

**ROTARY DISCS REACTOR FOR ENHANCED PRODUCTION OF
MICROBIAL CELLULOSE**

NORHAYATI BINTI PA'E

UNIVERSITI TEKNOLOGI MALAYSIA

ROTARY DISCS REACTOR FOR ENHANCED PRODUCTION OF MICROBIAL
CELLULOSE

NORHAYATI BINTI PA'E

A thesis submitted in fulfilment of the
requirements for the award of the degree of
Master of Engineering (Bioprocess)

Faculty of Chemical and Natural Resources Engineering
Universiti Teknologi Malaysia

OCTOBER 2009

Specially dedicated to my beloved and supporting family:

Pa'e Derman and Mariam Juri,

Asrul Asmawi bin Abdu Rahim,

Hardi and Imme Zuzana, Harris and Norhana,

Harizan and Muhammad Firdaus,

Nur Fatin Najwa and Nazhatul Saadiah.

ACKNOWLEDGEMENTS

First and foremost, I would like to take this opportunity to express my deepest gratitude and appreciation to my supervisor, PM Dr. Ida Idayu Muhamad for her advice, encouragement and dedicated guidance. Without her advice and consult, this thesis project would not be produced on time.

Furthermore, my heartfelt appreciations are also extended to Bioprocess Engineering Department Laboratory staff for providing technical help, and guidelines throughout the whole research. Pn. Siti Zalita Bt. Ad. Talib, En. Yaakob Sabudin, En. Abd. Malik Yusop, En. Nur Muhammad El Qarni Md. Noradin, and Pn. Nadia. Not forgetting, to En Yahya Khalid for the fabrication of Rotary Discs Reactor.

To all my friends; Nozieana Khairuddin, Nurul Asyikin Md Zaki, Pn. Eraricar Salleh, Iryatie Ishak, Nooranis Mustapha, Siti Nur Hidayah Mohamad and Norazlina Mohd Nawi, thank you for always being there, sharing every moment together. To all of them, I want to express my sincere gratitude.

Last but not least, I would like to express my earnest appreciation to my beloved parents, family and all people who involved directly and indirectly for their support, understanding and love throughout this unpredictable and meaningful journey.

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 advantageous 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 sebagai '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.

TABLE OF CONTENTS

CHAPTER	TITLE	PAGE
	DECLARATION	ii
	DEDICATION	iii
	ACKNOWLEDGEMENTS	iv
	ABSTRACT	v
	ABSTRAK	vi
	TABLE OF CONTENTS	vii
	LIST OF TABLES	xii
	LIST OF FIGURES	xiii
	LIST OF ABBREVIATIONS	xviii
	LIST OF APPENDICES	xx
1	INTRODUCTION	
	1.1 Background of the Problem	1
	1.2 Objective of Study	3
	1.3 Scope of Study	3
	1.4 Scope of Thesis	3
	1.5 Significant of Study	4

2	LITERATURE REVIEW	
2.1	Cellulose	6
2.2	Microbial Cellulose	7
2.2.1	Microbial Cellulose Content	8
2.2.2	Features of Bacterial Cellulose	9
2.2.3	Application of Microbial Cellulose	10
2.3	Production of Microbial Cellulose	14
2.3.1	<i>Acetobacter aceti</i> sp	14
2.3.1.1	<i>Acetobacter xylinum</i>	15
2.3.1.2	Taxonomy	17
2.3.2	Factors Affected Production of Microbial Cellulose by <i>A. xylinum</i> .	18
2.3.2.1	pH	18
2.3.2.2	Temperature	19
2.3.2.3	Oxygen Concentration	19
2.3.2.4	Surface Area	20
2.3.2.5	Biochemical Factor	21
2.3.3	Cellulose Biosynthesis by <i>Acetobacter Xylinum</i>	23
2.3.4	Production of Microbial Cellulose Under Different Condition	26
2.3.4.1	Static Production	27
2.3.4.2	Agitated Production	29
2.3.4.3	Rotary Discs Reactor.	30
2.4	Summary	34
3	MATERIAL AND METHODOLOGY	
3.1	Introduction	35
3.2	Design of Experiment	37

3.2.1	Physical Design	37
3.2.2	Pre – Fermentation	37
	3.2.2.1 Medium Preparation	37
	3.2.2.2 Agar Plate Preparation	38
	3.2.2.3 Inoculums Preparation	38
3.2.3	Fermentation Process	39
	3.2.3.1 Precautions Steps and Limitation during Fermentation	39
	3.2.3.2 Fermentation Condition	39
3.3	Physical Design of Rotary Discs Reactor (RDR)	40
3.3.1	Trough	41
3.3.2	Discs	42
3.3.3	Motor and Shaft	43
3.4	Pre – Fermentation	43
3.4.1	Colony Forming Unit (CFU) Test	43
3.4.2	Preliminary Study for Discs Selection	44
3.5	Phase 3: Fermentation Process	46
3.5.1	Fermentation Process using Static Culture and Rotary Disc Reactor (RDR)	46
	3.5.1.1 Preparation of Starter Medium for <i>Acetobacter Xylinum</i>	46
	3.5.1.2 Preparation of Medium	47
	3.5.1.3 Fermentation using Static Culture	47
	3.5.1.4 Fermentation using RDR	47
3.5.2	Data Analysis	48
	3.5.2.1 Measurement of Wet Weight and Dry Weight of Microbial Cellulose	48
	3.5.2.2 Statistical Analysis	49
	3.5.2.3 Dissolve Oxygen Measurement	49

	3.5.2.4 Glucose Analysis	50
3.6	Summary	51

4 RESULTS AND DISCUSSIONS

4.1	Introduction	52
4.2	Designing The Rotary Discs Reactor (RDR)	52
	4.2.1 Trough	53
	4.2.2 Discs	54
	4.2.3 Driven Motor and Shaft	56
	4.2.4 Overall Basic Design of Developed Rotary Discs Reactor.	57
4.3	Preliminary Study of Fermentation in RDR	58
	4.3.1 Colony Forming Units Test	58
	4.3.2 Preliminary Study for Discs Selection	60
4.4	Fermentation Process	63
	4.4.1 Fermentation with Static Culture	63
	4.4.2 Fermentation using Rotary Discs Reactor (RDR)	64
	4.4.2.1 Effect of Rotation Speed to Microbial Cellulose Production in RDR	65
	4.4.2.2 Effect of Initial pH of medium to Microbial Cellulose Production in RDR	67
	4.4.3 Comparison between Static and RDR Fermentation	69
	4.4.3.1 Comparison of yield between RDR (at speed 7 rpm) and Static Culture for 5-days Fermentation	70
	4.4.3.2 Dissolved Oxygen Measurement	73
	4.4.3.3 Glucose Analysis	75
	4.4.3.4 pH Drop in Static and RDR	

	Fermentation.	77
	4.4.3.5 Summary of Comparison between Static and RDR Fermentation.	79
4.5	Summary	81
5	CONCLUSIONS AND RECOMMENDATIONS	
5.1	Introduction	82
5.2	Summary and Conclusions	82
5.3	Recommendations for future works	85
	REFERENCES	86
	APPENDICES	91
	PUBLICATION	96
	ABOUT THE AUTHOR	97

LIST OF TABLES

TABLE NO.	TITLE	PAGE
2.1	Products which can be Manufacture from Microbial Cellulose (Brown Jr.R.M., 1986)	13
2.2	Microbial Cellulose Producers. (Jonas and Farah, 1997)	17
2.3	Major Elements and Their Functions in Bacterial Cellulose Production	22
2.4	Production of Microbial Cellulose using Different Method.	26
2.5	Summary of Previous Work on RDR	31
3.1	Shigeru Yamanaka Medium	38
3.2	Types of discs for Rotary Discs Reactor	46
4.1	Specifications for RDR	57

LIST OF FIGURES

FIGURE NO.	TITLE	PAGE
2.1	Cellulose as polymer of β -D-glucose	6
2.2	SEM micrograph of the wet structure of microbial cellulose at 2 μ m	7
2.3	Microbial cellulose compositions	8
2.4	Products from microbial cellulose	12
2.5	<i>Acetobacter xylinum</i> under electron microscope	15
2.6	Secretion of glucan into micro fibrils by <i>Acetobacter</i> cells (Brown, 1986).	16
2.7	Effect of surface area to production of microbial cellulose using different carbon sources (Holmes, 2004)	21
2.8	Differential between Bacterial and Plant Cellulose	23
2.9	Biochemical pathway from glucose to synthesize cellulose (Jonas and Farah, 1997)	25

2.10	Cellulose growing in static culture.	28
2.11	Microbial cellulose formed at the interface of air-liquid medium	29
2.12	Cellulose formed in agitated culture fermentation	30
2.13	Example of Rotating Biological Contactor (RBC) used in waste water treatment	31
2.14	Biosynthesis of microbial cellulose in the RDR (Krystynowicz <i>et al.</i> , 2002)	34
3.1	Schematic Diagram of Operational Framework.	36
3.2	Two dimensions drawing for RDR	41
3.3	Active surface area and submerged area for disc	43
3.4(a)	Polypropylene disc use for preliminary study	45
3.4(b)	Stainless Steel disc use for preliminary study	45
3.4(c)	Polyethylene (0.6cm mesh) disc use for preliminary study	45
3.4(d)	Polyethylene (0.3cm mesh) disc use for preliminary study	45
3.5	Rotary Discs Reactor for microbial cellulose production.	48
3.6	Hanna Oxy-Check for DO measurement.	50
3.7	YSI Glucose Analyzer for checking glucose content	51

4.1	Three dimension-view of RDR	53
4.2	Trough as a medium container in Rotary Discs Reactor	54
4.3	Discs for Rotary Discs Reactor	55
4.4	Shaft was attached to driven motor that provide rotation for discs.	56
4.5	Rotary Discs Reactor	57
4.6	Standard Curve for bacterial growth	58
4.7	Graph of log cfu/ml vs days for <i>Acetobacter xylinum</i> growth	59
4.8	Yield of microbial cellulose by using different disc types for RDR in preliminary study of fermentation	61
4.9 (a)	Microbial cellulose attached to different Polypropylene disc.	62
4.9(b)	Microbial cellulose attached to different Stainless Steel disc.	62
4.9 (c)	Microbial cellulose attached to different Polyethylene (0.6cm mesh) disc.	62
4.9(d)	Microbial cellulose attached to different Polyethylene (0.3cm mesh) disc.	62

4.10	Microbial cellulose weight <i>versus</i> pH for microbial cellulose production after 5 days fermentation in static culture.	64
4.11	Microbial cellulose production in RDR after 5 days Fermentation	65
4.12	Microbial cellulose weight <i>versus</i> rpm (after 5 days fermentation in RDR)	66
4.13	Microbial cellulose weight <i>versus</i> pH after 5 days fermentation in RDR	68
4.14(a)	Wet microbial cellulose from RDR fermentation	69
4.14(b)	Wet microbial cellulose from static culture fermentation	69
4.15(a)	Dried microbial cellulose from RDR fermentation	70
4.15(b)	Dried microbial cellulose from static culture fermentation	70
4.16	Comparison of cellulose produced between static and RDR fermentation after 5 days.	71
4.17	Summary of ANOVA	72
4.18	Dissolve Oxygen (DO) <i>versus</i> rpm for Static (data at 0 rpm) and RDR fermentation (data at 7, 9 and 11 rpm).	73
4.19	Dissolve Oxygen (DO) and Microbial Cellulose weight <i>versus</i> speed of rotation (rpm) for RDR fermentation after 5 days fermentation.	74

4.20(a)	Glucose content <i>versus</i> fermentation day for RDR fermentation	76
4.20 (b)	Glucose content <i>versus</i> fermentation day for static fermentation	76
4.21	Initial and final pH for fermentation in Static and RDR.	78
4.22	Value of pH drop in RDR and static fermentation after 5 days.	78
4.23	Comparison of cellulose wet weight using static and RDR fermentation at different initial pH.	79
4.24	Comparison of air and food diffusion in static and RDR fermentation.	80

LIST OF ABBREVIATIONS

$(\text{NH}_4)_2\text{SO}_4$	Ammonium Sulphate
<i>A.xylinum</i>	<i>Acetobacter xylinum</i>
<i>A.xylinus</i>	<i>Acetobacter xylinus</i>
ANOVA	Analysis of Variance
$\text{C}_{12}\text{H}_{22}\text{O}_{11}$	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

LIST OF APPENDICES

APPENDIX	TITLE	PAGE
A	Calculation: Surface Area for Discs	91
B	Proceeding: International Conference & Exhibition on Waste to Wealth (W2W) 2007 at Putra World Trade Centre (PWTC), Kuala Lumpur, Malaysia, 26 – 28 November 2007	94
C	Poster presented at Innovation, Art & Technology Exhibition (INATEX) 2007, UTM Skudai, 15-21 Aug 2007	95

CHAPTER I

INTRODUCTION

1.1 Background of the Problem

Microbial cellulose is produced by bacteria from the species of *Aerobacter*, *Acetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *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 CO₂ concentration can be stopped only by the absorption of CO₂ 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.