ROTARY DISCS REACTOR FOR ENHANCED PRODUCTION OF MICROBIAL CELLULOSE

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Specially dedicated to my beloved and supporting family:

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ABSTRACT

Production of microbial cellulose is receiving great attention since microbial cellulose is comparable to the synthetic cellulose, source of medium is abundant and cellulose has wide applications. However, microbial cellulose is produced in Malaysia in the form of 'nata' as food using traditional method only. Furthermore, the use of trays for static fermentation in traditional method is not economical, laborious and the up-scale process for high yield productivity is limited. This study aims to develop a practical methodology for enhanced production of microbial cellulose by designing a Rotary Discs Reactor (RDR). One of the major factors that determine the success of fermentation process is aeration during fermentation. Therefore, RDR applies the concept of Rotating Biological Contactor (RBC) that widely used in wastewater treatment in order to exposing the bacteria to oxygen for better aeration. This reactor consists of an array of discs that is mounted to a shaft. The shaft is connected to a driven motor so that the rotation of the shaft together with the discs is achievable and controllable. The discs on the shaft are positioned in a horizontally set trough that contains a biological medium in which at least a portion of the contained discs are being submerged. In the preliminary study of discs selection, discs made from stainless steel fabricated with 0.3cm mesh sizes gave the highest result compared to others. In addition, it was found that smallest mesh in stainless steel type of discs was advantagous in assisting the *Acetobacter xylinum* attachment onto the discs which resulted in better aeration and higher cellulose production. To study effect of rotation speed in RDR, fermentation in prepared sucrose medium had been carried out at the rotational speeds of 7, 9 and 11 rpm. It was found that rotational speed gives significant effect towards microbial cellulose production where fermentation in RDR using 7 rpm gave the highest microbial cellulose production of 149.12gram per liter substrate. A series of static and RDR fermentation had been run in a fixed condition in order to compare the production yields. Results showed that fermentation carried out using RDR gave 86.78% higher production of microbial cellulose compared to static fermentation after 5 days of fermentation. This indicated that RDR could give better aeration process compared to static fermentation. However, too much Dissolved Oxygen resulted from too high rotational speed resulted in decrease of microbial cellulose production in RDR as this affected the stability of the culture. Hence, it can be concluded that fermentation using RDR did not depend solely on dissolved oxygen in the medium as the rotation of discs permitted direct exposure to air for *A.xylinum* during the fermentation process.

ABSTRAK

Penghasilan selulosa mikrobial mula mendapat perhatian ramai berdasarkan sifat-sifatnya yang setara dengan selulosa sintetik, sumber medium yang mudah diperolehi dan penggunaannya yang meluas dalam berbagai bidang. Walaubagaimanapun, di Malaysia, selulosa mikrobial dihasilkan sebagai bahan makanan yang dikenali senagai 'nata' menggunakan kaedah tradisional sahaja. Lebih dari itu, penggunaan dulang dalam kaedah tradisional ini dilihat sebagai tidak ekonomi, memerlukan tenaga buruh yang ramai dan pengeluaran secara besar-besaran adalah terhad. Kajian ini dijalankan bertujuan untuk menghasilkan kaedah yang lebih praktikal bagi meningkatkan pengeluaran selulosa mikrobial dengan mereka-bentuk 'Rotary Discs Reactor' (RDR). Satu faktor yang menentukan keberhasilan proses penghasilan selulosa mikrobial adalah faktor pengudaraan semasa fermentasi. Justeru itu, RDR menggunakan konsep 'Rotating Biological Contactor' (RBC) yang digunakan secara meluas dalam rawatan air kumbahan bagi membantu bakteria mendapatkan oksigen untuk pengudaraan yang lebih optimum. Reaktor ini mengandungi susunan cakera yang dilekatkan pada satu pemegang. Pemegang tersebut bersambung dengan motor bagi membolehkannya di kawal semasa berputar bersama cakera. Cakera pada pemegang ditempatkan dalam satu takung yang mengandungi medium di mana sebahagian daripada cakera dibiarkan terendam. Kajian awal bagi pemilihan cakera untuk RDR menunjukkan penggunaan keluli tahan karat dengan permukaan bergrid seluas 0.3cm memberi jumlah penghasilan selulosa mikrobial tertinggi berbanding yang lain. Lebih dari itu, kajian mendapati permukaan bergrid yang lebih kecil pada keluli tahan karat membantu *A.xylinum* melekat pada cakera untuk pengudaraan yang baik sekaligus meningkatkan penghasilan selulosa mikrobial. Untuk mengkaji kesan kelajuan motor terhadap penghasilan selulosa mikrobial, fermentasi menggunakan *A.xylinum* dengan sukrosa sebagai medium telah dijalankan pada kelajuan motor 7, 9 dan 11. Kajian menunjukkan kelajuan motor memberi kesan besar terhadap pembentukan selulosa mikrobial di mana fermentasi pada kelajuan motor 7 rpm memberikan penghasilan selulosa mikrobial tertinggi sebanyak 149.12gram per liter medium. Fermentasi menggunakan kaedah statik dan RDR juga telah dijalankan pada keadaan yang sama bagi membandingkan penghasilannya. Hasil selepas 5 hari menunjukkan fermentasi menggunakan RDR memberi 86.78% lebih penghasilan selulosa berbanding fermentasi statik. Ini membuktikan bahawa RDR dapat memberi pengudaraan yang lebih baik berbanding fermentasi statik. Walaubagaimanapun, kandungan oksigen terlarut yang meningkat akibat peningkatan kelajuan motor telah menyebabkan penurunan dalam penghasilan selulosa mikrobial kerana gangguan terhadap kestabilan *A. xylinum* itu sendiri. Oleh itu, dapat disimpulkan bahawa fermentasi menggunakan RDR tidak bergantung sepenuhnya kepada kandungan oksigen terlarut memandangkan putaran cakera membolehkan *A. xylinum* mendapat bekalan oksigen terus dari udara semasa fermentasi.

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$(NH_4)_2SO_4$	Ammonium Sulphate
A.xylinum	*Acetobacter xylinum*
A.xylinus	*Acetobacter xylinus*
ANOVA	Analysis of Variance
$C_{12}H_{22}O_{11}$	Sucrose
Cel^-	Cellulose Non-producer
CFU	Colony Forming Unit
CMC	Carboxymethyl Cellulose
CO_2	Carbon dioxide
CSTR	Continuous Stirred Tank Reactor
DO	Dissolved Oxygen
GDH	Glucose Dehydrogenase
H^+	Hydrogen Ion
H_2S	Hydrogen Sulfide
KH_2PO_4	Pottasium Dehydrogen Phosphate
MARDI	Malaysian Agricultural Research and Development Institute
$MgSO_4$	Magnesium Sulphate
N_2	Nitrogen
NaOH	Natrium Hydroxide
NH_3	Ammonia
NO_3	Nitrate
OH^+	Hydroxyl Ion
PMMA	Poly-(methyl matacrylate)
PO_4	Phosphate

RBC	Rotating Biological Contactor
RDR	Rotary Discs Reactor
SEM	Scanning Electron Microscopy
SO_4	Sulphate
S	Sulfur

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CHAPTER I

INTRODUCTION

1.1 Background of the Problem

Microbial celulose is produced by bacteria from the species of *Aerobacter, Acetobacter, Achromobacter, Agrobacterium, Alacaligenes, Azotobacter, Pseudomonas, Rhizobium* and *Sarcina*. However, only the *Acetobacter* species produce enough cellulose to justify commercial interest. The most extensively studied member of the *Acetobacter* species is *A. xylinus*, formerly known as *A. xylinum*.

Microbial cellulose has finer structure compared to plant cellulose. Besides that, it does not have hemicellulose or lignin that need to be removed and can be grown to virtually any shape. In industries, microbial cellulose was produced for products such as dessert, wound dressing, high strength paper and diet foods.

American Chemical Society in Science daily on February 2007 reported that biotechnology's next high-value product could be microbial cellulose (Science Daily, 2007). The unique properties of microbial cellulose make it suitable to be used in different fields. Recently, many studies had been done to use microbial cellulose especially in medical field. R. Malcolm Brown Jr from Poland is one of the famous researcher that studied about microbial cellulose properties and used it for new purposes.

Some of his significance findings are to use microbial cellulose as wound dressing and as electronic display paper (Shah and Brown, 2004; Czaja *et al.,* 2006)

However, there are some issues that prevent larger scale commercialization such as high price of substrates, low volumetric yields and also lack of large scale production capacity.

The current study was done to enhance production of microbial cellulose by designing a Rotary Discs Reactor (RDR). The RDR uses concept of Rotating Biological Contactor (RBC) that expose bacteria to air for better aeration. The RDR uses multiple discs that rotate to give better aeration to the bacteria. Bungay and Serafica (1999) in their patent reported that material for discs give effect in microbial cellulose production. In this research, some focus had been given to find out what kind of discs and configurations that suitable for the fermentation.

A. xylinum was used in this study as cellulose producer. It is gram negative bacteria that can be found naturally in ripened and spoilage fruits. The inert surroundings of these bacteria make it very sensitive to harsh environment. Krystynowicz *et al.* (2002) reported that too harsh environment did not affect *A. xylinum* growth. However it does effect cellulose production since cellulose negative mutant of *A. xylinum* will be produced. Therefore, it is important to make sure RDR used in this experiment gives better aeration without disturbing the nature of these bacteria.

The rotational speed of the discs during cellulose production has a noticeable effect on the production rate of cellulose during the fermentation (Kim *et al.,* 2007). This research also includes an experiment to study the effect of rotational speed to microbial cellulose production.

1.2 Objective of Study

The objective of this study is to find an alternative way to produce higher yield of microbial cellulose compared to conventional static fermentation using tray method (surface culture). A Rotary Discs Reactor (RDR) is designed so that optimum conditions could be provided for cellulose production. This also includes manipulation of the parameters involved in the preparation methods and fermentation in the RDR.

1.3 Scope of Study

The scopes of this study are:

- To design a Rotary Discs Reactor (RDR) for the production of microbial cellulose
- To investigate the optimum parameter of culture/inoculums before fermentation in the RDR
- To compare production of microbial cellulose using traditional method (static fermentation using tray) and using the RDR

1.4 Thesis Outline

The work reported in this thesis focused on designing a reactor that expected to enhance production of microbial cellulose. Chapter 2 begins by introducing microbial cellulose, methods of production and the applications of microbial cellulose in different fields. The chapter also reviews bacteria that being used in this research i.e. *A. xylinum* and factors that affect its growth.

The design of RDR is described in detail in chapter 3. This chapter also list

out the materials and methods, equipments used and procedures for the experiment conducted in the research.

In chapter 4, the results are presented and discussed. In each section, the results are analyzed graphically. Each result is well summarized at the end of each sub-section in this chapter.

Finally, chapter 5 concludes the findings of this study. Few recommendations are listed with the intention that other researchers can make further improvements in the future.

1.5 Significance of Study

Preservation of forest resources is essential to prevent global warming because the increase in CO_2 concentration can be stopped only by the absorption of CO_2 by plants and trees. However, the use of trees for the production of paper and construction materials has continuously depleted forest resources. In the era of declining forests, global climate changes, continuing expansion of industrialization, it is reasonable to consider the consequences of an alternative source of cellulose. Bacterial cellulose is an alternative for plant cellulose where bacteria produce bacterial cellulose within a few days, while a tree needs in average more than 30 years to realize full growth. In this respect, bacterial cellulose is the key material for preventing global warming and preservation of the nature.

Interestingly, microbial cellulose was proven to be a remarkably versatile biomaterial and can be used in a wide variety of applied scientific products, such as paper products, electronics, acoustics, and biomedical devices. In world market, cellulose price ranges from RM 259.85 – RM 380.93 per kilogram in 2006 and keep increasing every year. Annual worldwide demands for cellulose are 50,000 tons. The highest cellulose worldwide demands are in pharmaceutical sector with annual demand of 30,000 tons (FMC Annual Report, Year 2002).

Traditional methods using static culture in trays has a problem especially for production in large scale. Based on the review, it was concluded that microbial cellulose is poised for use in a wide variety of medical devices and consumer products. Interest in Nata and other microbial cellulose products now is fueled by the demand for the product. It is ironical that demand now outpaces the supply for microbial cellulose, largely because of lack of investment in fermentation research and development to optimize microbial cellulose production on a large scale (Brown, 1996). Hence it is important to find method to scale up the production of microbial cellulose to justify commercial interest.