ABSTRACT

Brewery spent grain (BSG) which was used as the main organic material in this research is the by-product of brewing industries. Every 100 liter of beer generates 20kg of BSG. A Malaysian brewery (Carlsberg (M) Sdn. Bhd) alone generates 31,000 tonnes of BSG yearly. Almost all the BSG are being dumped into landfills, since there is no proper and separate guideline for industrial organic waste management in Malaysia. BSG materials are rich in nutrients and high in fermentable sugar as well as lignin, cellulose and hemicellulose. Dumping this high nutritional substrate into the landfills could contribute to adverse environmental impacts, especially greenhouse gas emission and global warming in long term. It is also can be said useful resourced are being wasted. In order to avoid environmental impacts and resources wastage, BSG can be exploited as a raw material in solid state fermentation (SSF), to produce enzymes with the help of filamentous fungi. The objective of this study is to investigate the potential utilization of BSG in producing enzymes and to identify the fungi that yield higher enzyme activity at different fermentation period and incubation temperature. Chemicals at different concentrations were used in this study to identify the effect of it in inducing the enzyme activity. Five types of filamentous fungi (*Aspergillus niger, Fusarium* sp., *Penicillium chrysogenum, Schizophyllum commune* and *Trichoderma* sp.) were used to determine the production of five types of enzymes including laccase, lignin peroxidase, xylanase, cellulase and amylase. Among the enzymes tested, laccase showed highest activity followed by lignin peroxidase, xylanase, cellulase and amylase in all the experiments conducted. A comparative study between BSG, spent mushroom compost (SMC) and sugarcane bagasse (SCB) was conducted to compare the ability of these substrates to produce higher enzyme activity at similar condition. Results showed BSG exhibited higher enzyme activity compared to other substrates. Enzyme activity in BSG fermented with different types of filamentous fungi was tested at different incubation period (day 1 to day 10) and at different incubation temperature (20^oC, 25^oC, 30^oC, 35^oC and 40^oC). Seven day fermentation at 30^oC yield higher enzyme activity for all the enzymes except for amylase activity. Amylase activity was almost twice higher at 35^oC compared to

30^{o}C. BSG was mixed with different percentage of SCB and SMC separately, to study the effect of mixed substrates in enzyme activity. However, the mixed substrate showed lowed enzyme activity compared to single substrate. Four chemicals namely, $CoCl_2$, $CuSO_4$, $MnSO_4$ and $FeSO_4$ with five concentrations (2 g/l, 4 g/l, 6 g/l, 8 g/l and 10 g/l) used in BSG cultivated with *A. niger* and *S. commune* to study the effect of chemicals in enzyme activity induction. Both fungi showed a positive result for the chemical induction. However, *S. commune* showed higher enzyme activity for cellulase, laccase, lignin peroxidase and xylanase compared to *A. niger*. BSG fermented with *A. niger* and *S. commune* with 10 g/l $CoCl_2$ showed highest amylase and laccase activity respectively. $CuSO_4$ at 4 g/l and 6 g/l showed highest xylanase and cellulase activity respectively in BSG fermented by *S. commune*. While 6 g/l $MnSO_4$ showed highest lignin peroxidise activity in BSG fermented by *S. commune*. Therefore, the optimal chemical concentration for each enzyme varies. Fungal biomass was determined by estimating the glucosamine content to perform kinetics of SSF. Consequently the fungal growth rate and fungal half-life was also determined by using first-order kinetic equation. The growth of fungus was correlated with laccase and lignin peroxidase activity, where *A. niger*, *S. commune* and *Fusarium* sp. showed a strong linear correlation with these enzymes. It is evident that, BSG can be utilized as a substrate in SSF for enzyme production.

ABSTRAK

"Brewery spent grain"(BSG) yang digunakan sebagai bahan utama dalam ujikaji adalah produk sampingan dari industri pembuatan bir. Setiap 100 liter bir mengahsilkan 20kg BSG. Industri pembuatan bir Malaysia (Carlsberg (M) Sdn. Bhd) menghasilkan 31,000 ton BSG setahun. Hampir semua BSG dibuang ketapak pelupusan, disebabkan ketiadaan garis panduan yang sesuai untuk pengurusan sisa industri organik di Malaysia. BSG ialah bahan yang kaya dengan nutrisi dan mengandung gula yang tinggi seperti lignin, selulosa dan hemiselulosa. Pembuangan substrat yangkaya dengan nutrisi di tapak pelupusan boleh menyumbang kepada kesan sampingan bagi persekitaran, khususnya pembebasan gas rumah kacadan pemanasan global dalam jangkamasa panjang. Dengan kata lain, sumber daya yang berguna sedang dibazirkan. Untuk mengelakkan kesan persekitaran dan pembaziran sumber, BSG dapat dimanfaatkan sebagai bahan baku dalam fermentasi padat (SSF), untuk menghasilkan enzim dengan bantuan kulat. Tujuan kajian ini adalah untuk mengetahui pemanfaatan potensi BSG dalam menghasilkan enzim dan juga untuk mengenalpasti kulat yang menghasilkan aktiviti enzim yang lebih tinggi pada tempoh inkubasi and suhu inkubasi yang berbeza. Lima jenis kulat (Aspergillus niger, Fusarium Sp., Penicillium chrysogenum, Schizophyllum commune dan Trichoderma sp.) telah digunakan untuk menentukan lima jenis enzim iaitu 'laccase', lignin peroksidase,'xylanase', selulase dan amilase. Diantara enzim yang diuji,'laccase' menunjukkan aktiviti yang lebih tinggi diikuti dengan lignin peroksidase,'xylanase', selulase dan amilase dalam kesemua ujikaji yang dijalankan. Ujikaji perbanding diantara BSG, kompos cendawan terpakai (SMC) dan pulpa tebu (SMC) dijalankan untuk membandingkan kebolehan substrat-substrat ini untuk menghasilkan enzim yang tinggi dalam keadaan yang serupa. Hasil keputusan menunjukan BSG menghasilkan enzim aktiviti yang lebih tinggi berbanding dengan substrat ysng lain. BSG telah diperam untuk tempoh satu hingga 10 hari pada suhu yang berbeza (20^{o}C, 25^{o}C, 30^{o}C, 35^{o}C dan 40^{o}C). Hasil kajian menunjukkan BSG yang diinkubasi untuk tujuh hari pada suhu 30^{o}C mengahsilkan aktiviti enzim yang tertiggi, kecuali bagi enzim amilase yang menunjukkan aktiviti yang tinggi pada suhu 35^{o}C. BSG yang

telah dicampur dengan SMC dan SCB dengan peratusan yang berbeza untuk mengkaji kesan substrat campuran dalam menghasilkan aktiviti enzim tertinggi. Namun aktiviti enzim di substrat tunggal adalah lebih tinggi berbanding dengan susbtrat bercampur. Empat jenis bahan kimia ($CoCl_2$, $CuSO_4$, $MnSO_4$ dan $FeSO_4$) dengan kepekatan yang berberza 2 g/l, 4 g/l, 6 g/l, 8 g/l dan 10 g/l telah digunakan dalam BSG yang diperam dengan A. niger dan S.commune untuk mengkaji kesan bahan kimia ke atas enzim aktiviti. Kedua-dua kulat telah menunjukkan keputusan yang positif dengan bahan kimia. Bagaimanapun, S. commune telah menunjukan hasil yang lebih memberangsangkan bagi enzim 'laccase', lignin peroxidase, selulase dan 'xylanse' berbanding dengan A. niger. BSG yang diperam dengan A. niger dan S. commune menunjukan aktiviti amilase dan laccase yang lebih tinggi dengan 10 g/l $CoCl_2$. $CuSO_4$ dengan kepekatan 4 g/l dan 6 g/l menunjukan aktiviti 'xylanase' dan selulase yang tertinggi bagi BSG yang diperam dengan S. commune. Manakala 6 g/l $MnSO_4$ menunjukkan aktiviti lignin peroxidase yang lebih tinggi di BSG yang diperam dengan S. commune. Oleh kerana itu, optimum kepekatan bahan kimia untuk menghasikan aktiviti enzim yang tinggi adalah berbeza. Biojisim kulat ditentukan dengan menganggarkan kandungan glukosamin untuk digunakan dalam kinetik SSF. Selanjutnya, kadar pertumbuhan kulat dan 'half-life' bagi kulat juga boleh ditentukan dengan mengaplikasikan persamaan 'First-order kinetics'. Kadar pertumbuhan kulat dikorelasikan dengan aktiviti enzim (laccase dan lignin peroxidase) dimana A. niger, S. commune dan Fusarium sp. menunjukkan korelasi yang tinggi dengan aktiviti enzim. Jelaslah bahawa, BSG boleh dimanfaatkan sebagai substrat dalam SSF untuk pengeluaran enzim cendawan.

ACKNOWLEDGEMENT

This dissertation is written based on the bench works conducted from July 2008 to April 2010.

First of all I would like to thank the God and my 'Guruji' for showering me with wisdom and strength in completing this degree of Master.

I would like to express my sincere gratitude and deep appreciation to my research supervisor, Professor Dr. Agamuthu Periathamby, Institute of Biological Sciences, University of Malaya for his boundless guidance and support from the preliminary to the concluding level enabled me to develop an understanding of the project. Insightful knowledge, encouraging attitude and hardworking characteristics of Prof. Dr. Agamuthu greatly influenced me. Thank you for taking the responsibility of being my supervisor and thank you for your critical instructions.

This research could not happen without the support and co-operation of Mr Siaw Yon Miaw and Mr. Gunnar Hepp (General Manager) and friendly staffs of Carlsberg Brewery (Malaysia) Sdn. Bhd. Thank you for all your help. My personal gratitude is extended to my beloved family, especially my mom Sarada Kanniappan, for her support, perseverance, concern, understanding and my dad Mr. Arumugam Doraisamy for always keeping my feet stand on ground. Special thanks for my dear sisters Ananthy Arumugam, Shamala Arumugam and Subasini Arumugam for all the good spirits, encouragement and support. Thanks you all.

I am deeply indebted to all the members of Solid and Hazardous Waste Laboratory, University of Malaya, past and present. I owed my deepest gratitude to Dr. Fauziah Shahul Hamid, for her sisterly advises and moral support. Dr Fauziah greatly encouraged me during the hard period.

Not to forget, my project partner as well as lab mate, late Mr. kahlil bin Mohammad Khidzir for his companionship, encouragement, guidance and moral support from the beginning of the project. I would like to share the credit of my work with you as I learned many things from you. Thank you for your availability and your advises. Also, to his parents, thank you so much for your support and love.

Author wishes to offer her sincere appreciation to University of Malaya (Postgraduate Research Grant PS183/2008B and PS461/2010B) and Ministry of Science, Technology and Innovation Malaysia (MOSTI), e-Science Research Grant (02-02-03-SF0363) for providing the fund for this project.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

LIST OF EQUATIONS