PINEAPPLE PEEL AS AN ALTERNATIVE SUBSTRATE FOR PRODUCTION OF BACTERIAL NANOCELLULOSE

By

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Chair : Professor Ts. Suraini Abd Aziz, PhD
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Bacterial nanocellulose (BNC) is a nano fibrillar polymer produced by nanocellulose producing bacteria such as *Acetobacter xylinum*. The diverse characteristics of BNC such as chemical purity, high mechanical strength, high flexibility, high absorbency, and the ability to create any shape or size and others that have attracted a lot of interest. The BNC's broad usage in food technology, paper, and electronics as well as the architecture in use, is due to its vast number of benefits. However, using commercial glucose as a carbon source in a media such as Hestrin and Schramm (HS) medium in large-scale synthesis of BNC is expensive. As a result, most researchers are looking for alternative substrates from accessible wastes to reduce the cost of BNC production. Thus, this research is aimed to investigate the synthesis of BNC by an isolated bacterial strain using an agro-industrial waste which is the pineapple peel extract. Six bacterial strains namely F8, F5, M1, M6, H7 and H11 were screened and identified for potential BNC producer. The selected bacterial strain was identified as *Bacillus cereus* MMS1 using 16S rRNA nucleotide sequences. Then, the production of BNC was done by *B. cereus* MMS1 using pineapple peel extract, while *A. xylinum* ATCC2376 was acted as a control. The BNC production in this study was attained at 2% (w/v) glucose concentration, 12 days of incubation period and 150 rpm agitation speed which was 5.83 g/L by *A. xylinum* ATCC2376 in HS medium using commercial glucose as carbon source. Meanwhile, 4.42 g/L and 2 g/L of BNC were produced by *B. cereus* MMS1 after 12 days of incubation with an initial concentration of 2% (w/v) using commercial glucose and pineapple peel extract, respectively. The fermentation effects of BNC production concludes that the optimum condition to produce BNC by using pineapple peel extract as an alternative substrate is 2% (w/v) carbon source concentration, 12 days of incubation period and 150 rpm agitation. This study showed that agro-industrial wastes such as the pineapple peel may be used in place of costly substrates to produce BNC.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
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KULIT NANAS SEBAGAI SUBSTRAT ALTERNATIF UNTUK PENGHASILAN NANOSELULOSA BAKTERIA

Oleh

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Nanoselulosa dari bakteria (BNC) ialah polimer fibrilar nano yang dihasilkan oleh bakteria penghasil nanoselulosa seperti *Acetobacter xylinum*. Ciri-ciri kepelbagaian BNC seperti ketulenan kimia, kekuatan mekanikal yang tinggi, fleksibiliti tinggi, daya serap yang tinggi, dan keupayaan untuk mencipta sebarang bentuk atau saiz dan lain-lain telah menarik minat ramai. Disebabkan oleh banyak faedahnya, penggunaan BNC adalah meluas dalam teknologi makanan, kertas dan elektronik serta seni bina. Walau bagaimanapun, penggunaan glukosa komersial sebagai sumber karbon dalam media seperti medium Hestrin dan Schramm (HS) dalam sintesis BNC berskala besar adalah mahal. Akibatnya, kebanyakan penyelidik mencari substrat alternatif daripada sisa yang boleh dicapai untuk mengurangkan kos pengeluaran BNC. Oleh itu, penyelidikan ini bertujuan untuk mengkaji sintesis BNC oleh strain bakteria terpencil menggunakan sisa agro-industri iaitu ekstrak kulit nanas. Enam strain bakteria iaitu F8, F5, M1, M6, H7 dan H11 telah disaring dan dikenal pasti sebagai pengeluar BNC yang berpotensi. Strain bakteria terpilih dikenal pasti sebagai *Bacillus cereus* MMS1 menggunakan jujukan nukleotida rRNA 16S. Kemudian, penghasilan BNC dilakukan oleh *B. cereus* MMS1 menggunakan ekstrak kulit nanas, manakala *A. xylinum* ATCC2376 bertindak sebagai kawalan. Pengeluaran BNC dalam kajian ini telah dicapai pada kepekatan glukosa 2% (b/i), 12 hari tempoh inkubasi dan kelajuan goncangan 150 rpm iaitu 5.83 g/L oleh *A. xylinum* ATCC2376 dalam medium HS menggunakan glukosa komersial sebagai sumber karbon. Manakala, 4.42 g/L dan 2 g/L BNC dihasilkan oleh *B. cereus* MMS1 selepas 12 hari inkubasi dengan kepekatan awal 2% (b/i) masing-masing menggunakan glukosa komersial dan ekstrak kulit nanas. Kesan penapaian pengeluaran BNC merumuskan bahawa keadaan optimum untuk menghasilkan BNC dengan menggunakan ekstrak kulit nanas sebagai substrat alternatif ialah 2% (b/i) kepekatan sumber karbon, 12 hari tempoh inkubasi dan goncangan pada 150 rpm. Kajian ini menunjukkan bahawa sisa agro-industri

seperti kulit nanas boleh digunakan sebagai pengganti substrat yang mahal untuk menghasilkan BNC.

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

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LIST OF ABBREVIATIONS

$(C_6H_{10}O_5)_n$	Bacterial nanocellulose formula
A.	*Acetobacter*
AAB	Acetic Acid Bacteria
B.	*Bacillus*
BC	Bacterial cellulose
BNC	Bacterial nanocellulose
BNCPVA	BNC-based polyvinyl alcohol
C60	Buckminsterfullerene
CETDEM	Centre for Environment, Technology and Development, Malaysia
CPPE	Citrus peel and pomace
DNS	3,5-Dinitrosalicylic Acid
dNTPs	Deoxynucleoside triphosphate
FAO	Food and Agriculture Organization
$FBTB_F$	Fermented black tea broth brewed from fresh tea leaves
FTIR	Fourier transform infrared spectroscopy
g	Gram
G.	*Gluconacetobacter*
GRAS	Generally Recognized as Safe
h	Hour
H_3PO_4	Phosphoric acid
HPLC	High Performance Liquid Chromatography
HS	Hestrin-Schramm
HSL	Lignosulfonate
K.	*Komagataeibacter*

L.	*Listeria*
min	Minute
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
O_2	Oxygen
OD	Optical density
OFAT	One factor at a time
OPFJ	Oil palm frond juice
P.	*Pseudomonas*
PP	Pineapple peel
PPE	Pineapple Peel Extract
rpm	Rotation Per Minute
rRNA	Ribosomal RNA
S.	*Salmonella*
S.	*Staphylococcus*
SCOBY	Symbiotic culture of bacteria and yeast
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscopy
SIFB	Static intermittent fed-batch
sp.	Species
T.	*Trichoderma*
TAE	Tris-acetate-EDTA
TC	Terminal Complexes
TE	Tris-EDTA
UDPGlc	uridine diphosphoglucose
XRD	x-ray diffraction

CHAPTER 1

INTRODUCTION

1.1 Research Background

Bacterial nanocellulose (BNC) is a linear polysaccharide made up of β-D-glucopyranose monomers connected by β-1,4-glycosidic bonds (Jacek *et al.*, 2019). A researcher named Adrian J. Brown was the first one to discover cellulose in 1886, when he observed *Acetobacter xylinum* cells producing cellulose with the existence of oxygen and glucose (Brown, 1886). It is a biopolymer with advanced technological characteristics generated by aerobic acetic acid bacteria. However, acetic acid bacteria have been reclassified, with *Acetobacter* being classed as *Gluconacetobacter*, which has just been reclassified as a new type of *Komagataeibacter*. Yuzo Yamada was the one to propose the present generic name, which is named by a Japanese scientist Kazuo Komagata (Yamada *et al.*, 2012). Numerous bacterial cellulose makers include nitrogen-binding bacteria like *Rhizobium leguminosarum*, plant pathogens (*Dickeya dadantii*), *Agrobacterium tumefaciens, Escherichia coli, Salmonella enterica, Pseudomonas putida*, and bacteria of the *Komagataeibacter* genus. (Römling & Galperin, 2015).

Owing to its amazing physical and chemical features, including as green processing, cheap manufacturing costs, enhanced tensile capabilities, hydrophilicity, good biocompatibility, and biodegradability, bacterial nanocellulose (BNC) has piqued global attention in recent decades (Gao *et al.*, 2019). High purity (free of lignin, hemicellulose, and pectin), high crystallinity, high elasticity and conformability, low density, high specific surface area, high degree of polymerisation, excellent permeability, high porosity and water content, and high mechanical strength in the wet state are all advantages of BNC over plant-derived cellulose, all of which contribute to BNC's multi-functionality (Mishra *et al.*, 2018; Bacakova *et al.*, 2019; Shoseyov *et al.*, 2019).

To fully leverage the features of BNC in specific applications, BNC can be changed via physical, chemical, or physicochemical means (Abraham *et al.*, 2020). For starters, it can be moulded *in situ* during fermentation to create diverse shapes like as tubes, membranes, spheres and layers of thin fibres, allowing it to satisfy the demands of functional materials for a variety of applications with a one-step synthesis (Reiniati, 2017). Secondly, it has a multihydroxyl molecular structure that may be functionalised through the use of functional compounds or polymer coating (Lee *et al.*, 2011). Bioprocessing, biomedical and pharmaceutical applications, wastewater treatment, electro-conductive materials, packaging, and food processing are just a few of BNC's application areas (Abol-Fotouh *et al.*, 2020).

The BNC production is becoming highly significant because of its environmentally friendly properties (Jozala *et al.*, 2016) and because of that benefit, other researchers also started to determine the capacity of cellulose production via microbial hosts. However, owing to reduced BNC productivity of known strains and the use of delicate and expensive culture medium ingredients, the entire BNC production process still needs to be improved significantly to be more competitive. It seems to be worth noting that the cost of the culture medium accounts for around 30% of the overall cost of BNC manufacturing (Revin *et al.*, 2018).

As a result, among the most frequently reported issues was the decision to partially replace the expensive culture medium elements with ones that is low-cost in the hopes of increasing BNC output in a short period of time (Hussain *et al.*, 2019). In this study, the challenge would be to identify a cost-effective medium component that can facilitate high production of BNC by using pineapple peel extract (PPE) as an alternative substrate replacing commercial D-glucose in Hestrin-Schramm medium. It is considered a cost-effective culture medium substrate because the pineapple peels will be utilised as it is an abundant agricultural waste in Malaysia especially in Johor where it is rich with pineapple plantation.

In recent years, various efforts have been dedicated to developing sustainable and ecologically friendly materials as a result of growing worries about severe environmental degradation. Thus, academic and industry research into the use of biomass wastes as a feedstock for energy and materials applications has expanded (Dai *et al.*, 2018). With an annual production of 16-19 million tons, pineapple is among the most famous tropical fruits (Dai & Huang, 2016). However, pineapple peel is produced during the processing of the pineapple, representing for 35% of the total weight of the pineapple (Dai & Huang, 2017a). It's worth mentioning that pineapple peel removal is expensive, and its large-scale waste disposal can affect the environment (Hu *et al.*, 2010). In a nutshell, from the perspective of waste processing, pineapple peel should be a viable biomass source for industrial applications that requires no extra cost input.

Pineapple peel is mostly made of cellulose, hemicellulose, lignin, and pectin, with cellulose accounting for 20-25% of the dry weight (Dai & Huang, 2017b). Besides containing all those indigestible carbohydrate, pineapple peel also contains digestible carbohydrates such as starch and sugar (Chongkhong & Tongurai, 2019). The goal of this study was to determine the potential of pineapple peel as an alternative substrate for the production of bacterial nanocellulose and to add value to pineapple harvests by utilising their peel. The microorganism used in this study is *Acetobacter xylinum* ATCC2376 and several local isolates. Previous study had stated that *A. xylinum* is the main microbial producer of BNC and it became a model system for biosynthetic mechanisms of BNC study (Jozala *et al*., 2016) and because it generates cellulose as an extracellular product, the bacterium *A. xylinum* has been utilised as a model system for studying cellulose production (Yu & Atalla, 1996). That cellulose producing bacterium, contains linear TCs that are made up of a row of particles on the plasma membrane (Tokoh *et al.*, 1998).

1.2 Significant of study

The benefits of a safe environment, as well as the responsibility to preserve it, are currently becoming universal concerns for our planet. In this context, the chemical sector must examine the adoption of greener technology and environmentally friendly supplies while maintaining competitive costs. In this way, industrial ecology concepts strongly advocate an economy in which solid wastes may be employed as raw materials for new goods and applications while waste disposal in the environment is minimised (Corujo *et al.*, 2016). Solid waste, also known as biomass waste, is a result of pineapple or any other fruits processing or after the plants have been harvested. Many sectors have found new and appealing waste management methods as a result of economic, social, and environmental concerns. Clean biotechnology is a platform that allows wastes to be converted into profitable and less harmful end products.

BNC has a long history of use as a biomaterial for a variety of purposes. However, due to expensive culture media and high cost of fermentation but with low productivity, its industrialisation on a wide scale remains a hurdle (Hussain *et al.*, 2019). Low-cost substrates and big waste biomass outputs from many sectors have been explored for BNC production to alleviate this difficulty. In this study, using pineapple peel waste does not only benefit the economy and the environment, but also the preservation of pineapple fields. The main research questions that contributed to the study are how to identify the potential bacterial nanocellulose (BNC) producer and what are the factors affecting the production of BNC. Therefore, the objectives of this research study are:

1. To screen and identify the potential bacterial nanocellulose (BNC) producers.
2. To study the feasibility of fermentation effects on bacterial nanocellulose (BNC) production from pineapple peels as an alternative substrate.

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