BACTERIAL CELLULOSE PRODUCTION BY
ACETOBACTER XYLINUM IN AERATED, AGITATED
AND ATTACHED SYSTEM

Dissanayake Mudiyanselage Susantha Chandana Dissanayake

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Degree of Master of Science in Sustainable Process Development

Department of Chemical and Process Engineering

University of Moratuwa
Sri Lanka

September 2013
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D. M. S. C. Dissanayake

Dissertation submitted in partial fulfillment of the requirements for the degree of Master
of Science in Sustainable Process Development

Department of Chemical and Process Engineering

University of Moratuwa
Sri Lanka

September 2013
Declaration of the candidate and supervisor

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Dr. (Mrs.) Marliya Ismail,
Senior lecturer,
Department of Chemical and Process Engineering,
University of Moratuwa,
Sri Lanka.
Abstract

Cellulose is the most common biopolymer on earth and has been identified as a major building material of all plants. It is common not only in the higher order plants, but also in microorganisms. Plant Cellulose formed to be structural materials for higher order cell, while bacterial cellulose (BC) plays a protective action in its cell. Most common genera which produce BC are *Acetobacter*, *Rizobium*, *Agrobacterium* and *Sarcina*. It was found that *Acetobacter xylinum* has the highest capability to produce cellulose rather than other species performed in the same condition. BC could play a very important role as a versatile biomaterial in modern industries as it has high purity, high mechanical strength, high water holding capacity and high crystalline ability compared to plant cellulose. Most of the BC studies have been carried out with Static Fermentation (SF) techniques. However static systems have the drawback when it comes to an industrial usage, due to the reduction of dissolved oxygen (DO) and pH of the media with the increase of cell mass increment and cellulose production. Therefore agitated and aerated systems were developed to overcome the limitations of the SF. Rotating Biological Fermentor (RBF) could be considered as both an agitated and aerated system. This was developed to overcome problems which hindered BC production in SF system.

In this research, lab scale RBF was designed and fabricated to operate in three different agitator speeds. Substrate media was prepared using sterilized coconut water inoculated with *Acetobacter xylinum*. pH and DO variations in RBF and in SF were recorded for 7 to 8 days. Yield of cellulose production and the cell mass was also investigated during the fermentation period for different agitator speeds on both systems. Initial pH of the SF and RBF was 5.3 and with time it reached a steady value of 3.4 whereas in SF the pH decreased further. In the case of DO, the initial value was 1.47 mg/l. There was a continuous drop of DO in SF while in RBF it fluctuated within the range of 0.25 to 0.43 mg/l. Cellulose production was 0.889×10^-10 g CFU^-1 ml^-1 for SF and 1.92×10^-10 g CFU^-1 ml^-1 for RBF respectively after 8 days. These investigations indicate that the RBF system could supply air to the culture medium in a continuous manner and was able to regulate DO when compared to a SF system. Further pH variation was also minimized in RBF compared to SF favoring the growth of cell mass and thereby yield of cellulose. A mathematical model for the synthesis of BC in a RBF system was also developed. The growth of cellulose is considered as a cellulose film from a mono culture. Glucose depletion, cellulose production and microbial growth in the fermentation medium were explained using the developed models. It was shown that the simulated and experimental results were in close agreement. In addition, the model was successful in predicting yield of cellulose at different rotational speeds of the RBF unit. On conclusion it could be said that RBF is a better system to generate cellulose when compared to SF and the developed model could explain the cell mass and cellulose growth profiles which could be useful in mass scale production.

Keywords: Bacterial Cellulose, *Acetobacter xylinum*, Rotating Biological Fermentation, Mathematical model
Dedication

Lady who,
Always by my side,
Motivating me every time,
Dreaming with me,
Financially assisting me,
My beloved wife,

Niduka Nadeeshani Dissanayake,
I bestow this effort,
To you
Acknowledgement

My deepest appreciation is conveyed to my first teacher and my elementary school Grade three class teacher, my dearest mum, D.M.D. Dassanayake, and to my dearest dad, D.M.D.B. Dissanayake. Without their kind dedication, I might not be grace my place in this world. I am happily reminiscing my brother’s D.M.C.K. Dissanayake, and sister’s D.M.C.D. Dissanayake co-operations since my childhood.

I am grateful to my research supervisor, Dr. (Mrs.) Marliya Ismail. Without her kind support and comments, this effort would not have been successful. She paid her fullest attention every time I needed; she understood my abilities, talents and weakness. She helped me to develop myself and improved everything she could. I appreciate her kind dedication.

I appreciate laboratory technicians, Mrs. I. K. Athukorala, Mrs. S. M. N. D. Martino and laboratory attendant Mr. W. L. D. Fernando, M. P. A. J. Kumara for the assistance given to fulfill this effort. I also thank the great support given by Mechanical Engineering workshop, workers including Mr. M.D. Sarath Ananda.

I would like to convey my appreciation to Mr. Dilhara Wengappuli Arachchige, for his kind support and technical advice in constructing the RBF unit.

I convey my heartfelt gratitude to Head, academic staff, non academic staff of Department of Chemical and Process Engineering-University of Moratuwa, my dear friends, colleagues, and my family members that gave me a peaceful environment, thus stimulating the progress of my work.

I highly appreciate Mr. Anushka Perera’s support, encouragement and his attention given while I was visiting Norway for the conference paper presentation based on this research.

Finally I wish to convey my special thanks to the authorities of the M.Sc. funds given by NOMA, Norway and for the collaboration of Telemark University College, Norway.
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<td>BC</td>
<td>Bacterial cellulose</td>
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<tr>
<td>BOD</td>
<td>Biochemical oxygen demand</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
</tr>
<tr>
<td>DC</td>
<td>Direct current</td>
</tr>
<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
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<tr>
<td>RBC</td>
<td>Rotating biological contactor</td>
</tr>
<tr>
<td>RBF</td>
<td>Rotating biological fermentor</td>
</tr>
<tr>
<td>rpm</td>
<td>Round per minute</td>
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<td>SF</td>
<td>Static fermentation</td>
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