New Approach for Observation of Bacterial Cellulose Sheet Formation Method using Image Processing

D A Nugroho¹, L Sutiarso², E S Rahayu³, R E Masithoh²

¹ Department of Agricultural Industrial Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia
² Department of Agricultural Engineering and Biosystems, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia
³ Department of Food and Agricultural Products Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Yogyakarta 55281, Indonesia

*Email: ari.nugroho@gmail.com

Abstract. The thickness of the bacterial cellulose (BC) sheet is an important parameter that determines the end of the fermentation process. During the fermentation process, BC sheets produced will be visually visible. Commonly, the end of the fermentation process is determined using manual observation based on fermentation time and approximation of BC thickness which are subjective and susceptible to error especially for routine and large samples. To overcome those limitations, a new approach for accurate and real-time observation system to monitor the formation of BC thickness is developed in this research. The system can perform several tasks from image capturing and processing, image conversion to BC thickness, until data collection. The system is also able to send notification of fermentation conditions including BC thickness through the email system during the fermentation process regularly. The system consists of USB camera to capture image, the Python programming language to process image, and Raspberry Pi 3 installed with MySQL database to store the BC thickness data. Thickness calculation algorithm is compiled using python programming language and has succeeded in calculating various thickness of BC sheets during the fermentation process every 15 minutes for 8 days. The BC thickness data is automatically sent to the MySQL database and at the same time sent to user's email.

1. Introduction

Bacterial Cellulose (BC) is a product of the fermentation of the Acetobacter xylinum bacteria, in the form of a gram-negative rod which became known as Gluconacetobacter xylinum [1]. The formation of BC in static fermentation is determined by the presence of oxygen in the fermenter, while the amount of BC obtained is determined by the number of carbon sources used as the fermentation medium [2]. The fermentation process generally takes about 6-8 days, marked by the formation of a cellulose sheet on the surface of the fermentation medium with a thickness of 1-1.5 cm. In general, the fermentation process will be stopped after the BC sheet reaches a certain thickness.
BC is widely used in various fields such as cosmetics, drug delivery and food [3]. For this reason, a BC sheet with a certain thickness is required. Therefore, fast, and accurate observation of the thickness of the BC sheet formed during the fermentation process is very important to meet the requirement.

During the fermentation process, BC sheets produced will be visually visible as the fermentation time passes [4]. This visual appearance can be captured using a camera to produce an image that shows an increase in thickness over time [5]. For this reason, a series of digital image processing is required. Image processing or often called digital image processing, is a process of filtering the original image into another image according to our wishes [6]. The use of digital images has been widely used for sorting techniques for several horticultural products such as strawberries and tomatoes [7-9]. Data processing can be done by a computer, either in the form of a simple microcomputer (microcomputer-based computer) or large computer (mainframe computer), depending on the amount of data and the type of processing. The Raspberry Pi 3 was used for this study because of its ability to be similar to a desktop computer [10] with a small shape and size so that it is practical to carry and be placed in various conditions of limited space.

2. Material and Method

The fermenter is built using acrylic material painted with a non-reflective black color 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 area facing the USB camera [5]. A LED lamp 145 lux is positioned in front of the fermenter to provide lighting assistance and increase object contrast. The transparent part of the fermenter measuring 4 cm x 6 cm is placed right in front of the USB camera (Alloet, made in China, maximum resolution: 1280x960 pixels, sensor type CMOS, auto focus capability).

The growth medium consisted of 1.5L coconut water, 5% sucrose, 0.5% ammonium sulfate and acetic acid to adjust the pH condition of the fermentation medium to 4. The medium then boiled and allowed to cool. Inoculation has done by adding 5% of the culture stock to the fermentation medium and then pouring it into the fermenter. The fermenter is then covered with paper and fermented for 8 days.

Raspberry Pi 3 is installed with Raspbian OS and several programs needed for the research process such as python, OpenCV library, Apache web server, phpMyAdmin and MySQL database which was connected to a USB camera. A table is prepared in the database with columns in the form of date, time, fermentation code, and thickness. The date and time are automatically filled in according to the time and date of the Raspberry Pi 3, the fermentation code is initiated in the program file call command at the beginning of fermentation, and the thickness is the calculation result of the programming script.

The process of capturing images via a USB camera is carried out every 15 minutes during fermentation through a series of commands arranged in the Python programming language. Image processing stages are programmed also in python language in the same file by using the OpenCV library to convert image information into thickness information. The thickness reading results are then sent to the MySQL database which is already installed in the Raspberry Pi 3. The results of reading the data records are sent to the user's e-mail using the http server and php mailer function that have been installed on the Raspberry Pi 3 via the internet network every 15 minutes. The condition of the reading is monitored via a monitor screen which is also connected to the Raspberry Pi 3. All the process as seen on Figure 1.

3. Result and Discussion

The fermenter is operated without stirring and agitating. The dimension of fermenter gives a wide surface area for contact with the oxygen. The fermenter lid also made from paper which oxygen can still pass through. The decreasing activity occurs due to a decrease in the amount of the substrate content. During the fermentation process, microorganisms will slowly secrete BC fibers starting from the bottom where the biomass settles and slowly rises to the surface to form a solid white sheet on the surface of the fermentation medium. The BC formed during the fermentation process will provide a contrasting colour to the environment in the fermenter, because of the application of dark paint around the fermenter.
Changes in colour contrast will be more visible as the thickness of BC increases during the fermentation process. Python is programmed to recognize BC objects that are formed separate from the medium and environment. To calculate the thickness of BC, a workflow is arranged that describes the programming stages from the stages of image capture, image processing, calculation to obtain the thickness data results as shown in Figure 2.

**Figure 1.** The fermenter and detection equipment.

**Figure 2.** Workflow of image processing programming stages in BC fermentation.
The process stage begins with the initiation of the OpenCV library and statistics library, then continues with a database connection. The next stage is the initiation of the USB camera and the determination of several image parameters. The results of image capturing are shown in Figure 3.

In Figure 3, the painting around the fermenter wall in black colour provides a very good contrast so that the BC layer is clearly visible. However, the BC fibers that are formed also contribute to contrasting colour. The fibers can be clearly seen after changing the RGB mode to Grayscale which increases the threshold to get black and white images followed by edge detection step as shown in Figure 4.

![Figure 3. Image capturing results (a) air, (b) BC layer (c) fermentation medium (d) BC fibers.](image1)

![Figure 4. Edge detection of BC image.](image2)

Figure 4 showed the BC fibers formed at the bottom are detected as edges which are included in calculating the BC sheet caused by contrast of BC fibers. BC fibers on the top have relatively flat and stable edge due to direct contact with air. Based on the previously reported method [5] with improvement in the calculation stages, the first stage done is the determination of the detection points for each upper limit and lower limit. The detection points on the upper limit are coloured red, while the detection points on the lower limit are coloured blue. In this study, 100 detection points for each upper limit and lower limit are used. Calculation of the BC thickness starts from reading the top of the image until it touches the edge of the first contrast boundary, then continues reading until it finds the second edge of the contrast boundary. The flow of reading is as in Figure 5.
In Figure 5, it is assumed that the thickness of BC provides a flat contrast area at the upper limit and lower limit, although in fact, as in Figure 4, the BC sheet does not follow a horizontal pattern, especially for the lower limit readings. The BC fibers formed at the bottom will also be counted as detection points, so the calculation of the difference between the upper limit and lower limit results in an incorrect thickness value. The incorrect number gives the thickness calculation result that is bigger than it should be. For this reason, it is necessary to make calculation adjustments that can eliminate the BC fiber object formed at the bottom from being included in the calculation as shown in Figure 6.

![Figure 5. The direction of the reading process for thickness calculation](image)

![Figure 6. Illustration calculation steps to eliminate non-BC sheet objects.](image)

The adjustment begins by calculating the modus number of the detection point at the upper limit by calling the statistic library in the python script. This modus number shows an outline of the tendency of the object's contrast value to the upper limit. The deviation value of the modus is determined by 50 pixels (px) to calculate the upper detection point that is around 50px up and down from the modus number. The next stage is to calculate the average of the detection points that fall within the deviation range of the upper limit modus. The result of this average value is called the average upper line. The second reading is carried out with the same steps for determining the average lower line. The thickness is obtained from the difference between the average upper line and the average lower line. The detection points on the upper limit, lower limit and average upper line and average lower line are then plotted back into the RGB image to obtain the accuracy of the thickness determination based on the actual image, as shown in Figure 7.
Figure 7 shows that the calculation step provides accurate detection of BC sheet which succeeded in separating the BC sheet from the medium, BC fibers, and air. Some detection points appear to deviate from the BC sheet object indicated by the blue dots, but do not change the thickness detection of BC sheet. To evaluate the detection accuracy, a test was carried out on the fermenter during fermentation, starting from the formation of a thin sheet of BC until the BC sheet thickened along with the fermentation time. Figure 8 shows the BC sheet object during fermentation which was successfully measured at several thickness differences. In can be concluded that the thickness calculation stage can detect the formation of BC sheet thickness from the beginning of its formation to the end of fermentation, as well as separating the BC sheet object from other objects.

![Figure 7. Plotting detection result into actual image](image)

Figure 8. BC sheets were measured on different days during the fermentation process.

The results of the thickness reading are then entered into a table in the MySQL database through the execution of the SQL command which is also contained in the same python script for BC thickness detection. The steps of image capturing process, image processing, and calculation step to obtain thickness data is done every 15 minutes also scripted into the same python script. The Apache server
that is already installed in the Raspberry Pi 3 is used to run the php mailer function. This function will call the last data record in the table of database to be sent to the user's email address via the internet network using a Wi-Fi module that is already installed and connected to the Raspberry Pi 3. An e-mail notification process is used as a monitoring report on the condition of the fermenter by comparing the last data with the previous data. The process flow from the Raspberry Pi 3 is sent to the user's mailbox as shown in Figure 9, while the example of display email from a user who receives a report from a fermenter is shown in Figure 10. From Figure 10, the report display is continuously sent from the Raspberry Pi 3 while simultaneously providing notification of the fermenter condition to the user.

![Diagram](image1.png)

**Figure 9.** The process flow from the Raspberry Pi to the user's mailbox.

![Email Example](image2.png)

**Figure 10.** Display email reports sent from the Raspberry Pi 3 to the user's smartphone.

4. **Conclusion**

Image processing can be used as an approach to measuring the thickness of BC sheet during the fermentation process. The more frequent image shooting processes will require a larger storage capacity on the Raspberry Pi.

**Acknowledgment**

The authors express their gratitude to LPDP (Indonesia Endowment Fund for Education), the Ministry of Finance, and the Republic of Indonesia for providing financial support for this research.
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