MATHEMATICAL MODELING OF BACTERIAL CELLULOSE PRODUCTION BY *ACETOBACTER XYLINUM* USING ROTATING BIOLOGICAL FERMENTOR

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KEYWORD

Mathematical model, Rotating Biological Fermentor, Bacterial Cellulose

ABSTRACT

Bacterial cellulose (BC) has a basic cellulose structure that gives high purity, high crystalline ability, high mechanical strength, and high water holding capacity. During the last few decades, BC has gained as an important biomaterial because of these unique physical and chemical characteristics. BC is synthesized by *Acetobacter xylinum* extracellularly in a suitable substrate media. Researchers have produced BC using a synthetic media or using coconut water and inoculating with *Acetobacter xylinum* in a static fermentation system. In order to eliminate shortcomings in static fermentation and to achieve increased cellulose production, agitated and aerated fermentation systems were experimented. Rotating Biological Fermentor (RBF) is an is aerated and agitated system, which gives continuous oxygen flux to the fermentation medium thereby increasing the yield of biomass and cellulose synthesized.

In this study, a mathematical model for the synthesis of BC in a RBF system was developed. The growth of cellulose is considered as a biofilm 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.

INTRODUCTION

Cellulose is one of the most abundant polymers in nature. It is found as a structural component in the cell wall of plants. In addition, a few bacterial species, taxonomically related to the genus *Acetobacter xylinum* (acetic acid bacteria), extracellularly secrete bacterial cellulose (BC) as fibres. BC produced by *Acetobacter* species, displays unique properties, including high mechanical strength, high water absorption capacity, high crystallinity, and an ultra-fine and highly pure fibre network structure. It is expected to be a new commodity with diverse applications, if its mass production process could be improved. Presently BC is used as a fibre reinforcement material, as a wound dressing material, as a dietary fibre and as diaphragm of speakers (Lina et al. 2005 and Yoshinaga et al. 1997). Many researchers have produced BC using a synthetic media while most Asian researchers have produced BC using coconut water inoculated with *Acetobacter xylinum*. Coconut water can be recognized as a waste material in desiccated coconut industry with high BOD and COD values (Jayakody et al. 2011). Considering the level of natural sugar content in coconut water, it can be directly used to produce BC as a culture medium (Gamage 2012).

Due to the numerous importance of BC, researchers have focused on producing BC using different technologies (Bungay and Serafica 1999; Verschuren et al. 2000). Currently the technique of producing commercial scale BC has been limited to static fermentation system. However, research is in progress to use aerated and agitated continuous systems to produce BC in large scale (Yang et al. 1997 and Cheng et al. 2009). Rotating Biological Fermenter (RBF) that could be considered as an agitated and aerated fermentation system is one of the innovative techniques developed to produce BC (Patel et al. 2008). An RBF unit consists of a set of discs connected to a shaft and immersed in a container having a suitable substrate media. A similar study carried out by Dissanayake and Ismail (2013) showed that BC production in the RBF unit using coconut water as the substrate medium produced a maximum cellulose yield when the discs were rotating at 30 rpm. In addition, this study showed that the biomass was entrapped in the secreted BC matrix that was attached to the rotating discs. Cell growth and product formation are significantly influenced by mass –transport phenomena of nutrients and oxygen to the interface.

BC attached to a rotating disc can be considered as a biofilm. According to Wilderer and Characklis (1989), biofilm is a layer of prokaryotic and eukaryotic cells anchored to a substratum surface and embedded in an organic matrix of biological origin. One dimensional

Proceedings 27th European Conference on Modelling and Simulation ©ECMS Webjørn Rekdalsbakken, Robin T. Bye, Houxiang Zhang (Editors)
ISBN: 978-0-9564944-6-7 / ISBN: 978-0-9564944-7-4 (CD)

mixed culture biofilm model for flow of water was investigated by Lee and Park (2007). A similar experiment was conducted by Warner et al (2006) and different models were introduced to the analysis of biofilm growth. Erkmen and Albane, (2002) experimented the citric acid production using *Aspergillus Niger* and a mathematical model was developed to simulate the microbial growth and metabolic byproduct formation in an accurate manner. However prediction of growth of microbial culture for some bioprocess systems could be a complex task as it can be hindered by factors such as endogenous respiration, accumulation of toxic byproduct in the medium, decrease of oxygen penetration and spore formation in the medium (Novick 1955; Kenneth 2008).

The objective of this study is to develop a mathematical model which can describe the RBF system. For the convenience of developing the mathematical models and calculations, RBF unit was considered as a batch reactor and the cellulose growth was considered as a biofilm. In order to simplify the complex system, few assumptions were made. Later model was simulated using AQUASIM numerical software (Reichert P. 1998) and simulated results were compared with the experimented results.

ASSUMPTION

1. Bio film substratum is inert
2. Bacteria consume only dissolve oxygen as the source of oxygen
3. For a given microorganism, metabolic reaction rate is depends on one single rate limiting substrate.
4. Even though liquid is prominent in the system, solid biofilm is the main focus on modeling
5. System is a completely mixed system with batch type reactor
6. Density of the system is assumed to be constant.
7. Cellulose growth and microbial reaction decrease when turbulence flow occurs.

MODEL DISCRIPTION

RBF unit that is considered as a batch reactor, consist of coconut water inoculated with *Acetobacter xylinum* and operated for 8 days continuously.

Table 1: Model variables

Symbol	Notation	Values/ Units
$\mathbf{mass_{in}}$	Mass inflow	g day^{-1}
$\mathbf{mass_{out}}$	Mass outflow	g day^{-1}
$\mathbf{Biomass_{gene}}$	Growth of microorganism	g COD l^{-1} day^{-1}
$\mathbf{Biomass_{deca}}$	Decay of microorganism	g COD l^{-1} day^{-1}
$\mathbf{D_{decay}}$	Decaying rate	g COD l^{-1} day^{-1}
$\mathbf{m_G}$	Glucose weight	g
$\mathbf{m_X}$	Weight of microorganism	g
$\mathbf{m_C}$	Cellulose weight	g
$\mathbf{C_G}$	Concentration of Glucose	g l^{-1}
$\mathbf{C_{Gini}}$	Initial glucose concentration	32.567 g l^{-1} Jayakody et al. 2011
$\mathbf{C_X}$	Micro organism concentration	g COD l^{-1}
$\mathbf{C_{Xin}}$	Initial microorganism concentration	1500 g COD l^{-1} Grady et al. 1972
$\mathbf{C_C}$	Concentration of cellulose	g l^{-1}
$\mathbf{K_G}$	First-order rate	0.336 day^{-1} Alpkvist et al. 2006
$\mathbf{K_S}$	Concentration giving one half of the maximum rate	20 g l^{-1}
$\mathbf{K_D}$	Decay rate constant	0.1 day^{-1} Alpkvist et al. 2006
$\mathbf{K_{DO}}$	Flux of dissolve oxygen	rpm^{-1}
$\mathbf{K_{DO,\omega}}$	Rate of dissolve oxygen due to disc rotation	rpm^{-1}
$\mathbf{K_{DO,static}}$	Dissolve oxygen in very low rpm	rpm^{-1}
$\mathbf{K_{BF}}$	Proportional constant	$mm^4 l\, g^{-2}$
$\mathbf{\mu_{max,C}}$	Maximum specific cellulose growth rate	0.475 day^{-1}
$\mathbf{\mu_{max,X}}$	Maximum specific growth of microorganism	4.707 day^{-1} Alpkvist et al. 2006
$\mathbf{\omega}$	Rotational speed	rpm
$\mathbf{BF_L}$	Thickness of biofilm	mm
$\mathbf{BF_{L,\omega}}$	Thickness of biofilm with agitation	mm rpm^{-1}
$\mathbf{BF_A}$	Biofilm attachment	g l^{-1} day^{-1}
$\mathbf{BF_D}$	Biofilm detachment	g l^{-1} day^{-1}
$\mathbf{R}$	Rates	
$\mathbf{R_G}$	Rate of glucose depletion	g l^{-1} day^{-1}
$\mathbf{R_X}$	Rate of micro organism generation	g COD l^{-1} day^{-1}
$\mathbf{R_{X,\omega}}$	Rate of micro organism generation with agitation	g COD l^{-1} day^{-1} rpm^{-1}
$\mathbf{R_C}$	Rate of cellulose production	g l^{-1} day^{-1}
$\mathbf{R_{C,\omega}}$	Rate of cellulose production with agitation	g l^{-1} day^{-1} rpm^{-1}
$\mathbf{\rho_C}$	Density of cellulose	g l^{-1}
$\mathbf{a}$	Constant specific for RBF unit	0.009 rpm^{-3}
$\mathbf{b}$	Constant specific for RBF unit	1 rpm^{-2}
$\mathbf{c}$	Constant specific for RBF unit	0.36 rpm^{-1}

For this batch reactor, a general mass balance can be given as;

$$\frac{dm}{dt} = mass_{in} - mass_{out} + R \quad (1)$$

If the system density is assumed to be constant, then;

$$\frac{dm_G}{dt} = \frac{dC_G}{dt} = R_G \quad (2)$$

For a batch reactor, $mass_{in} - mass_{out}$ is equal to zero. In other words net flux is zero.
Similarly, the microbial growth in the system can be given as;

$$\frac{dm_X}{dt} = \frac{dC_X}{dt} = R_X \quad (3)$$

Therefore rate of biomass generation can be shown as

$$\frac{dC_X}{dt} = Biomass_{generation} - Biomass_{decay} \quad (4)$$

$Biomass_{decay}$ can be seen in microbial culture as due to endogenous respiration.

Cellulose growth in the system can also be represented by using a similar kind of equation

$$\frac{dm_C}{dt} = \frac{dC_C}{dt} = R_C \quad (5)$$

Rates of the three different reactions can be modeled for the system as given below.

BC production is affected by DO in the fermentation medium. Therefore, a new parameter K_{DO} that considers the variation in DO in the system due to the rotational speed of discs ω was defined.

Since, K_{DO} depends on ω ;

$$K_{DO} \propto \omega \quad (6)$$

At low rotational speeds, the flow streams in the substrate medium would behave in the laminar region. However, increase in rotational speeds will create turbulence that would disturb cellulose attachment to the discs while supplying excessive DO to the system. Therefore this explains that the rotational speed that changes the flow regimes would give the maximum cellulose production.
Then mathematically, function of K_{DO} for culture medium can be written as a quadratic equation which describes the influence of DO.

$$K_{DO} = -a\omega^2 + b\omega + C \quad (7)$$

$$K_{DO} = K_{DO,\omega} + K_{DO,static} \quad (8)$$

At very low rpm it can be assumed,

$$K_{DO,\omega} = 0 \quad (9)$$

Then,

$$K_{DO} = K_{DO,static} = C \quad (10)$$

Value of C can be experimentally obtained. Values of 'a' and 'b' were coefficients which describe the contribution from other factors such as disc and container characteristics in the system are given in Table 1.

Rate of glucose depletion in the system is considered as behaving as first order kinetics.

$$R_G = -K_G \times C_G \quad (11)$$

Negative sign is due to the depletion of glucose with time.

Increase in biomass and formation of cellulose depends largely on glucose and DO level. In addition to the major dependent factors pH, concentration of growth inhibitors and concentration of byproducts can have an effect on cellulose growth. However for the modeling purposes only major components were taken into account. Then as the initial step in the derivation, the rates of biomass and cellulose could be taken as proportional to glucose substrate utilization so that;

$$R_X \propto R_G \text{ and } R_C \propto R_G \quad (12)$$

Further, rate of biomass generation can be expressed using Monod equation (1949). However, Monod equation was modified to express heterotrophic micro organism growth with heterotrophic decay and the dependency of DO by including the term K_{DO}, as defined previously. Then, rate of biomass generation in dynamic system could be expressed as,

$$R_X = \left\{\left[\mu_{max,X}\left(\frac{C_G}{K_S + C_G}\right)C_X\right] - D_{decay}\right\} \quad (13)$$

$$R_{X,\omega} = \left\{\left[\mu_{max,X}\left(\frac{C_G}{K_S + C_G}\right)C_X\right] - D_{decay}\right\}K_{DO} \quad (14)$$

D_{decay} kinetics could be given by

$$D_{decay} = K_D \times C_X \quad (15)$$

An expression for the cellulose production can be derived similar to Eq. 12 -14. Development of model for cellulose production was taken as an ordinary concentration of culture which contains mix of both living and dead cells where heterotrophic decaying has been eliminated. Moreover, Monod equation can be used to describe a microbial system with byproduct formation. However rate of maximum production of

byproduct is obviously a lower value than the rate of maximum heterotrophic microorganism growth. Rate of maximum production of byproduct; cellulose, is represented by $\mu_{max,C}$. Then $\mu_{max,X}$ in general Monod equation (1949) could be replaced with $\mu_{max,C}$. Further, BC production too is affected by DO and glucose concentration in the fermentation medium. Then the growth of bacterial cellulose in the dynamic system could be given as;

$$R_C = \left[\mu_{max,C} \left(\frac{C_G}{K_S + C_G}\right) C_C\right] \quad (16)$$

$$R_{C,\omega} = \left[\mu_{max,C} \left(\frac{C_G}{K_S + C_G}\right) C_C\right] K_{DO} \quad (17)$$

In the RBF system, generated cellulose continuously attach to the discs. Attached cellulose can be considered as a biofilm. According to Wanner et al (2004) growth of biofilm is proportional to the biofilm attachment rate and detachment rate. Then;

$$R_{C,\omega} \propto BF_A - BF_D \quad (18)$$

Then;

$$\frac{dBF_L}{dt} = \rho_C \times K_{BF}(-BF_D + BF_A) \quad (19)$$

For a batch reactor, inflow and out flow net fluxes are equal to zero. Then BF_D can be neglected as produced cellulose that is retained on dices or sedimented inside the reactor liquid volume. Then;

$$BF_A = R_{C,\omega} \quad (20)$$

Then biofilm conversion can be written as;

$$\frac{dBF_{L,\omega}}{dt} = \rho_C \times K_{BF} \times R_{C,\omega} \quad (21)$$

The physical meaning of K_{BF} is mm of biofilm growth per unit concentration per unit density.

RESULTS AND DISCUSSION

Mathematical model that described the BC synthesis in a RBF system was solved to obtain solutions for glucose depletion, micro organism growth and for cellulose production. Simulated numerical solution that was obtained by solving equation 11, which describes glucose depletion in the system, is as shown in Figure 1. This result was compared with the experimented results of Yang et al (1997). According to Yang et al (1997), depletion of glucose starts after 10-15 hours of inoculation and then it begins to decrease drastically. At the end of the 3rd day of inoculation, glucose concentration becomes closer to a zero level. Similarly, simulated results were derived with an initial glucose concentration (C_{Gini}) of 32 g l^{-1} which is the case in coconut water. Then it shows the continuous depletion of glucose in the media until the 14th day where it becomes zero. The two conditions have given similar trends with a shift in time scale. This could be due to the difference in the systems, culture volume and the specific microbial culture strain used.

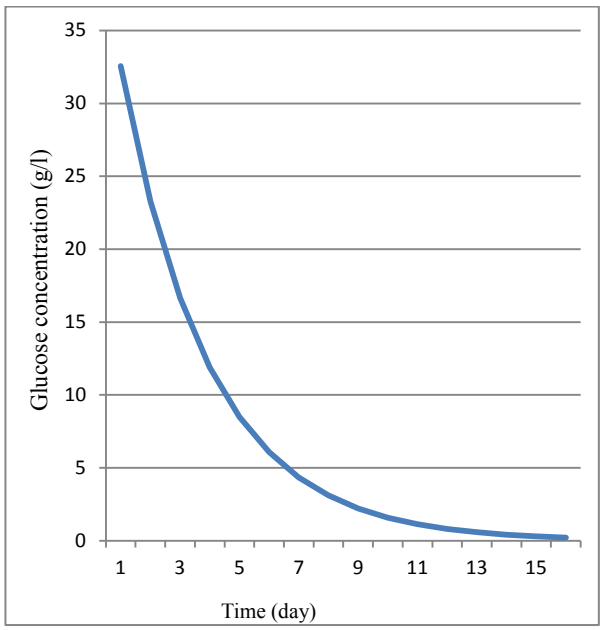

Figure 1: Simulated results of depletion of Glucose in the RBF unit

Figure 2 shows the simulated microbial growth with time which was obtained by solving equation 13. It shows an initial lag phase and an exponential growth phase which closely resembles Monod kinetics. A similar bacterial growth pattern was observed in the modeled results of Erkmen and Albane (2002). Moreover, Hammes et al (2007) investigated a monoculture micro organism and used a biofilm model to simulate the experimented results successfully. Even though growth rates and other parameters vary in different experiments (Erkmen and Albane. 2002; Hammes et al. 2006), a generalized growth curve was derived in this study and made it specific for the bioprocess under consideration. Further, Yang et al (1997) and Verschuren et al (2000) experimentally proved that microbial growth can be increased by increasing the dissolved oxygen in the media through aeration and agitation. In this study, this effect was proved through mathematical modeling of the microbial growth at different rotational speeds of the RBF system.

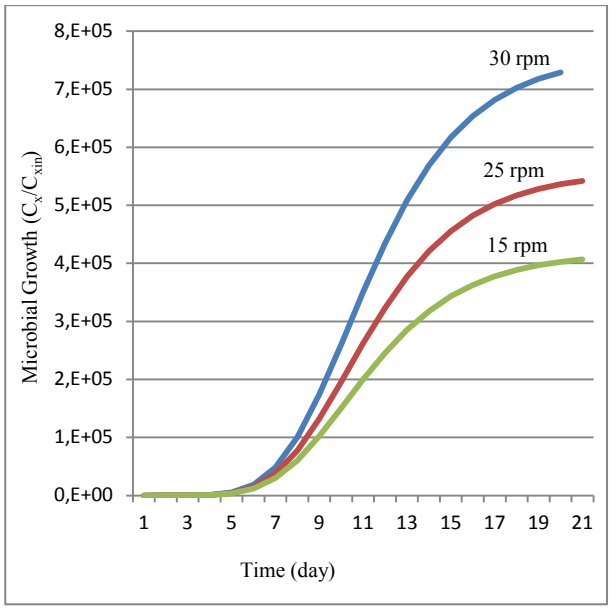

Figure 2: Microbial growth in RBF system for 30, 25, 15 rpm

Further, it is important to understand the yield of cellulose when the RBF system is operated at different rotational speeds. The simulated results obtained by solving equation 15 which gives the yield of cellulose when the rotational speeds are 30, 25, and 15 rpm for a batch volume of 2.75 liters are given in Figure 3. Initial cellulose concentration was considered as very small at the beginning of the experiments. Hornung et al (2009) did a similar study for static fermentation where they derived a model and verified with experimental results. They too showed that the yield of cellulose increase with time and reaches a maximum.

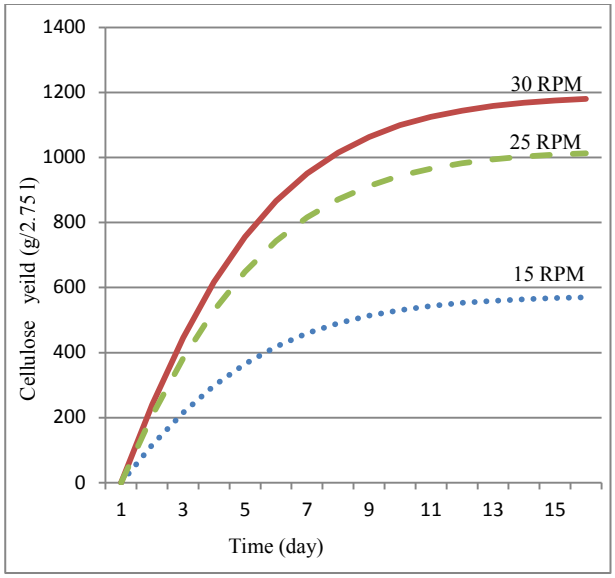

Figure 3: Simulated results of cellulose growth at three different rotational speeds in the RBF system

Moreover Figure 3 shows that the yield of cellulose could be increased when the rotational speed is increased. In a previous study, Dissanayake and Ismail (2013) experimented the yield of cellulose in a RBF system at the three different rotational speeds that are under consideration. These experimented results are in close agreement with simulated results at the 8th day of fermentation as shown in Table 2. Many researchers attempted to increase the cellulose production by increasing the oxygen content in the medium by aeration and agitation (Tantratian et al. 2005, Verschuren et al. 2000, Kauda et al. 1997). This is because the growth of *Acetobacter,* a typical aerobic BC producer, is dependent on oxygen and supply of oxygen is directly associated with BC productivity in an aerated and agitated culture system.

Therefore, the simulated and experimentally validated results support this argument. However, there is a significant percentage error when the rotational speed was 15 rpm. According to Dissanayake and Ismail (2013), at low rotational speeds, the experimental system behaves similar to a static medium. Hence it could be that the developed mathematical model does not take into account the limitations in diffusion of oxygen and glucose at low rpm values to the disc surface where the growth of cellulose occurs. This could also be the reason, for simulated results to show a maximum yield of cellulose on 10th – 11th day, although the experimental maximum and simulated values that are compared after 8 days are coherent.

Table 2: A comparison of simulated results of yield cellulose with experimented results of Dissanayake and Ismail (2013) at 8 days

rpm	Experimented results (g)	Simulated results (g)	Difference	Percentage error %
30	1098.25	1065	33.25	3.02
25	892	915.1	-23.1	(-2.58)
15	721	516.7	204.3	(-28.33)

Further, the derived model was tested to obtain the yield of cellulose at five different rotational speeds as given in Figure 4. Accordingly it shows that there is an optimum rotational speed 55- 65 rpm that would give a maximum yield of cellulose. Tantratian et al (2005) who studied the effect of dissolve oxygen on cellulose production by *Acetobacter Sp.* in an agitated system has also experienced similar results. They have obtained a maximum cellulose yield at an optimum rotational speed of 100 rpm and further increase in rotational speed have resulted in low yields of cellulose. According to Tantratian et al (2005), the excess oxygen that dissolves in the medium increases the accumulation of gluconic acid and adversely affects the cellulose production. On the other hand, too low an amount of dissolved oxygen content in the medium could not provide enough oxygen for the culture to grow and that caused the reduction of cellulose production. Further, Kauda et al (1997) mention that microbial cells could

oxidize due to excessive aeration. Thus these explanations are clearly in line with the simulated results.

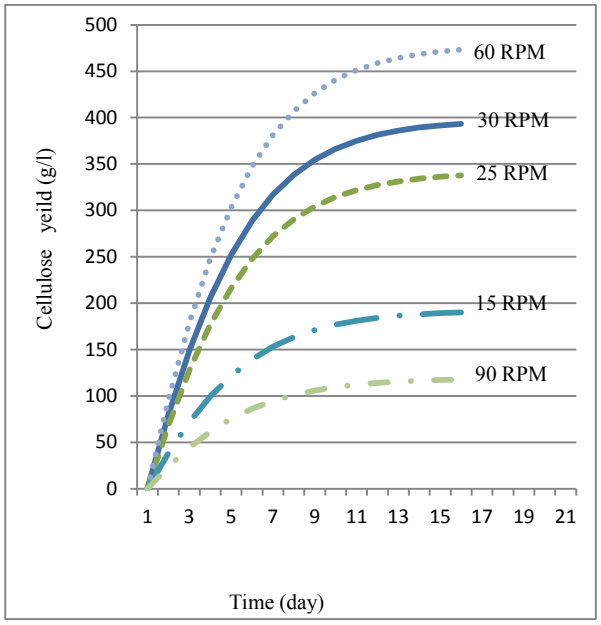

Figure 4: Simulated results of yield of cellulose at different rotational speeds

CONCLUSION

The mathematically derived model to explain the synthesis of BC in a RBF system is closely related to the experimented values. Developed models and constant could be used to describe depletion of glucose, AX growth and cellulose production in a coconut water based culture medium. This also means that the assumptions that were made in deriving the model are within acceptable limits. Model on the other hand could be used to predict the yield of cellulose, at any given rotational speed. However, there were limitations in using the model when the RBF unit closely resembles a static fermentation system.

RECOMMENDATION

Further analysis should be done in order to describe the BC synthesis in static fermentation.

AKNOWLEDGEMENT

I wish to convey my special thanks to the M.Sc. funds which was given by NOMA, Norway and for the collaboration of Telemark University College, Norway.

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