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BACTERIAL CELLULOSE FROM A. XYLINUM FERMENTATION USING HYDROLYSATE OF BANANA-PEEL WASTE AS CARBON SOURCE

MUSTAPHA BIN AKIL

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by

MUSTAPHA BIN AKIL

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

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Chair: **Dayang Radiah Bt. Awang Biak, PhD**

Faculty: **Faculty of Engineering**

The high cost of utilizing commercial sugar as carbon source in bacterial cellulose (BC) production has become an issue in the industry. Discovery of alternative carbon source from cheap sources, *e.g.* agricultural (food) waste is needed to overcome this problem. Two key objectives are set in this study, namely (i) to investigate the reducing sugar yield obtained from the diluted acid hydrolysis of agricultural (food) waste and (ii) to synthesize and characterize BC using hydrolysate obtained in (i) as the carbon source.

Sago effluent, potato peel and banana peel were selected as raw material. The dilute acid hydrolysis method was used to hydrolyze these wastes. Three hydrolysis parameters, sulphuric acid concentration (2%, 4% and 6%), reaction time (20, 40 and 60 minutes) and temperature (105°C and 121°C) were studied. The highest glucose yield for each waste's hydrolysate was used in the fermentation experiment to screen the best carbon source for BC production.

Hydrolysis of potato produced the highest glucose yield, *i.e.* 43.44 mg/ml using 4% (v/v) acid at 20 minutes reaction time and 121°C reaction temperature. The highest glucose yield, *i.e.* 19.15°C from banana peel hydrolysis was obtained after 20 minutes using 6% (v/v) of acid at 121°C whilst for sago effluent, as reaction time was extended to 40 minutes using 4% (v/v) acid at 105°C, only 2.64 mg/ml of glucose was obtained.

Banana peel hydrolysate was selected as the carbon source used in this study because it produced the highest yield of BC, *i.e.* 2.89 g/l after 21 days of incubation period compared to other hydrolysate. For the selection of the suitable initial pH and initial carbon source concentration, it was found that initial pH 6 and 2.0% of carbon source concentration produced 2.81 g/l of BC after 16 days incubation. SEM analysis shows that BC has ultrafine fibrils and a pore-like reticulated structure. TGA result shows that BC was rapidly degraded at 250°C. The existence of common functional group *i.e.* OH group, C=O group, C-H bending, C-H stretching, C-H wagging, C-C stretching, C-O-C vibration and C-O stretching confirms the purity of BC. From BET and BJH analysis, BC has mesopore range of porosity with maximum pore size of 24.629Å during adsorption and 19.070Å for desorption process. The total surface area of the BC was 10.891 m^2/g. Investigation of tensile strength shows that BC has maximum tensile stress and Young's modulus values at 55.84 MPa and 733.61 MPa, respectively.

In conclusion, this study provides information on converting agricultural (food) waste into fermentable sugar via dilute acid hydrolysis where it can be utilized for BC production.

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SELULOSA BAKTERIA DARIPADA FERMENTASI *A. XYLINUM* MENGGUNAKAN HIDROLISAT SISA KULIT PISANG SEBAGAI SUMBER KARBON

Oleh

MUSTAPHA BIN AKIL

Disember 2011

Pengerusi: **Dayang Radiah Bt. Awang Biak, PhD**

Fakulti: **Fakulti Kejuruteraan**

Kos yang tinggi apabila menggunakan gula komersial sebagai sumber karbon dalam penghasilan selulosa bakteria (BC) menjadi satu isu kepada industri. Pencarian sumber karbon alternatif daripada sumber yang murah seperti sisa tanaman (makanan) adalah perlu untuk mengatasi masalah ini. Dua objektif utama telah ditetapkan dalam kajian ini iaitu (i) mengkaji hasil gula terturun yang diperoleh daripada hidrolisis asid cair terhadap sisa tanaman (makanan) dan (ii) mensintesis BC menggunakan hidrolisat yang diperoleh daripada objektif (i) sebagai sumber karbon.

Efluen sagu, kulit kentang dan kulit pisang telah dipilih sebagai bahan mentah. Kaedah hidrolisis asid cair telah digunakan untuk menghidrolisis sisa-sisa tersebut. Tiga parameter hidrolisis iaitu kepekatan asid sulfurik (2%, 4% dan 6%), masa tindak balas

(20, 40 dan 60 minit) dan suhu (105°C dan 121°C) telah dikaji. Kandungan glukosa tertinggi bagi setiap hidrolisat daripada sisa telah digunakan dalam eksperimen penapaian untuk menyaring sumber karbon terbaik untuk penghasilan BC.

Hidrolisis bagi kulit kentang menghasilkan glukosa tertinggi iaitu 43.44 mg/ml dengan menggunakan asid berkepekatan 4% (v/v) selama 20 minit masa tindak balas dengan suhu tindak balas pada 121°C. Nilai glukosa tertinggi iaitu 19.15mg/ml diperoleh daripada hidrolisis kulit pisang setelah 20 minit masa tindak balas berlaku menggunakan asid berkepekatan 6% (v/v) pada suhu 121°C sementara bagi efluen sagu, setelah masa tindak balas dilanjutkan ke 40 minit menggunakan asid berkepekatan 4% (v/v) pada suhu 105°C, hanya 2.64mg/ml glukosa telah diperoleh.

Hidrolisat kulit pisang telah dipilih sebagai sumber karbon yang digunakan dalam kajian ini kerana ia menghasilkan BC dengan hasil tertinggi iaitu 2.89 g/l selepas 21 hari tempoh inkubasi berbanding hidrolisat lain. Oleh itu, hidrolisat kulit pisang telah dipilih sebagai sumber karbon dalam kajian ini. Dalam pemilihan pH permulaan dan kepekatan sumber karbon permulaan, pH 6 dan sumber karbon berkepekatan 2.0% menghasilkan 2.81 g/l BC selepas 16 hari tempoh inkubasi. Analisis Mikroskop Elektron Pengimbas (SEM) menunjukkan bahawa BC mempunyai fibril yang sangat halus dan struktur liang yang bersaring. Analisis TGA menunjukkan BC didegradasi dengan cepat pada suhu 250°C. Kehadiran kumpulan berfungsi yang biasa seperti kumpulan OH, kumpulan C=O, lenturan C-H, regangan C-H, goyangan C-H , regangan C-C, getaran C-O-C dan regangan C-O memastikan keaslian BC. Daripada analisis BET dan BJH, BC mempunyai keliangan pada julat mesoliang dengan saiz liang maksimum adalah

24.629Å semasa proses penjerapan dan 19.070Å bagi proses penyahjerapan. Jumlah luas permukaan BC adalah 10.891m^2/g. Kajian terhadap kekuatan tegangan menunjukkan BC mempunyai nilai tegasan tengangan maksimum dan modulus Young pada 55.84 MPa dan 733.61 MPa masing-masing.

Kesimpulannya, kajian ini memberi informasi dalam menukarkan sisa tanaman (makanan) kepada gula boleh tapai melalui hidrolisis asid cair yang mana ia boleh digunakan dalam penghasilan BC.

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LIST OF ABBREVIATION

ATR	Attenuated total reflectance
BC	Bacterial cellulose
BET	Brunauer-Emmett-Teller
BH	Banana peel hydrolysate
BJH	Barret-Joyner-Halenda
Ca	Calcium
$CaCO_3$	Calcium carbonate
$CaSO_4$	Calcium sulphate
CM-BC	Carboxymethylated-bacterial cellulose
CMC	Carboxymethylated cellulose
DTG	Derivative Thermogravimetric
EABC	Diethylenetriamine-bacterial cellulose
EDX	Energy Dispersive X-ray
ELSD	Evaporative Light Scanning Detector
FTIR	Fourier Transform Infrared
H_2SO_4	Sulphuric acid
H_3COOH	Acetic acid
HPLC	High Performance Liquid Chromatography
HS	Hestrin and Schramm
MARDI	Malaysian Agricultural Research and Development Institute
Na	Sodium
NaOH	Sodium hydroxide

OD	Optical density
PES	Millipore Express PLUS
SEM	Scanning Electron Microscope
SME	Small and medium enterprises
TGA	Thermogravimetric Analysis
UDP-Glucose	Uridine-5'-diphosphate glucose
UV	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Background

Cellulose mainly is produced by plant as one of the core component of cell walls (Somerville, 2006). However, to obtain cellulose from plants, tree and woods are sacrificed and it costs adverse impact to the environment. Hestrin & Schramm, (1954) had found an alternative way to obtained cellulose, *i.e.* which produced by *Acetobacter xylinum* and named it bacterial cellulose (BC). Since then, BC became an interesting biomaterial to be studied by researchers all over the world either to increase its production or to be used in a few applications as reviewed later.

Production of BC requires carbon source as the main nutrient to be used by *Acetobacter xylinum*. Initially, glucose was used as the main carbon source for BC production (Hestrin & Schramm, 1954). Later, researchers had shifted to other saccharides and alcohol (Masaoka *et al.*, 1993; Keshk & Sameshima, 2005). Abundant amount of waste biomass in Malaysia might be utilized as a new energy source for BC production. Malaysia has many natural resources available especially from forestry and agriculture sectors (Tock *et al.*, 2010). In addition, municipal solid waste also generated about 1.7

kg/person/day where about 60 % was contributed by food and organic waste (Kathirvale *et al.*, 2004). Those wastes have potentials to be converted into valuable material, *e.g.* bioethanol (Del Campo *et al.*, 2006), biofuel (Lenihan *et al.*, 2010) and new carbon source that can be used to produce BC. For example, in sago starch production, the factory generated approximately 1000 m^3 effluent per day in the process (Vikineswary *et al.*, 1994). Since the effluent contains sago pith and also starch, it has potential to be used as carbon source for BC production. Banana peel also can be used as carbon source in BC production since it is a lignocellulosic material and has high amount of starch. The peel can be purchased or obtained from various places like food processing plants, markets, food and beverages shops and etc (Tock *et al.*, 2010). In addition, potato peel can also be used as carbon sources to produce BC. Since Malaysia has a few products that are potato-based product, *e.g.* Twist Potato and Jacker, the peels supplies exist. The main hypothesis of this work is that those materials are expected to have potentials to become alternative carbon source due to they consist complex sugar that can be treated. Treatment of those materials is needed so that it is suitable and can be used by *Acetobacter xylinum* for BC production.

1.2 Problem statements

In order to obtain fermentable sugar by utilizing agricultural (food) wastes, hydrolysis of those wastes is needed. Diluted acid hydrolysis is widely used and preferable to treat wastes compared to enzymatic hydrolysis due to its low cost and effective process for energy production (Aguilar *et al.*, 2002; Sun and Cheng, 2002; Herrera *et al.*, 2004; Del Campo *et al.*, 2006; Lenihan *et al.*, 2010). The high cost of enzyme, the need for specialized labor and the requirement for sophisticated laboratories (Tasic *et al.*, 2009) become a major problem to use enzymatic hydrolysis. Agro waste such as potato peel, banana peel and sago effluent which has high content of carbohydrate such as cellulose and starch that can be manipulated. The hydrolysis of wastes may give two benefits *i.e.* production of new alternative of fermentable sugar and at the same time elimination of the wastes.

Investigation of carbon source content to improve BC production was conducted by using a few type of biochemical (Keshk & Sameshima, 2005). The high cost of using commercial sugar, *i.e.* glucose, fructose, mannitol etc limit its industrial production. Discovery of new carbon source had been done by Hong and Qiu (2008) by preparing alternative carbon source from konjac powder to replace the commercial sugar. In addition, a few researchers had used fruit juices in the production of BC (Kongruang, 2008; Kurosumi *et al.*, 2009). Hence, hydrolysate from agro waste especially from sago effluent, banana peel and potato peel which is not available in literature is investigated in this study to produce BC.

1.3 Objectives

The main objective of this study is to synthesize BC from alternative carbon source. To accomplish this objective, two key objectives were set, namely:

i. To investigate the reducing sugar yield obtained from the diluted acid hydrolysis of agricultural (food) waste.
ii. To synthesize and characterize BC using hydrolysate obtained in (i) as the carbon source.

1.4 Scope of work

The scope of this work can be divided into three main sections:

i. Hydrolyze and characterize the agricultural (food) wastes that will be used as carbon source
ii. Synthesize the BC using the selected carbon source
iii. Analyze BC and media of fermentation

1.5 Thesis Outline

The thesis consists of five chapters. Chapter 1 covers the background, the problem statement and the research objectives of the study.

The literature review is covered in Chapter 2 where the basis to the production of fermentable sugar and BC production are elucidated. Published works are also described, summarized and discussed.

Chapter 3 briefly describes the research approach of this work. The methodology is explained in detail. Here, the method used to convert waste into fermentable sugar and production of BC from the selected alternative carbon source is presented.

In Chapter 4, the results and discussions were explained in detail. Findings for first and second objective were discussed.

Chapter 5 concludes the major finding of this work and provides recommendation for future work.

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