UNIVERSITI PUTRA MALAYSIA

PRODUCTION OF POLYHYDROXYBUTYRATE BY *Cupriavidusnecator* CCGUG 52238 USING ORGANIC ACIDS RECOVERED FROM FERMENTED KITCHEN WASTE

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FBSB 2011 21

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MASTER OF SCIENCE

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By

FARAH NADIA OMAR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in fulfillment of the Requirement for the Degree of Master of Science

JULY 2011

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

PRODUCTION OF POLYHYDROXYBUTYRATE BY *Cupriavidusnecator* CCGUG 52238 USING ORGANIC ACIDS RECOVERED FROM FERMENTED KITCHEN WASTE

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July 2011

Chairman : Nor 'Aini Abdul Rahman, PhD

Faculty : Faculty of Biotechnology and Biomolecular Sciences

The amount of solid waste generated was gradually increased and currently in Malaysia, all the waste is disposed in the municipal landfill. Solid wastes generated aremainly characterized by high concentration of organic matter primarily from kitchen waste. In this study, the organic acids recovered from naturally fermented kitchen waste was utilized as a sole carbon source for the production of polyhydroxybutyrate (PHB). PHB production was achieved through two stage processes: organic acids recovered from naturally fermented kitchen wastes followed by PHB production utilizing the recovered organic acidsby

Cupriavidusnecator CCGUG 52238 in batch and fed-batch mode of fermentation. Kitchen waste was fermented using 50 L bioreactor at 37°C with agitation of 150 - 200 rev/min. The initial pH of fermentation was adjusted to pH 7. After seven days ofkitchen waste fermentation, about 60 g/L of total organic acids could be produced where it comprised lactic acid (98%), acetic acid (1.8%) and formic acid (0.2%). Organic acids were recovered after undergo few treatment processes which were freezing and thawing, centrifugation, filtrationand evaporation methods. The effect of the treatments on the organic acids concentration, nitrogen content as well as total suspended solids (TSS) removal was also investigated. As a result, about 223.9 g/L of organic acids had been successfully recovered and concentrated from initial 25.7 g/L of total organic acids produced, 93% of TSS had been removed after filtration process and about 98% of total nitrogen content had reduced. This indicates that the stepwise recovery process was able to recover high concentration of organic acids as well as removes the suspended solids.

The recovered organic acids from kitchen wastes were then utilized for PHB production by *C. necator* CCGUG 52238 in series of batch and fed batch experiments. In batch culture, fermentation was carried out in 2 L bioreactor and the pH and temperature was controlled at 7 and 30°C, respectively. The dissolve oxygen level (DO) was maintained at 30%, via agitation 300-700 r/min. The maximum PHB content produced from kitchen wastes organic acids was 52.79% (w/w) withdry cell weight of 4.62 g/L. The PHB yield and

productivitywere obtained at 0.382 g/g and 0.1091 g/L/h, respectively. In fed batch culture, experiment was conducted in 7 L of bioreactor with condition was set up as same as in batch fermentation. About five timesincrement in PHB production was achieved by applying feeding strategy for every 10 hours. The PHB yield and productivity obtained were 0.79 g/g and 0.49 g/L/h, respectively. Therefore, organic acids derived from kitchen waste have a potential to be used as one of a favorable substratefor PHB production by *C.necator* CCGUG 52238.

Abstraktesis yang dikemukakankepadaSenatUniversiti Putra Malaysia sebagaimemenuhikeperluanuntukIjazah Master Sains

PENGHASILAN ASID POLIHIDROKSIBUTIRIKOLEH *Cupriavidusnecator* CCGUG 52238 MENGGUNAKAN ASID ORGANIK YANG DIPEROLEHI DARIPADA FERMENTASI SISA BUANGAN DAPUR

Oleh

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Jumlahsisabuanganpepejal yang dihasilkan di Malaysia semakinmeningkatdankesemuasisabuangantersebutdilupuskan di tapakpelupusansampahperbandaran.Sisapepejal yang dihasilkandikategorikansebagaiorganikdankebanyakannyaterdiridaripadasisabuangandapur.Di dalamkajianini, asidorganik yang diperolehidaripadasisabuangandapurtelahdigunakansebagaisumberkarbontunggaluntukmenghasilkanasidpolihidroksibutirik(PHB).Penghasilan PHB melaluiduafasaiaitu: pemerolehanasidorganikdaripadafermentasisemulajadi sis

buangandapurdiikutidenganpenghasilan PHB menggunakanasid organic oleh*Cupriavidusnecator* CCGUG 52238dengankaedahfermentasisesekelompokdansuapansesekelompok. Fermentasisisabuangandapurdilakukandenganmenggunakan 50 L tangkiberpengadukpadasuhu37°Cdengankelajuanmengadukantara 150 – 200 rev/min. pH permulaanfermentasidilaraskankepada pH 7. Selepastujuhharifermentasi, kira-kira 60 g/L asidorganikyangdihasilkanterdiridaripadaasidlaktik (98%), asidasetik (1.8%) danasidformik (0.2%).Pemerolehanasidorganikmelaluibeberapapra-rawataniaitupenyejukbekuandanpencairan, pengemparan, penapisandanpemekatan.Kesanrawatan-rawatanituterhadapkepekatanasidorganik, kandungan nitrogen danpepejal yang terampaiturutdikaji.Sebanyak 223.9 g/L asidorganikberjayadipekatkandandiperolehidaripada 25.7 g/L jumlahasidorganik yang dihasilkanpadapermulaannya, sebanyak 93% pepejalterampaiberjayadibuangdansebanyak 98% kandungan nitrogen berjayadikurangkan. Inimenunjukkankajian proses pemerolehanasidsecaraberturutandapatdilakukanuntukmemperolehiasidorganik berkepakatantinggidisampingdapatmembuangpepejalterampai.

Asidorganik yang diperolehidaripadsisabuangandapurtadikemudiannyadigunakanuntukpenghasilan PHB oleh*C. necator* CCGUG 52238

menggunakankaedahfermentasisesekelompokdansuapansesekelompok.Dalam suapansesekelompok, fermentasidilakukan di dalamtangkiberpengaduk 2L denganpH dansuhupadamasing-masing 7 dan30°C. Level oksigenterlarutdilaraskankepada 30%menggunakanpengadukberkelajuan300 – 700 r/min. Penghasilan PHB maksimum yang dihasilkandariasidorganiktersebutadalah 52.79% denganberatselkeringialah 4.62 g/L. Penghasilandanproduktiviti PHB yangdidapatimasing-masingsebanyak 0.382 g/gdan 0.1091 g/L/h. Dalamfermentasisuapansesekelompok,eksperimendilakukan di dalamtangkipengaduk 7 L dengankeadaansepertidalamfermentasisesekelompok. PHB telahdihasilkanhampirlima kali gandapeningkatandenganmenggunakanstrategisuapanberkalasetiap 10 jam. Penghasilandanproduktiviti PHB yang dihasilkanmasing-masingadalah 0.79 g/g dan 0.49 g/L/h. Kesimpulannya, asidorganik yang diperolehidaripadasisabuangandapurberpotensisebagaisubstrat yang sesuaiuntukpenghasilan PHBoleh*C.necator* CCGUG 52238 .

ACKNOWLEDGEMENTS

Alhamdulillah, thank a lot to merciful Allah for giving me the strength and the courage for me to get through during my entire life.

I would like to express my great appreciation and gratitude to my chairman, Dr. Nor 'Aini Abdul Rahman and co-supervisor, Dr. Phang Lai Yee, for full guidance, comments and encouragements through out this study.

My big gratitude and thanks also to all my laboratory mates HalimatunSaadiah, Fadzillah, SitiBalkhis, Yee LianNgit, Noor Azman, MohdRafein, Mior Ahmad Khushairi, Mumtaz for their valuable discussions, helps and cooperation during this project.

I gratefully acknowledge the financial support by Universiti Putra Malaysia. Also my heartiest thanks to all research officers, lab staffs and technicians for their kindness and helps throughout my study.

I am sincerely grateful to my loving parents and my sisters for their understanding, love and endless support during my graduate degree. May Allah bless them.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment for the requirement for the degree of Master of Science. The members of Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at UniversitiPutra Malaysia or other institutions.

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Date: 25 July 2011

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