et al.*, 2011).

Sampling of substrates and enzyme extraction

SSF process for the production of CMCase by *A. niger* USM AI 1 was carried out for 6 to 7 days. About 10 g of substrate was sampling out from each of the sampling port (3) for every 24 h interval. Prior to the sampling process, the substrate was mixed for 5 min. The sample was used to determine the CMCase activity, moisture content and glucosamine content (Lee *et al.*, 2011). The extraction of enzyme was performed by mixing the fermented substrates with distilled water containing 0.1% (w/v) Tween-80. The mixture was stand still for 2 h under room temperature. The solid residue was separated from the enzymic solution by filtration through Whatman 1 filter paper (Aikat and Bhattacharyya, 2000).

Determination of CMCase activity, fungal growth and moisture content

The enzyme of interest to be determined in this study is carboxymethyl cellulase activity (CMCase) activity. The enzyme activity was expressed as unit (U) per g of fermented substrate. Enzymes production was express as unit (U) per mg of glucosamine content of the fungal growth. All experiments were carried out in triplicates and the results were presented as mean of the triplicate results. The CMCase activity was assay according to the method described by Gessesse and Gashaw (1999). The released sugar was measured spectrophotometrically at 575 nm using glucose as the standard. One unit of CMCase activity was defined as the amount of enzyme that releases 1 µmol of glucose per minute under the above assay conditions. While, the growth of *A. niger* USM AI 1 was examined by determined the glucosamine content of the fungus as described by Swift (1972). The glucosamine was measured spectrophotometrically at 530 nm using glucosamine as standard. The moisture content of the substrate was determined by measuring the change of weight of approximately 4.0 g of fermented substrate before and after dried in an oven at 80 °C for at least 48 h (Nagel *et al.*, 2001).

Optimization of CMCase production

Factors that affecting the growth of *A. niger* USM AI 1 and the production of CMCase were examined and optimized throughout this study using developed FERMSOSTAT. These were including amount of substrates, moisture content, incubation temperature, aeration rate and aeration time, mixing rate and mixing intensity. The optimum condition obtained from each experiment was used in the following experiments unless otherwise stated (Lee *et al.*, 2011).

Comparison of CMCase production with *Trichoderma reesei*

Trichoderma reesei was used as a benchmark for the production of CMCase by *A. niger* USM AI 1 because it is an established culture for cellulases and xylanases

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

production. In order to compare the enzymes production, the determined optimum SSF conditions were used by *T. reseei* on the production of CMCase.

Statistical method

The significance of difference between each test variable were determined using one way ANOVA analysis and Least Significance Test, computed using SPSS version 11.5 software. All tests were done with a confidence interval of 95%.

RESULTS AND DISCUSSION

Effect of amount of substrates

CMCase activity was decreased significantly with the increasing amount substrate used in the fermentation process (Figure 1). The highest CMCase activity of 47.0 U/g was obtained when 0.5 kg of substrate was used in the fermentation process. When the amount substrate used increased 50% to 0.75 kg and 100% to 1.0 kg, about 73% and 86% dropped in the CMCase activity was observed, respectively. In addition, statistical analysis indicated significant differences (p<0.05) in CMCase activity using 0.5 kg of substrate compared with 0.75 kg and 1.0 kg of substrate. The results was in agreement with Roussos *et al.* (1993), who reported about 60 U/g or 44% reduction in CMCase activity when the amount of substrate used in the fermentation process in *Zymotis* was increased from 4 kg to 12 kg. Less than 7.0 U/g of CMCase activity was detected when 1.0 kg of substrate was used in the fermentation process. Also shown in Figure 1 regardless the different amount of substrate used, CMCase activity was increased and reaching maximum level after 4 days of fermentation process. However, unlike 0.75 kg and 1.0 kg of substrate used CMCase activity increased marginally from day 1 and reaching maximum level after day 4 of fermentation when 0.5 kg of substrate was used. However, Kang *et al.* (2004) obtained the highest CMCase activity of about 130 U/g after 5-6 days of fermentation process using mixture of rice straw and wheat bran. Enzymes production was expressed as unit (U) per mg of glucosamine content of the fungal growth. CMCase production of 14.4 U/mg_G glucosamine was obtained at the highest CMCase activity (Figure 1). Nevertheless, the highest CMCase production was not parallel with the highest CMCase activity detected. While, the highest CMCase production of 3.4 U/mg_G and 1.9 U/mg_G glucosamine was obtained when 0.75 kg and 1.0 kg of substrate were used, respectively. Enzyme production profiles were quite similar with enzymes activity profiles. These possibly maybe due to the enzymes produced by *A. niger* USM AI 1 are growth dependent.

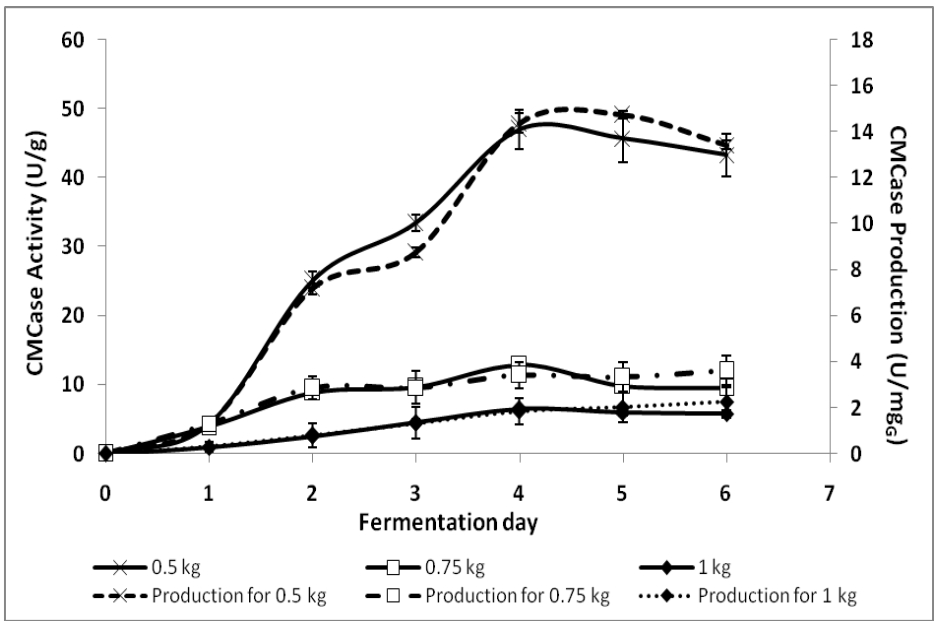
Effect of moisture content

Moisture content plays a crucial role in any SSF process because this variable had influenced on growth and biosynthesis as well as secretion of different metabolites such as enzymes. Distinctively different in CMCase activity was observed when the moisture content used ranged from 65 to 75% (w/w) (Figure 2). As shown in the Figure, different moisture content resulted in different time course required for CMCase activity to achieve maximum level. These can be seen when 65, 70 and 75% (w/w) of moisture content were used, CMCase production achieving maximum level after 3, 5 and 4 days of fermentation process, respectively. Under the optimum moisture content of 70% (w/w), the highest CMCase activity of about 61.0 U/g was gained against 46.0 U/g when 75% (w/w) of moisture content was used. In addition, statistical analysis indicated significant differences (p<0.05) in CMCase activity at 70% (w/w) moisture content compared with other moisture content used in fermentation process. On the other hand, Krishna (1999) also obtained the highest CMCase activity of 9.6 U/g under optimum moisture content of 70% (w/w) when *Bacillus subtilis* (CBTK 106) was used in SSF process. However, the result obtained in this work was in disagreement with Panagiotou *et al.* (2003), who obtained optimum CMCase activity of 210.0 U/g at 80% (w/w) moisture content. In addition, marginally different in CMCase activity was detected at lower moisture content of less than 70% (w/w) or higher than 70% (w/w). This can be seen when about 19% and 24% dropped in CMCase activities were detected when less than 70% (w/w) and higher than 70% (w/w) of moisture content were used, respectively. However, no significant differences (p>0.05) in CMCase activity obtained using moisture content of 65% (w/w) and 75% (w/w). On the other hand, CMCase production of about 18.1 U/mg_G and 15.6 U/mg_G glucosamine were obtained after 6 days of fermentation process when 70% and 75% (w/w) of moisture content were used, respectively. While, 65% (w/w) moisture content resulted about 13.0 U/mg_G glucosamine of enzyme production after 3 days of fermentation process.

Effect of incubation temperature

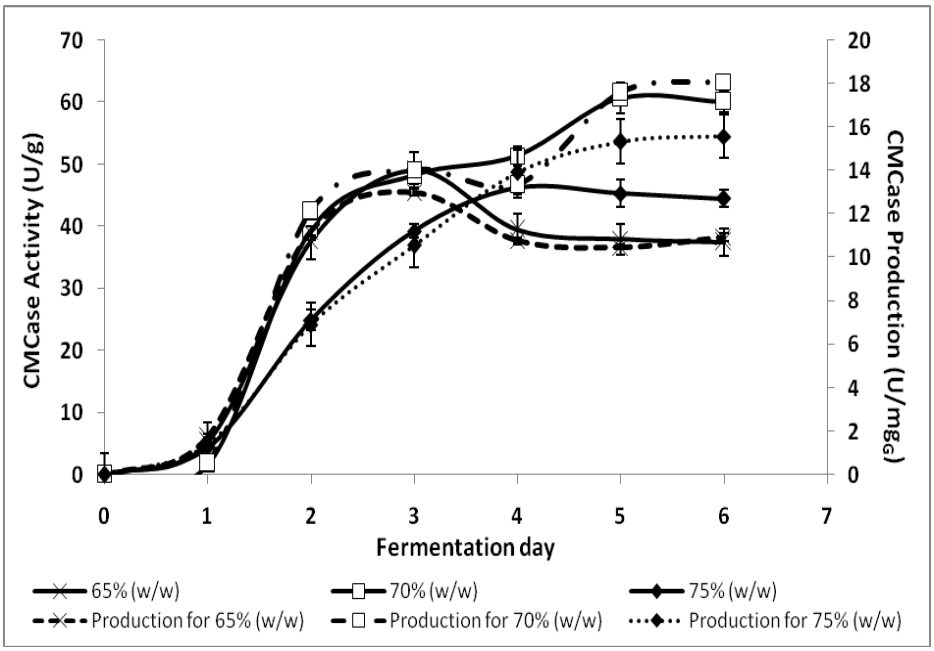
Temperature control of the substrate bed during the SSF process is very crucial as it ultimately affects the growth of the microorganism, spore formation and germination as well as product formation (Pandey, 2003). The result obtained in this work indicated that, CMCase activity increased and reaching maximum level after 4-5 days of fermentation process at temperature ranging from 28 °C to 32 °C (Figure 3). The highest CMCase activity of 67.5 U/g was detected after 4 days of fermentation process at 30 °C with CMCase production of about 17.8 U/mg_G glucosamine. These finding was in agreement with Jecu (2000) who reported the highest CMCase activity of 14.8 U/mL when *A. niger* 38 was grown on wheat straw and wheat bran (9:1) under the optimum incubation temperature of 30 °C for 4 days. While when the fermentation process was carried out at 28 °C and 32 °C, the maximum level of CMCase activities gained were 59.2

 ISSN (print): 1823-8262, ISSN (online): 2231-7538



Note: The SSF process was carried out at the indicated amount of substrate; 75% (w/w) moisture content; 28 °C; no aeration and mixing for 5 min at 0.5 rpm for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

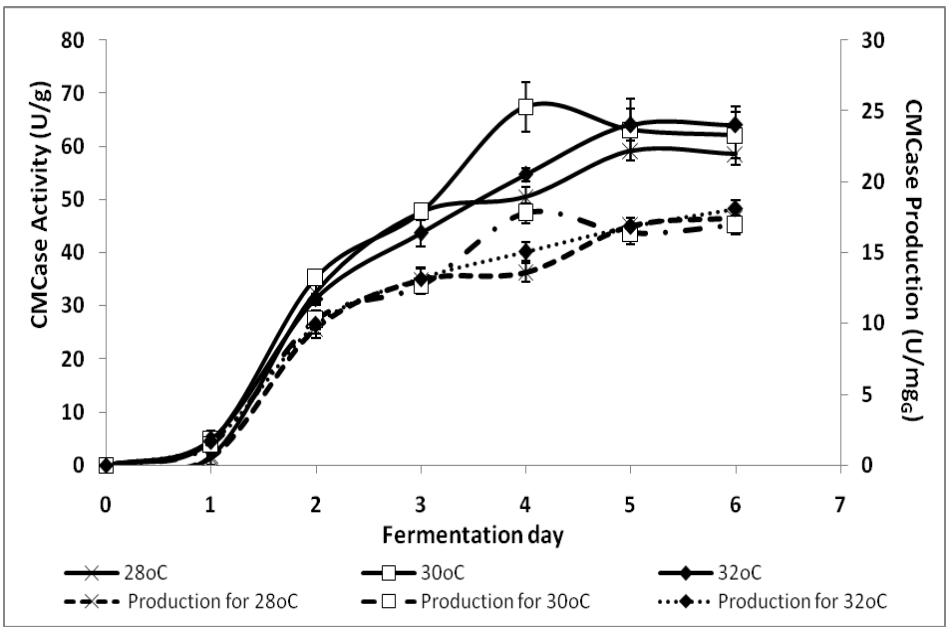
Figure 1: Effect of different amount of substrate on CMCase activity and production.



Note: The SSF process was carried out at the indicated moisture content, 0.5 kg substrate; 28 °C; no aeration and mixing for 5 min at 0.5 rpm for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

Figure 2: Effect of different moisture content on CMCase activity and production.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538



Note: The SSF process was carried out under the indicated incubation temperature, 0.5 kg substrate; 70% (w/w) moisture content; no aeration and mixing for 5 min at 0.5 rpm for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

Figure 3: Effect of different incubation temperature on CMCase activity and production.

and 64.1 U/g after 5 days of fermentation process, respectively. However, according to Krishna (1999), highest CMCase activity of 9.6 U/g was detected when *Bacillus subtilis* (CBTK 106) was used in SSF process for 3 days at 35 °C with media containing banana fruit stalk. No significant differences (p>0.05) in CMCase activity was exhibited for the different incubation temperature used. This is probably because the temperatures used were only differences 2 °C that not really can shows relatively high differences in the enzyme produced. Furthermore, for all the different incubation temperatures used in the fermentation process, only around 5-8% differences in CMCase activity. Unlike, the fermentation process carried out at 30 °C, CMCase activity remained constant and stable after achieving the optimum enzyme production days. In terms of enzyme production, about 18.1 U/mg_G and 17.4 U/mg_G glucosamine were detected for fermentation process carried out at 28 °C and 32 °C, respectively.

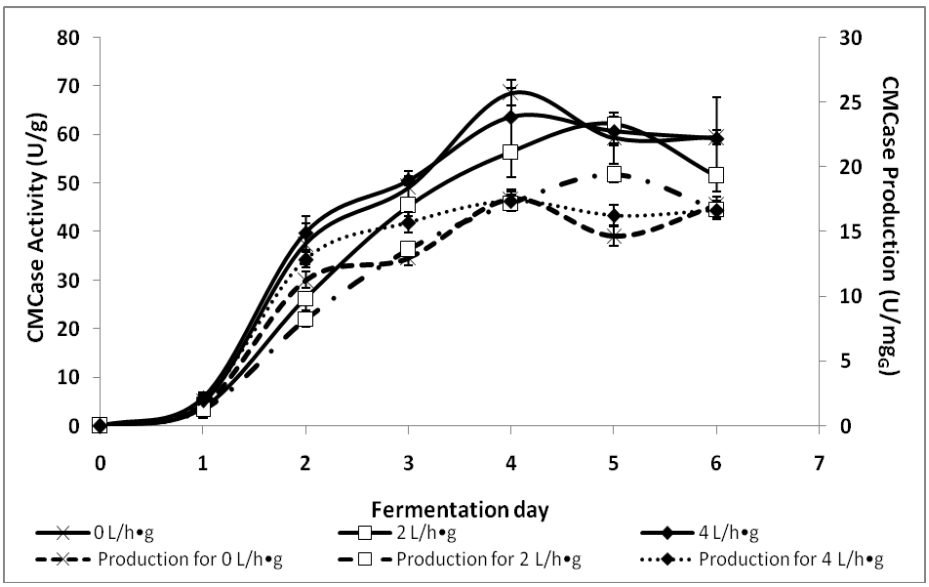
Effect of aeration rate

Aeration not only provides oxygen to the microorganism but also removes carbon dioxide and metabolites heat from bioreactors (Weiland, 1988). Figure 4 shows that no aeration was needed during the fermentation process for optimum CMCase activity. At the same time CMCase activity profiles also showed that the enzyme activity increased rapidly and reaching maximum level after 4 days of fermentation process when no aeration and aeration at 4 L/h.g fermented substrate were used during the fermentation process. CMCase production showed the highest activity of 69.0 U/g followed by 64.0 U/g and 62.0 U/g when no aeration, aeration at 4 L/h.g and 2 L/h.g fermented substrate were used during the fermentation process, respectively. However, statistical analysis showed no significant differences (p>0.05). These findings were not similar to Kalogeris *et al.* (2003), who obtained optimum endoglucanase activity of 1709 U/g when the fermentation process was carried out at the highest aeration rate of 0.9 L/h.g dry wheat straw. As shown in Figure 4 when the aeration rate increased 100%, the CMCase activity was only increased 3.2%. This showed that, higher aeration rate did not marginally increase the CMCase activity. However, about 20% higher in endoglucanase production was observed when the airflow rate used in the fermentation process increased 100% from 0.3 L/h.g to 0.6 L/h.g dry wheat straw (Kalogeris *et al.*, 2003). Although aeration at 2 L/h.g fermented substrate gives the lowest CMCase activity of 62.0 U/g but it shows the highest enzyme production of 19.0 U/mg_G glucosamine. At the same time, CMCase production of 17.6 and 17.4 U/mg_G glucosamine were obtained when no aeration and aeration at 4 L/h.g fermented substrate were used during the fermentation process, respectively.

Effect of aeration time

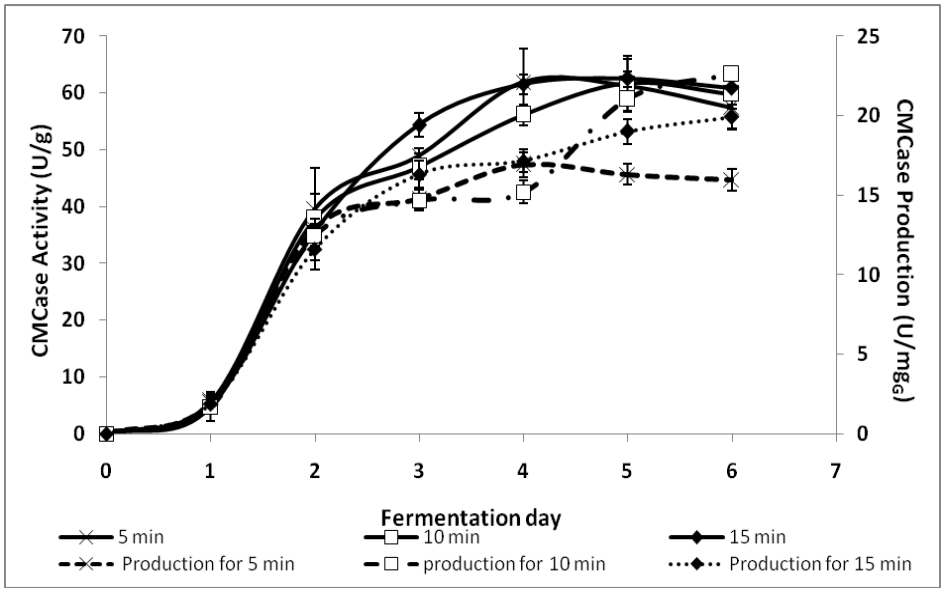
The different aeration time used during the fermentation process showed no significant differences (p>0.05) in CMCase activity (Figure 5). This was because less than 1% difference in the enzyme activity was obtained when

 ISSN (print): 1823-8262, ISSN (online): 2231-7538



Note: The SSF process was carried out under the indicated aeration rate for 5 min; 0.5 kg substrate; 70% (w/w) moisture content; 30 °C and mixing for 5 min at 0.5 rpm for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

Figure 4: Effect of different aeration rate on CMCase activity and production.

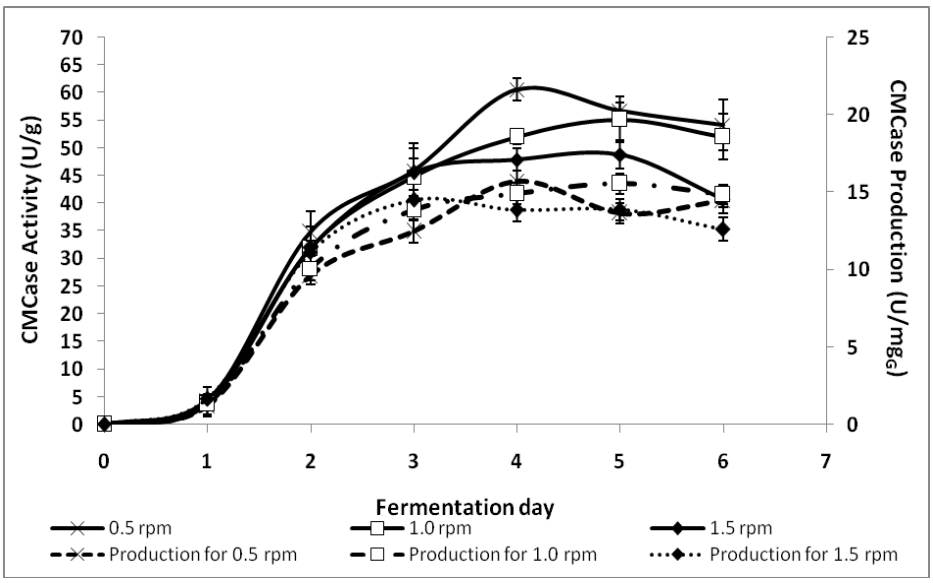


Note: The SSF process was carried out under the indicated aeration time; 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; 4 L/h.g fermented substrate of aeration rate and mixing for 5 min at 0.5 rpm for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

Figure 5: Effect of different aeration time on CMCase activity and production.

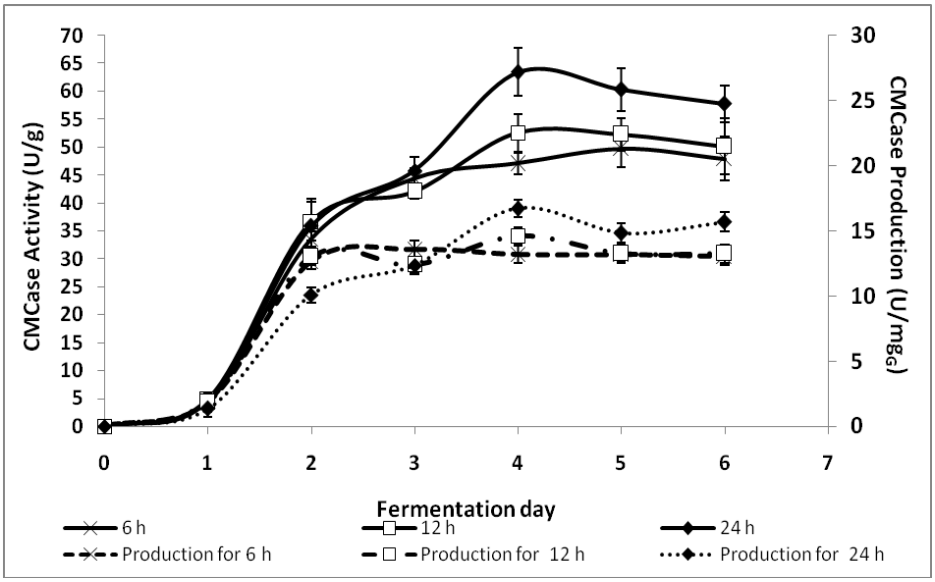
the aeration used varying from 5 to 15 min. CMCase activity reaching maximum level after 4 days of fermentation process using 5 min of aeration time. While, longer fermentation days (5 days) was required by the enzyme to reach maximum level with the increasing of aeration time used during the fermentation process. The highest CMCase activity of 62.5 U/g was obtained when 15 min of aeration time was used during the fermentation

 ISSN (print): 1823-8262, ISSN (online): 2231-7538



Note: The SSF process was carried out under the indicated mixing rate; 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; aeration at 4 L/h.g fermented substrate for 5 min and mixing for 5 min for every 24 h intervals. Arrow bars indicate means with standard error of three replicates.

Figure 6: Effect of different mixing rate on CMCase activity and production.

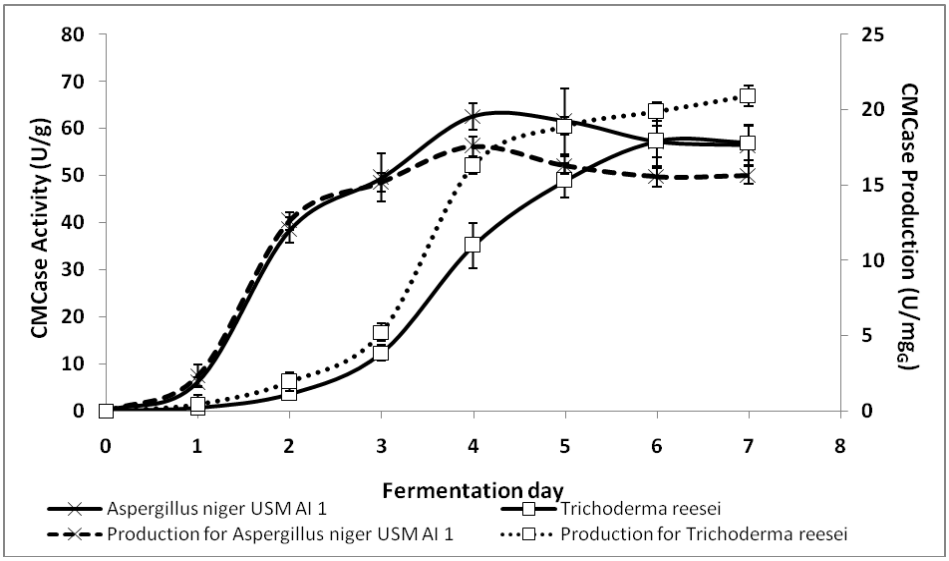


Note: The SSF process was carried out under the indicated mixing intensity; 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; aeration at 4 L/h.g fermented substrate for 5 min and mixing at 0.5 rpm for 5 min. Arrow bars indicate means with standard error of three replicates.

Figure 7: Effect of different mixing intensity on CMCase activity and production.

process compared with 61.8 U/g and 61.9 U/g when 10 min and 5 min of aeration time were used. On the other hand, Zhang *et al.* (2003) obtained maximum cellulase activity of 10.0 U/g when the fermentation process was carried out for 5 days at airflow rate of 5 L/min for 15 min under natural atmospheric pressure. Nevertheless, the authors observed optimum cellulase production (15.0 U/g) when carried out the fermentation process at 4 atmospheric pressures for 4 days under the same aeration. On the other hand, the highest enzyme activity

 ISSN (print): 1823-8262, ISSN (online): 2231-7538



Note: The SSF process was carried out under the indicated fungus; 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; aeration at 4 L/h.g fermented substrate for 5 min and mixing at 0.5 rpm for 5 min. Arrow bars indicate means with standard error of three replicates.

Figure 8: Production of CMCase enzyme by *A. niger* USM AI 1 and *T. reesei* under optimized fermentation conditions.

did not give the highest enzyme production. This can be seen when CMCase production of 22.6 U/mg_G and 20.0 U/mg_G glucosamine were detected when 10 min and 15 min of aeration time was applied during the fermentation process, respectively.

Effect of mixing rate

Mixing at 0.5 rpm for 5 min gave the highest CMCase activity and production of 60.5 U/g and 15.8 U/mg_G glucosamine after 4 days of fermentation process, respectively (Figure 6). In contrast to mixing at 0.5 rpm, CMCase activity required longer fermentation day to reach maximum level when higher mixing rate were used. CMCase activity dropped from optimum level at 60.5 U/g to 55.1 and followed by 48.8 U/g when the mixing rate used increased from 0.5 to 1.0 and 1.5 rpm, respectively. In addition, statistical analysis indicated that the decreased was significantly differences (p<0.05). On the other hand, Kalogeris *et al.* (1999) obtained CMCase activity of about 956.0 U/g when carried out the SSF process with mixing at 10 rpm for 1 min at 3 h interval. For mixing at 1.5 rpm, rapidly declined in the enzyme activity was detected after the enzyme reached maximum level. However, only slightly decreased in enzyme activity was detected for lower mixing rate used. These can be seen when 16.5% reduction in CMCase activity was observed using 1.5 rpm of mixing rate compared with less than 5% dropped using lower mixing rate. On the other hand, CMCase production of 14.5 U/mg_G and 15.6 U/mg_G glucosamine were obtained after 3 days and 5 days of fermentation process when 1.5 and 1.0 rpm of mixing rate were used during the fermentation process, respectively.

Effect of mixing intensity

The highest CMCase activity was detected when the mixing was carried out at 0.5 rpm for every 24 h interval followed by 12 h and finally 6 h interval for 5 min (Figure 7). In addition significant differences (p<0.05) in CMCase activity was observed when mixing was carried out every 24 h interval compared with every 12 h and 6 h interval. At the same time, when mixing intensity increased from 24 h to 6 h interval, the enzyme activity dropped about 22% to 49.7 U/g. In contrast to mixing at 6 h interval, CMCase activity achieved maximum level after 4 days of fermentation process. Under optimum mixing intensity of every 24 h internal, about 63.4 U/g of CMCase activity was detected compared with only about 49.7 U/g when mixing was carried out every 6 h interval. CMCase production of 17.7 U/mg_G glucosamine was observed when the mixing intensity was carried out for every 24 h interval during the fermentation process compared with about 15.5 U/mg_G glucosamine when mixing was carried out every 12 h interval.

Comparison of CMCase production using *Trichoderma reseei*

Trichoderma reesei was used as a benchmark for the production of CMCase by *A. niger* USM AI 1 because it is an established culture for the production. The SSF conditions used was the optimized conditions for enzymes produced by *A. niger* USM AI 1 but not for *T. reesei*. As indicated in the Figure 8, CMCase activity increased rapidly and reaching maximum level after 4 days of fermentation process using *A. niger* USM AI 1. While, CMCase enzyme produced by *T. reesei* was marginally

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

increased and reaching maximum level after 6 days of fermentation process. Slightly declined in enzyme activity was detected after optimum level. The highest CMCase activity of 62.6 U/g was produced by *A. niger* USM AI 1 with enzyme production of 17.5 U/mg_G glucosamine. On the other hand, as reported by Gao *et al.* (2008), the highest CMCase activity of 581.0 U/g was produced *Aspergillus terreus* M11 using corn Stover as substrate under the optimum fermentation conditions of 45 °C incubation, pH 3.0, 80% (w/w) moisture content and 0.8% yeast extract as carbon and nitrogen sources. However, maximum CMCase activity of 129.0 U/g was produced by *A. niger* KK2 mutant after 5-6 days of fermentation process using rice straw alone as substrate (Kang *et al.*, 2004). Maximum level of enzyme produced by *T. reesei* was 57.3 U/g, which was about 8.5% lower compared with *A. niger* USM AI 1. However, statistical analysis indicated no significant differences (p>0.05). On the other hand, optimum CMCase activity of 25.2 U/g was also detected after 3 days of fermentation process using *T. reesei* Rut C-30 grown on Kinnow pulp and wheat bran at 3:2 (w/w) ratio in Mandel Weber medium (Oberoi *et al.*, 2008). Although the enzyme produced by *T. reesei* was lower but the enzyme production obtained was higher compared with *A. niger* USM AI 1. CMCase production of about 20.9 U/mg_G glucosamine was obtained by *T. reesei* after 7 day of fermentation process, which was about 19% higher compared with *A. niger* USM AI 1.

CONCLUSION

Under optimum SSF conditions of 0.5 kg substrate; 70% (w/w) moisture content; 30 °C; aeration at 4 L/h.g fermented substrate for 5 min and mixing at 0.5 rpm for 5 min, the maximum CMCase activity obtained was 62.6 U/g, which was about 9.6 fold increased compared with before optimization process. On the other hand, the result obtained indicated that *A. niger* USM AI 1 was superior producer (8.5% higher) for CMCase compared with *T. reesei*. Many aspects pertaining to the bioreactor design are yet to be studied in detail. However, the results obtained in this study indicated that the developed solid state fermenter, FERMSOSTAT was able to produce CMCase with reasonable good titers. Furthermore, the results obtained were comparable with findings reported elsewhere. The developments of FERMSOSTAT may bring about easier collecting of lab scale data that may facilitate future up scaling studies. Thus, FERMSOSTAT has great potential in promoting transfer of SSF technologies for industrial exploitation.

ACKNOWLEDGEMENT

This work was mainly financed by Intensive Research Priority Area (IRPA) grant from Ministry of Science, Technology and The Environment, Malaysia. The author would like to thank USM for granting him USM Incentive grant and USM short term grant (304/PTEKIND/6311130).

REFERENCES

Aikat, K. and Bhattacharyya, B. C. (2000). Protease extraction in solid state fermentation of wheat bran by a local strain of *Rhizopus oryzae* and growth studies by the soft gel technique. *Process Biochemistry* **35, 907-914.**

Cannel, E. and Moo-Young, M. (1980). Solid state fermentation systems. *Process Biochemistry* **15, 2-7.**

Gao, J. M., Weng, H. B., Zhu, D. H., Yuan, M. X., Guan, F. X. and Xi, Y. (2008). Production and characterization of cellulolytic enzymes from the thermoacidopilic fungal *Aspergillus terreus* M11 under solid state cultivation of corn Stover. *Bioresource Technology* **99, 7623-7629.**

Gessesse, A. and Gashaw, M. (1999). High-level xylanases production by an alkaliphilic *Bacillus* sp. by using solid-state fermentation. *Enzyme and Microbial Technology* **25, 68-72.**

Jecu, L. (2000). Solid-state fermentation of agricultural wastes for endoglucanase production. *Industrial Crops and Products* **11, 1-5.**

Kalogeris, E., Fountoukides, G., Kekos, D., Macris, B. J. (1999). Design of solid state bioreactor of thermophilic microorganisms. *Bioresource Technology* **67, 313-315.**

Kalogeris, E., Iniotaki, F., Topakas, E., Christakopoulos, P., Kekos, D. and Macris, B. J. (2003). Performance of an intermittent agitation rotating drum type bioreactor for solid state fermentation of wheat straw. *Bioresource Technology* **86, 207-213.**

Kang, S. W., Park, Y. S., Lee, J. S., Hong, S. I. and Kim, S. W. (2004). Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. *Bioresource Technology* **91, 153-156.**

Krishna, C. (1999). Production of bacterial cellulases by solid state bioprocessing of banana wastes. *Bioresource Technology* **69, 231-239.**

Kumar, P. K. R. and Lonsane, B. K. (1990). Solid state fermentation: Physical and nutritional factors influencing gibberellic acid production. *Applied Microbiology and Biotechnology* **34, 145-148.**

Lee, C. K., Darah, I. and Ibrahim, C. O. (2011). Production and optimization of cellulase enzyme using *Aspergillus niger* USM AI 1 and comparison with *Trichoderma reesei* via solid state fermentation system. *Biotechnology Research International* **Doi: 10.4061/2011/658493.**

Lonsane, B. K., Saucedo-Castaneda, G., Raimbault, M., Roussos, S., Viniegra-Gonzalez, G., Ghildyal, N. P., Ramakrishna, M. and Krishnaiah, M. M. (1992). Scale up strategies for solid state fermentation systems. *Process Biochemistry* **27, 259-273.**

Nagel, F. J. J., Tramper, J., Bakker, M. S. N. and Rinzema, A. (2001). Model for online moisture content control during solid state fermentation. *Biotechnology and Bioengineering* **72(2), 231-243.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Oberoi, H. S., Chavan, Y., Bansai, S. and Dhilon, G. S. (2008). Production of cellulases through solid state fermentation using kinnow pulp as a major substrate. *Food Bioprocess Technology* **3(4), 528-536.**

Pandey, A. (2003). Solid state fermentation. *Biochemical Engineering Journal* **13, 81-84.**

Pandey, A., Soccol, C. R. and Larroche, C. (2008). General consideration about solid state fermentation process. *In:* Current Developments in Solid State Fermentation. (Pandey, A., Soccol, C.R. and Larroche, C. (eds). Springer Science Business Media, New York, USA. **pp. 13-25.**

Pang, P.K., Darah, I., Poppe, L., Szakacs, G. and Ibrahim, C. O. (2006). Xylanase production by local isolate, *Trichoderma* spp. FETL C3-2 via solid-state fermentation using agricultural wastes as substrates. *Malaysian Journal of Microbiology* **2, 7-14.**

Panagiotou, G., Kekos, D., Macris, B. J. and Christakopoulos, P. (2003). Production of cellulolytic and xylanolytic enzymes by *Fusarium oxysporum* grown on corn stover in solid state fermentation. *Industrial Crops and Products* **18, 37-45.**

Perez-Guerra, N., Torrado-Agrasar, A., Lopez-Macias, C. and Pastrana, L. (2003). Main characteristic and applications of solid substrate fermentation. *Electronic Journal of Environmental, Agricultural and Food Chemistry* **2(3), 343-350.**

Raimbault, M. (1998). General and microbiological aspects of solid substrate fermentation. *EJB Electronic Journal of* Biotechnology **1(3), 174-188.**

Raimbault, M. and Alazard, D. (1980). Culture method to study fungal growth in solid fermentation. *European Journal of Applied Microbiology and Biotechnology* **9, 199-209.**

Roussos, S., Raimbault, M., Prebois, J. P. and Lonsane, B. K. (1993). Zymotis, a large scale solid state fermenter: Design and evaluation. *Applied Biochemistry and Biotechnology* **42, 37-51.**

Smits, J. P., Rinzema, A., Tramper, J., van Sonsbeek, H. M. and Knol, W. (1996). Solid-state fermentation of wheat bran by *Trichoderma reesei* QM9414: Substrate composition changes, C balance, enzyme production, growth and kinetics. *Applied Microbiology and Biotechnology* **46, 489-496.**

Steinkraus, K. H. (1984). Solid state (solid substrate) food beverage fermentations involving fungi, *Acta Biotechnology* **4, 83-88.**

Swift, J. (1972). The estimation of mycelial biomass by determination of the hexosamine content of wood tissues decayed by fungi. *Soil Biological and Biochemistry* **5, 321-322.**

Wang, L. P. and Yang, S. T. (2007). Solid state fermentation and its applications. *In:* Bioprocessing For Value Added Products From Renewable Resources. Yang, S. T. (ed.). Elsevier Science Publishers, Amsterdam BV. **pp. 465-498.**

Weiland, P. (1988). Principles of solid state fermentation. *In:* Treatment Of Lignocellulosic With White Rot Fungi. Zadrazil, F. and Reiniger, P. (eds.). Elsevier, London. **pp. 64-76.**

Zhang, X. Y., Mo, H. T, Zhang, J. A. and Li, Z. H. (2003). A solid state bioreactor coupled with forced aeration and pressure oscillation. *Biotechnology Letters* **25, 417-420.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538