

## Kinetics observations of bacterial cellulose thickness formation using image processing approach during the fermentation process

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### Abstract

Bacterial cellulose is an exopolysaccharide that has a higher level of purity compared to cellulose from plants. Bacterial Cellulose (BC) is widely used for various uses so that it requires certain initial conditions, one of which is thickness. During the fermentation process, cellulose will be secreted into the medium to form BC sheets and visually visible over the time period. The aim of this research was to study the relationship between variables that influence during the fermentation and to fit the kinetic model of the BC thickness formation using image processing approach during the fermentation process. A USB camera was placed in front of the fermenter to capture the formation of BC thickness. Python programming language was used to process the image and calculated the thickness of the BC sheet from the beginning to the end of the fermentation process. Several supporting parameters were observed by placing the turbidity, pH, and medium temperature sensors. Observations were made in real time with a range of data collection every 15 mins during fermentation. The highest correlation value was obtained from the relationship between time and thickness. The fermentation process is divided into 2 clusters, a change in cluster occurs at the 61st hour. The model that describes the relationship between time and thickness was the Gompertz model.

## 1. Introduction

Bacterial Cellulose (BC) is an exopolysaccharide that has a higher level of purity compared to plant cellulose, produced by several types of microorganisms (Chawla *et al.*, 2009). It is a biopolymer with many purposes of uses. It has a better material property, including high purity, high degree of porosity, relatively high permeability to liquid and gases, high water-uptake capacity, tensile strength, and ultrafine network (Ullah *et al.*, 2016). BC is widely used for purposes in various fields. In the food sector, it is widely used as a dessert drink (Iguchi *et al.*, 2000), a low cholesterol diet (Chau *et al.*, 2008), a meat substitute for vegetarians with the addition of *Monascus purpureus* (Purwadaria *et al.*, 2010), as well as a food additive as an ingredient for enzyme immobilization (Wu and Lia, 2008).

The use of BC material for various applications requires different initial conditions for BC material, such

as shape, size, and thickness. BC membranes of various thicknesses ranging from 5 mm, 7 mm and 9 mm were used as guided bone regeneration (Lee *et al.*, 2017). In vitro drug release rate was dependent on the BC film thickness (Maver *et al.*, 2015). Modification of the fermentation process was carried out to obtain BC with a thickness of up to 30 mm compared to conventional methods (Hsieh *et al.*, 2016). For this reason, this study aims to monitor the rate of BC thickness formation using an image processing approach during the fermentation process.

The high yield cellulose production capacity is the most crucial parameter in BC production during the fermentation process (Bilgi *et al.*, 2016). The increase of growth time will increase the formation of BC along with hydrogen and C-H bonding (Sheykhnazari *et al.*, 2011). The growth of BC formation can be seen by the thickness over the time of fermentation passes (Nugroho *et al.*, 2020). Several conditions that influenced the

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formation of BC were fermentation time (Iguchi *et al.*, 2000), temperature (Mohammad and Rahman, 2014), turbidity, pH, and thickness of BC (Hsieh *et al.*, 2016).

The method of observing the changes in BC thickness in real time using image processing has been reported (Nugroho *et al.*, 2020). The thickness measurement algorithm has succeeded in separating BC sheet objects from other objects such as medium, non-sheet BC and air space between the medium and the fermenter cover (Figure 1). This research is aimed to study the relationship between variables that influence during the fermentation and to fit a kinetic model of the BC thickness formation using image processing approach during the fermentation process.

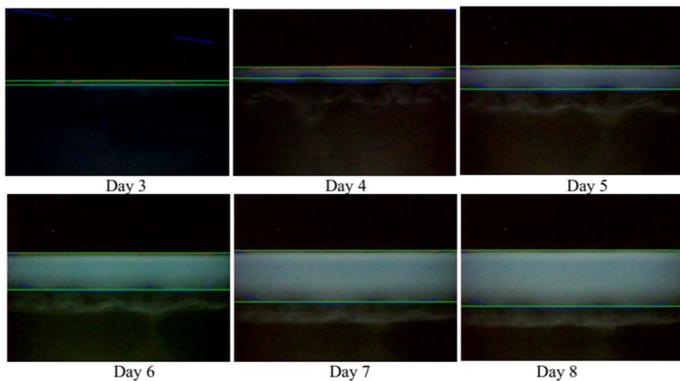


Figure 1. BC sheet thickness measured using image processing during fermentation at room temperature without agitating and stirring.

## 2. Materials and methods

### 2.1 Materials

The composition medium for this fermentation were consisted of 1.5 L coconut water, 5% sucrose, 1.5% ammonium sulphate, and acetic acid for adjusting pH to 4.0 (Nugroho *et al.*, 2020). The medium was sterilized for 15 mins and then allowed to cool. The fermentation process began by pouring the medium into the fermenter followed by inoculating 5% of the stock culture of *Gluconacetobacter xylinus* into the fermenter.

### 2.2 Fermenter

The fermenter was built using acrylic material that is given a non-reflective type of black paint on the outer surface with a length of 35 cm, a width of 25 cm and a height of 6 cm, and provides a transparent field facing the USB camera of 6 cm width and 4 cm height. A 145 lux LED lamp (measured with luxmeter LX-101) was positioned in front of the fermenter to provide lighting assistance and increase object contrast. An HD type USB camera used with the brand name Alloet (maximum resolution of 1280 × 960 pixels, CMOS sensor type, and autofocus) is placed right in front of the transparent field (Figure 2). The fermenter was operated without stirring

and agitating, at room temperature, in aerobic condition. The dimension of the fermenter gives a wide surface area for contact with the oxygen and the fermenter lid was also made from paper which oxygen could pass through.

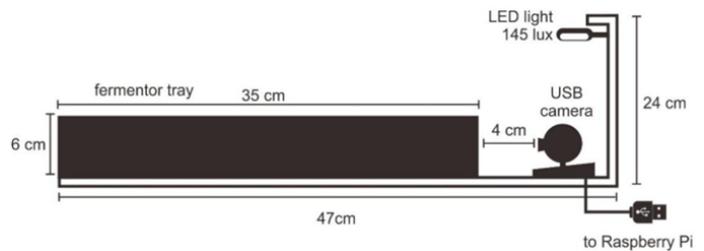


Figure 2. Arrangement of fermenter for image processing purpose (Nugroho *et al.*, 2020).

### 2.3 Equipment used

The sensor equipment was placed through the fermenter cover, which consists of DS18B20 Temperature Sensor Module Kit Waterproof with Digital Sensor Cable and Stainless-Steel Probe Terminal Adapter for Arduino, Keystudio Turbidity Sensor V1.0 Compatible with Arduino and PH4502C pH Meter Value Detector Module Detection with Module Monitoring Controller and BNC Block Electrode Probe. All the sensors were placed submerged in the fermentation medium 1 cm from the bottom of the fermenter. Those sensors were connected to the Arduino Uno. The Arduino Uno is a microcontroller board based on the ATmega328. It has six analog inputs, a 16-MHz crystal oscillator, a USB connection, a power jack, and an In-Circuit Serial Programming header (D'Ausilio, 2012). The sensors connected to Arduino Uno will provide current reading information, which will be converted into digital data that can be read by the Raspberry Pi 3. In contrast, the USB Camera is directly connected to the Raspberry Pi.

Raspberry Pi 3 was an economical, palm-sized computer. This board had 4x USB ports onboard, an Ethernet port, Wi-Fi capability, HDMI port, SD card slot, and 40 pins. It was installed with Raspbian OS, a free and open-source software. The OS was based on the Linux kernel. The BC thickness calculation algorithm and command insertion method into the MySQL database (Nugroho *et al.*, 2020) was developed in python language programming.

### 2.4 Data collection

Data collection of reading sensor values was done by sending the values from Arduino to Raspberry Pi 3, while data from the USB camera was directly sent to Raspberry Pi 3 through the BC thickness calculation method first (Figure 3). All the data were inputted into MySQL database that is also already installed on Raspberry Pi 3. Data was taken every 15 mins from the

beginning of fermentation until 8 days.

The reading data is stored in the MySQL database every 15 mins, and at the same time, the data was sent to the user's email using the http server, which was also installed on the Raspberry Pi 3 via internet access.

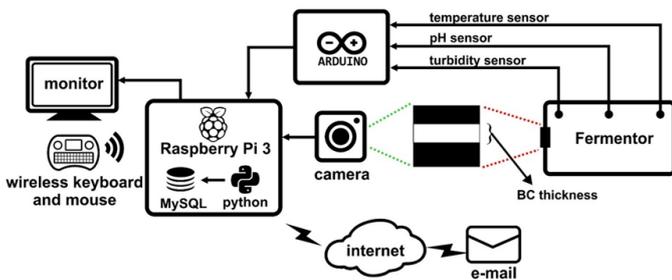


Figure 3. Dataflow process of BC fermentation monitoring

Data obtained during fermentation was then analyzed using the R programming language for time-period data reading, PCA analysis, and kinetic analysis. The R version is 3.6.3, downloaded from <https://www.r-project.org>, while the GUI application uses RStudio Desktop version 1.3.1093, downloaded from <https://download1.rstudio.org/desktop/windows/RStudio-1.3.1093.exe>.

### 3. Results and discussion

During the fermentation process, the four sensors installed on the fermenter, namely the temperature sensor, pH sensor, turbidity sensor and camera, provide data readings which were plotted in a graph as shown in Figure 4. During the fermentation process, the newly inoculated microorganisms need the amount of time for adaptation, increase the amount of biomass, and produce metabolites. However, because the amount of biomass is still small, the metabolite results in the form of BC sheets are not visually visible nor thickness figures can be read. The rate of increase in thickness of the BC sheet begins after 61 hours and continues to increase significantly until the end of the fermentation process.

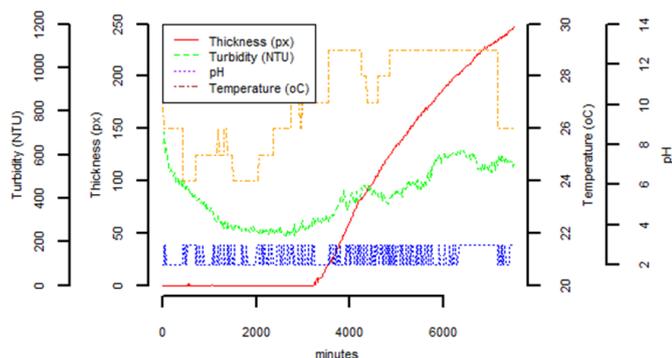


Figure 4. Thickness, turbidity, pH, and temperature during BC fermentation.

Turbidity data in Figure 4 shows a different pattern. At the time of the initial inoculation, it shows a high

number of turbidities, because, at the time of inoculation, the biomass that initially settles is shaken out before being poured. This shaking makes biomass is evenly distributed in the inoculum and when it is poured it will give a turbidity number to the medium in the fermenter. As shown in Figure 4, the biomass will begin to settle, which is indicated by a decrease in the turbidity number from 1000 to 3000 mins. However, along with the increase in thickness of the BC sheet, the turbidity rate increases, because the BC fibre produced by the inoculum begins to be secreted a lot into the medium before forming the BC sheet and influences increasing turbidity number.

Measurement of temperature in the medium shows a similar pattern with turbidity (Figure 4). At the beginning of fermentation, the temperature in the fermenter ranges from 24-26°C. While BC began to visually form, there are an increase in temperature ranging from 26-29°C. This is due to an increase in the metabolic rate of the inoculum when BC formed is accompanied by heat release which affects the temperature in the medium. Meanwhile, the pH reading data did not show any significant difference during the fermentation process.

During the fermentation process, the four sensors provide reading data and then analyse the relationship between time, thickness, turbidity, pH, and temperature variables. The relationship between each variable is shown in Figure 5. The relationship with the highest percentage is shown between time and thickness of 94%, followed by the relationship between thickness and turbidity by 80%. Figure 5 shows that the fermentation time is strongly related to the thickness of the BC sheet formed. This can be explained that the longer the fermentation time will provide an opportunity for *G. xylinum* to secrete more BC fibre so that the BC sheet is formed. The more BC fibre formed, the more BC sheet thickness is added as shown in relation value of 80% between thickness and turbidity.

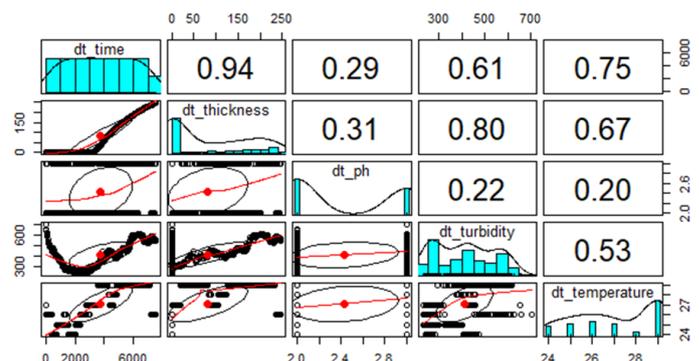


Figure 5. Variable relations of time, thickness, pH, turbidity, temperature during BC fermentation

PCA Analysis is used to reduce the dimensions of the variables used in this study. The PCA Analysis calculation uses the "prcomp" command by standardizing the data for each variable by entering the TRUE argument in the scale parameter. The results obtained 4 Principal Components in the form of PC1, PC2, PC3 and PC4 (Figure 6). From the figure, PC1 and PC2 occupy a cumulative proportion of 91.92%, which means that PC1 and PC2 can capture 91.92% of the diversity of data.

```
> summary(dataplot.pca)
Importance of components:
      PC1      PC2      PC3      PC4
Standard deviation  1.7787 0.7123 0.55984 0.12458
Proportion of Variance 0.7909 0.1268 0.07836 0.00388
Cumulative Proportion 0.7909 0.9178 0.99612 1.00000
> dataplot.pca
Standard deviations (1, ..., p=4):
[1] 1.7786645 0.7123289 0.5598402 0.1245760

Rotation (n x k) = (4 x 4):
      PC1      PC2      PC3      PC4
dt_time  0.5261107 -0.2228180  0.5456057 -0.6130857
dt_thickness 0.5427452  0.1799874  0.3718613  0.7312669
dt_turbidity 0.4646010  0.6958980 -0.4732981 -0.2754283
dt_temperature 0.4612819 -0.6585458 -0.5831145  0.1162491
```

Figure 6. The proportion of each principal components

Based on the relationship between time and thickness of BC in Figure 5, the data group was analysed during the fermentation process. Cluster analysis is used to estimate the number of data groupings based on the relationship between time and thickness of BC data. The step taken is to find the optimum cluster on the existing data relationship, using the "wss" and "silhouette" methods. The graph plot of the two methods is shown in Figure 7. The optimum number of clusters obtained between those two methods is 2. This means that during the fermentation process there are 2 data groupings based on time and thickness data.

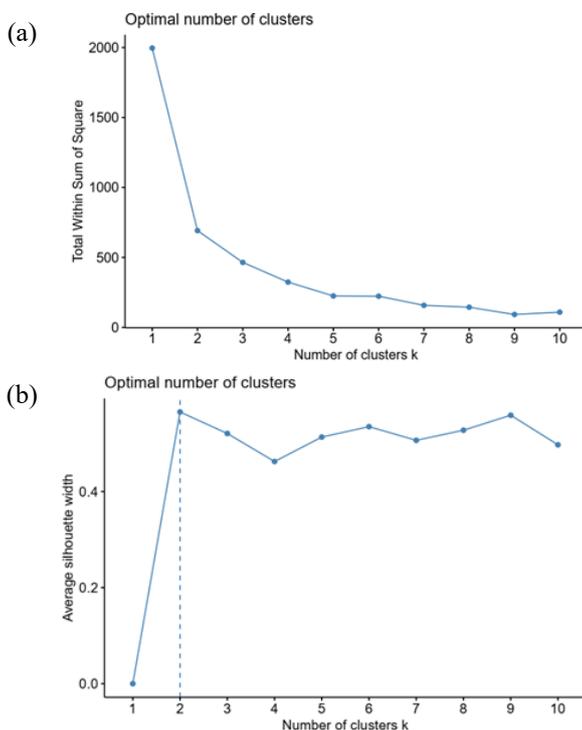


Figure 7. Optimum number of clusters obtained by (a) wss method and (b) silhouette method.

The results of the cluster analysis are used to identify the different phases in the BC sheet formation process. Each cluster is given a different colour attribute based on the cluster on the BC sheet thickness measurement graph as shown in Figure 8.

From Figure 8, during the fermentation process, there are two groups of process stages. The first cluster occurs from the stage from fermentation to the initial reading of the BC thickness data. This stage ends at the 61st hour and continues to the second cluster, namely the stage of the BC thickness formation process. This shows that up to the 61st hour the fermenter condition is still in the preparation phase leading to the formation of BC thickness, then after 61 hours, there is an increase in the formation rate of BC which is very different from the previous group. As shown in Figure 8, there is a sharp increase in thickness.

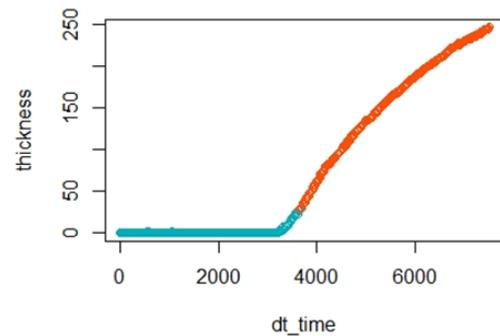


Figure 8. Graph of BC sheet thickness measurement based on clustering data during fermentation.

After obtaining a graph of the increase in thickness of BC sheet per unit time, an equation model that describes the speed of formation of BC sheet is determined. The choice of equation model is based on the largest  $R^2$  value. The Gompertz curve model using start iterations values of  $a = 100$ ,  $b = 2$  and  $c = 0.05$  gives  $R^2$  values 0.9972 is shown in Figure 9.

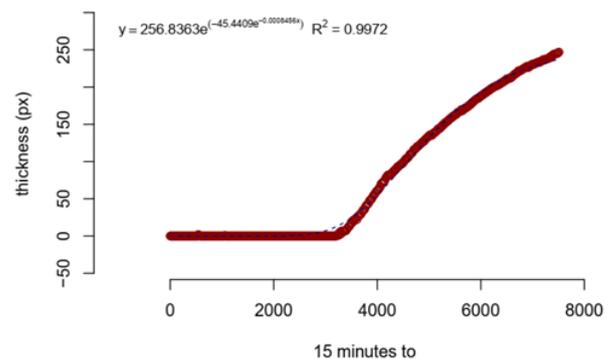


Figure 9. The plot of Gompertz curve model for BC thickness measurement during fermentation

The accuracy of the model is determined by the  $R^2$  value on the relationship between thickness and prediction of thickness based on the Gompertz model. These results are shown in Figure 10. Figure 10 shows that thickness data and thickness prediction data has a

value of  $R^2 = 0.9973$ . From this  $R^2$  showed, conclude that the Gompertz curve model is right to describe the rate of formation of BC sheet thickness during the fermentation process.

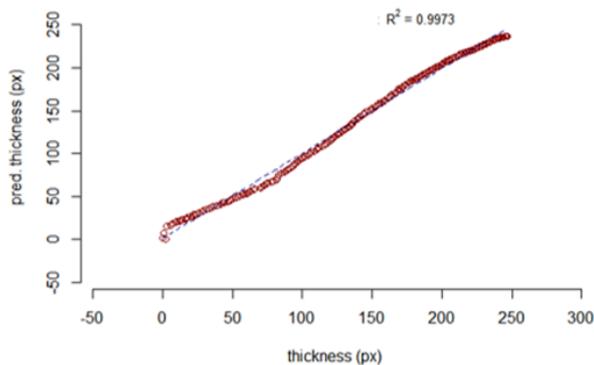


Figure 10. Relation between actual thickness and prediction of thickness during BC fermentation based on Gompertz model.

#### 4. Conclusion

The highest correlation value was obtained from the relationship between time and thickness. The rate of BC formation during the fermentation process best follows the Gompertz model, with an increase in the rate occurring after the 61st hour.

#### Conflict of interest

In this research and publication, there is no conflict of interest from all parties.

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