BIOSYNTHESIS OF POLY(3-HYDROXYBUTYRATE) BY *CUPRIAVIADUS NECATOR* DSM 428 UTILIZING WASTE DATE FRUITS AS A CARBON SOURCE

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by

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DECLARATION BY AUTHOR

This dissertation is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. The content of my dissertation is the result of work I have carried out since the commencement of my research project and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution.

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LIST OF SYMBOLS

Symbol	Caption
±	Plus-minus
%	Percentage
℃	Degree Celcius
℃/min	Degree Celcius per minute
X	Multiply

LIST OF ABBREVIATIONS

Symbol	Caption
PHB	Polyhydroxybutyrate
P3HB	Poly(3-hydroxybutyrate)
g/L	Gram per litre
PHA	Polyhydroxyalkanoate
K	Thousand
PE	Polyethelene
PP	Polypropelene
Kg	Kilogram
PLA	Polylactid acid
kg/m^3	Kilogram per Cubic Meter
mPa	Mega pascal
UV	Ultraviolet
P(3HB-co-3HV)	Poly(3-hydroxybutyrate-co-3-hydroxyvalerate)
Scl	Short chain length
CDW	Cell dry weight
3HV	3-hydroxyvalerate
Mmt	Million metric tonnes
Ml	Millilitre
Rpm	Revolutions per minute
HPLC	High Performance Liquid Chromatography
GC	Gas Chromatography
g	Gram
pH	Potention of hydrogen

Psi	Pound per square inch
µl	Microlit
µm	Micrometer
Mm	Millimeter
Min	Minutes
ml/min	Millilitre per minute
NaOH	Sodium Hydroxide
HCl	Hydrochloric Acid
Mg	Milligram
NB	Nutrient Broth
L	Litre
M	Molar
MSM	Mineral salt medium
$(NH_4)_2SO_4$	Ammonium sulfate
KH^2PO_4	Potassium dihydrogen phosphate
Na_2HPO_4	Sodium phosphate dibasic
$MgSO_4.XH_2O$	Magnesium sulphate hydrate
$FeSO_4.7H_2O$	Ferrous sulfate heptahydrate
$ZnSO_4.7H_2O$	Zinc sulphate heptahydrate
$CuSO_4.5H_2O$	Copper sulphate pentahydrate
$MnSO_4.H_2O$	Manganese sulphate monohydrate
$CaCl_2.2H_2O$	Calcium chloride dihydrate
$Na_2B_4O_7.10H_2O$	Sodium tetraborate decahydrate
$(NH4)_6Mo_7O_{24}.4H_2O$	Ammonium molybdate tetrahydrate
OD	Optical Density

v/v	Volume to volume
kPa	Kilopascal
TCA	Tricarboxylic acid
Nm	Nanometre
H	Hour
ANOVA	Analysis of variance

BIOSINTESIS POLI (3-HIDROKSIBUTIRAT) OLEH *CUPRIAVIDUS NECATOR* DSM 428 MENGGUNAKAN BUAH KURMA TERBUANG SEBAGAI SUMBER KARBON.

ABSTRAK

Banyak penyelidikan telah dilakukan untuk mencari pengganti plastik yang dihasilkan dari petroleum konvensional. Polihidroksibutirat (PHB) adalah polimer biodegradasi yang dihasilkan dari sumber yang boleh diperbaharui telah mendapat perhatian untuk menggantikan plastik yang dihasilkan dari petroleum konvensional kerana ia memiliki sifat kimia dan fizikal yang sangat baik, dengan kelebihan kebolehbiodegrdasi yang lengkap. Batasan dalam menghasilkan PHB dalam skala besar adalah kerana kos sumber karbonnya yang mahal. Kajian ini memberi tumpuan kepada penghasilan PHB oleh *Cupriavidus necator* menggunakan sumber karbon yang berkos rendah seperti hasil buangan buah kurma. Tiga nisbah kepekatan larutan buah kurma terhadap air disediakan iaitu 1:3, 1:5, 1:8 dengan keadaan penapaian 30 ℃, 200 rpm dan penapaian sehingga 29 jam. Analisis kandungan gula dalam larutan buah kurma dilakukan sebelum dan selepas proses penapaian. Kandungan glukosa menunjukkan jumlah tertinggi berbanding fruktosa dan sukrosa iaitu 48.5 ± 6.3% (v/v), 55.7 ± 3.8% (v/v) dan 60.6 ± 5.1% (v/v). Purata berat sel kering terhadap nisbah 1:3, 1:5 dan 1:8 adalah 0.179 ± 0.03g/L, 0.166 ± 0.01g/L dan 0.180 ± 0.01g/L. Manakala kandungan PHB adalah, 7.9 ± 3.0%, 4.1 ± 3.4% dan 10.4 ± 9.6%. Nisbah 1:8 mencatatkan berat sel kering dan PHB tertinggi dibandingkan dengan semua nisbah walaupun ia adalah larutan buah kurma yang paling cair. Pemerhatian ini adalah berdasarkan penggunaan sumber karbon yang berkesan kerana mengandungi glukosa tertinggi dibandingkan dengan nisbah lain, dan jumlah nitrogen yang terhad (1.45 ± 1.06%) yang meningkatkan kesan positif terhadap penghasilan PHB.

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ABSTRACT

Many researches have been conducted to find a replacement for conventional petroleum-derived plastics. Poly(3-hydroxybutyrate) (PHB) is biodegradable polymers produced from a renewable source have gained many interest to replace conventional plastic, as it possesses excellent chemical and physical properties, with an advantages of complete biodegradability. The limitation in producing huge amount of PHB is due to its expensive cost of carbon sources. This study focus on the production of PHB by *Cupriavidus necator* using low-cost carbon sources such as waste of date fruits. Three concentration ratios of date fruits to water was prepared which are 1:3, 1:5, and 1:8 with the fermentation condition of 30℃, 200 rpm and up to 29 hours of fermentation. Analysis of sugar content in date fruits solution was performed prior and after the fermentation process. Glucose content shows the highest amount compared to fructose and sucrose which are 48.5 ± 6.3%, 55.7 ± 3.8% and 60.6 ± 5.1%. The average CDW of ratio 1:3, 1:5 and 1:8 are 0.179 ± 0.03g/L, 0.166 ± 0.01g/L and 0.180 ± 0.01g/L. While the PHB content are, 7.9± 3.0%, 4.1± 3.4% and 10.4± 9.6% respectively. Ratio 1:8 state the highest CDW and PHB in comparison to all ratios even it is the most diluted date fruits solution. This observation is due to effective utilization of carbon sources as it contained the highest glucose compared to other ratios, and limited amount of nitrogen (1.45 ± 1.06%) which enhanced the positive effect on PHB production.