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

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

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Declaration of the candidate and supervisor

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Signature of the Supervisor:………………………. Date:……………………….

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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

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LIST OF ABBREVIATION

Abbreviation	Description
BC	Bacterial cellulose
BOD	Biochemical oxygen demand
CFU	Colony forming unit
COD	Chemical oxygen demand
DC	Direct current
DO	Dissolved oxygen
RBC	Rotating biological contactor
RBF	Rotating biological fermentor
rpm	Round per minute
SF	Static fermentation
UV	Ultra violet

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