

Utilizing Real-Time Image Processing for Monitoring Bacterial Cellulose Formation During Fermentation

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ABSTRACT

In general, nata is a bacterial cellulose results from bacterial fermentation of *Gluconacetobacter xylinus*. During the fermentation process, bacterial cellulose accumulates on the surface of the medium and is eventually visible. The parameter of the end of the fermentation process is indicated by the formation of bacterial cellulose sheets with a certain thickness. During this time, the determination of the success of the fermentation process is done by direct observation of the thickness formed. In this way, the failure of the fermentation process cannot be detected early. Real-time monitoring during the fermentation period will be very helpful to monitor the speed of bacterial cellulose formation and early failure detection of the fermentation process. Currently, image processing has been widely used for various purposes. This study describes how to utilize image processing to monitor bacterial cellulose formation during the fermentation process. For this reason, it is necessary to modify the fermentor by making an area to shoot and follow any thickness increase in the bacterial cellulose, as well as painting the fermentor in dark color to better contrast with the bacterial cellulose color. The device used is the Raspberry Pi, which has been connected to a web camera. Once the image has been captured, it is then processed to calculate the thickness, after which the thickness data are sent to the database.

Keywords: Bacterial cellulose; image processing; Raspberry Pi

INTRODUCTION

Bacterial cellulose (BC) is an environmentally friendly polymeric material, which is now receiving increased attention in society. It is an unbranched polymer with nanofibrils, made up of (1/4) β -glycosidic linked glucose units. These linear glucan chains form highly regular intra- and intermolecular hydrogen bonds (Shi, Zhang, Phillips, & Yang, 2014). *Acetobacter xylinum* is a gram-negative rod-shaped microorganism, which is also known as *Gluconacetobacter xylinus* (Mohammad & Rahman, 2014). Microbial cellulose is an exopolysaccharide produced by various species of bacteria, such as those of the genera *Gluconacetobacter*

(formerly *Acetobacter*), *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Rhizobium*, *Sarcina*, and *Salmonella* (Chawla, Bajaj, Survase, & Singhal, 2009). In general, nata is a BC produced during fermentation by *G. xylinus*. This type of bacteria produces two forms of cellulose: (i) cellulose I, a ribbon-like polymer, and (ii) cellulose II, a thermodynamically more stable amorphous polymer (Chawla et al., 2009). The differences in the assembly of cellulose I and cellulose II outside the cytoplasmic membrane are described in Figure 1. During the fermentation process, cellulose fibers are secreted out, which eventually accumulate on the surface of the medium until they become visible (Figure 2).

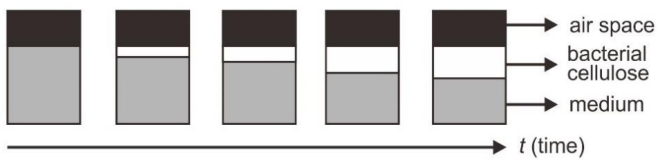


Figure 1. Assembly of cellulose microfibrils by *G. xylinum* (Iguchi, Yamanaka, & Budhiono, 2000)

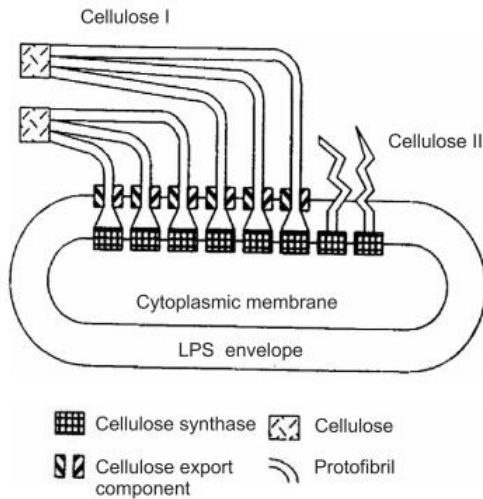


Figure 2. Change in thickness of BC against time

Traditionally, the fermentation process was carried out in a tray that was covered with newsprint and tied with rubber. The parameter of the end of the fermentation process is indicated by the thickness of the nata formed, usually ranging from 0.8–1 cm. The success of the fermentation process is determined by opening the lid of the tray and lifting a sheet of nata to determine the thickness.

At present, digital image processing has been widely used in various fields, such as photography, medicine, agriculture, health, and advertising. In the field of agriculture, image processing is widely used for the selection and sorting process. Several journals related to image processing in agriculture have been carried out, including the determination of tomato quality (Álvarez-Bermejo et al., 2017), the determination of the area of corn farming land (Burgos-Artizzu, Ribeiro, Guijarro, & Pajares, 2011), detection of soybean aphids (Maharlooee, Sivarajan, Bajwa, Harmon, & Nowatzki, 2017), and strawberry sorting (Durand-Petiteville, Vougioukas, & Slaughter, 2017).

During the fermentation process, the BC becomes visible over time (Nugroho & Aji, 2015). BC observations can be replaced by using a digital image processing approach. A camera will be needed for the image capture, and then image processing is done by using

digital software that can be run on Raspberry Pi. In general, any digital image processing algorithm consists of three stages: input, processor, and output. In the input stage, the image is captured by a camera. This particular system focuses on a pixel of image that gives the output as a processed image (Shilpashree, Lokesha, & Shivkumar, 2015). The object of image processing results is then stored in the database. The aim of this research is utilizing real-time image processing for monitoring BC formation during fermentation.

MATERIALS AND METHODOLOGY

The ingredients needed for the fermentation medium include 1.5 L of coconut water, 5% sucrose, 0.5% ammonium sulfate, and acetic acid for adjusting the pH to 4.0. The fermentation medium is then boiled for 15 minutes and allowed to cool. The medium is inoculated with 5% culture stock in a fermentor tray, which is then covered up with clean paper. This fermentor tray is then placed into main board with shooting area directly faces to a USB web camera, as shown in Figure 3. Microorganism counting was performed by colony-forming unit (cfu) to measure viable bacteria on a medium, which consists of 20 g of glucose, 5 g of yeast extract, 10 g of agar, 20 g of CaCO_3 , and 50 mL of 99% ethanol. The sampling process is carried out daily. A series of dilutions is made and inoculated into a petri dish containing the medium above and then incubated for 5 days at room temperature. Colonies are calculated, which form a clear zone on the medium.

Fermentors are built based on the dimensions commonly used in the nata de coco industry (i.e., 35 × 25 × 6 cm). However, for research purposes requiring transparent material for image capture, the fermentors were made using acrylic material. Modifications were made by providing a transparent field for the camera viewfinder, as well as a fermentor cover to make it easier to close and open. The fermentor cover uses paper that is secured by a frame. Black paint is used to coat around the fermentor, leaving 4 × 6 cm of transparent space for the camera's shooting area, as shown in Figure 4.

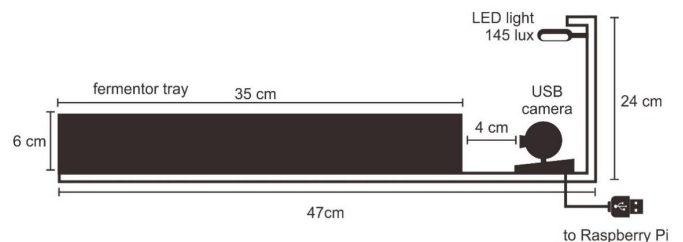


Figure 3. Arrangement of fermentor for image processing purposes

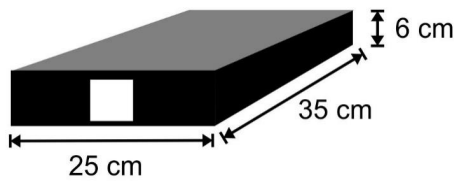


Figure 4. Design of the fermentor

A USB HD webcam with brand name Alloet (maximum resolution of 1280 × 960 pixels, CMOS sensor type, and auto focus) is placed right in front of the transparent field, which will capture images at intervals of 15 min for 8 days. This camera is directly connected to the Raspberry Pi using a USB port. LED light is placed above the shooting area to facilitate the process of image capture. A luxmeter LX-101 (Lutron) is used to measure the intensity of the LED light at 145 lux.

During those 8 days, the Raspberry Pi should be connected to a power source. To anticipate a power outage, the electricity source is first connected to an Uninterruptible Power Supply with 600-VA capacity before connecting to the Raspberry Pi.

One of the devices that can be used to provide periodic shooting sequences is a Raspberry Pi 3 model B+ (Raspberry Foundation, UK). Python is an integral part of the Pi's operating system (Donat, 2018) and runs seamlessly on Raspberry Pi. Python also provides the libraries needed in this study, including MSQldb, statistics, OpenCV, and NumPy. Image capture and digital image processing can be programmed using the Python language with the OpenCV library. The command module for taking pictures, changing to black and white images, and edge detection is already in the OpenCV library, whereas the object recognition algorithm to be transformed into thickness information of the nata object was developed in this study. The data obtained during processing are then sent to the MySQL database. This program consists of two main stages: the image processing program for color and the image processing program to calculate the thickness of the object on the image. The following steps in the image processing program are as follows: (1) image capture, (2) RGB image conversion to black and white, (3) determination of detection threshold, and (4) determination of object boundaries. Meanwhile, the image processing steps to obtain the image width are as follows: (1) processing the image calls with borders, (2) determination of the upper edge, (3) determination of the lower edge, and (4) calculation of the distance difference between the upper and lower margins. Thickness is expressed as the



Figure 5. Arrangement of equipment in the research

average difference between the upper and lower edge. The arrangement of research equipment is shown in Figure 5.

RESULTS AND DISCUSSION

The number of colonies was counted to determine the growth pattern of *G. xylinum* during the BC fermentation process. Figure 6 shows that the growth process of *G. xylinum* is still in the log phase after reaching day 6. Since BC material is continuously secreted during the fermentation process by microorganisms, it is possible to observe the fermentation process carried out with a minimum of 6 days or more.

The process of capturing digital images during fermentation is done by arranging equipment as in Figure 7. The Raspberry Pi board is connected to the web camera through a USB port. The web camera faces directly in front of the transparent area of the fermentor, so that the shooting area of the webcam is only the width of the transparent plane of the fermentor. All fermenter walls were colored with non reflective black color-type black paint to minimize the reflection effect of the light, as well as to increase the contrast with the object to be captured.

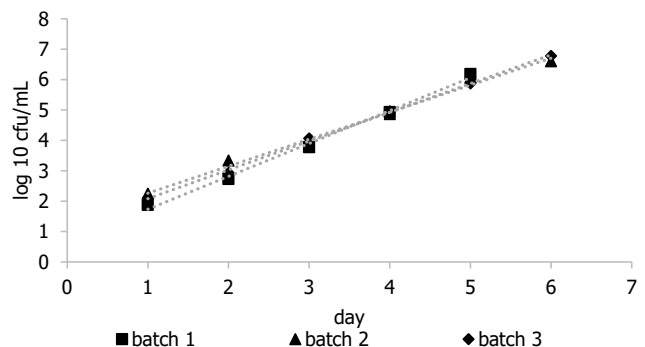


Figure 6. Growth of *G. xylinum* during BC fermentation

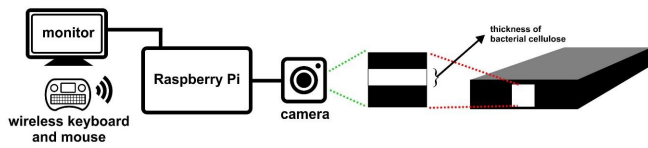


Figure 7. Digital image capture equipment series in the fermentor

During the fermentation process, an increase in the thickness of BC can be observed according to the time function. The results of image capturing are shown in Figure 8.

Figure 8 shows that, from day 1 to day 3, the background color, the outer color, and the color of the medium look dark and in contrast with the BC layer that begins to form on the surface of the medium. After day 4, an increase in *G. xylinum* activity was marked by an increase in BC thickness and an increase in turbidity from the fermentation medium. The increase in thickness and increase in turbidity will continue until the end of the fermentation period; however, the increase in turbidity contributes to the color, which is in contrast with the background color. This can interfere with the BC thickness measurement process against the background color.

The ability of image processing in measuring the dimensions of thickness is tested by using two images that are designed to describe the initial conditions and final conditions of BC fermentation. The initial condition of fermentation is depicted by a thin line that describes the initial formation of BC on the surface of the medium. This condition can also test the ability of image processing scripts in recognizing very fine objects. As a

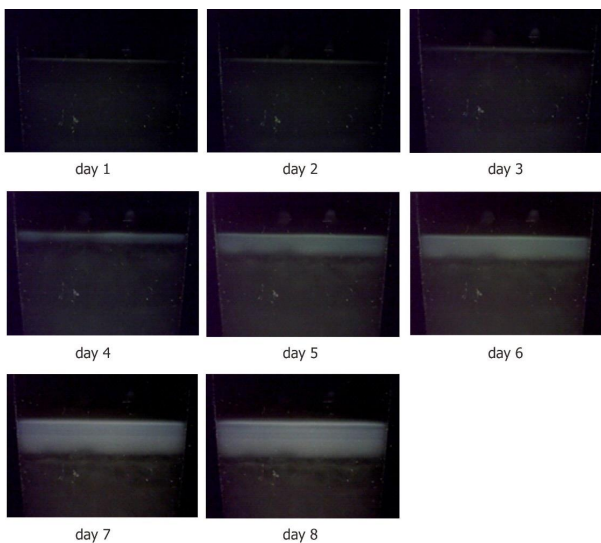


Figure 8. Change in thickness of nata for 8 days

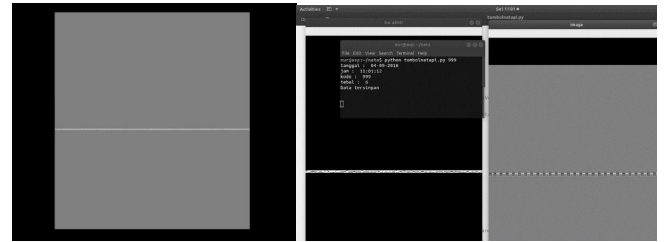


Figure 9. Illustration of the initial conditions of the formation of BC in the transparent plane of the fermentor and the appearance of black and white images with edge detection and sampling points

result, the script is able to recognize objects and convert them into binary images, which can then be detected by their edges. At the same time, it can also detect several sample points to calculate the average thickness (Figure 9). Successful detection would display this information in the form of thickness of nata, which can then be sent to the database.

Testing the ability of image processing scripts is also done for image samples that represent the final fermentation conditions, based on the results of shooting the transparent field of the fermentor (Figure 10). Successful results of the image processing script that was tested would include the following: changing to binary image; performing edge detection; and displaying information in the form of thickness, fermentation batch, and the time and date of supervision; as well as sending the information into the database.

During the fermentation process, the biomass will settle to the bottom and will release cellulose threads

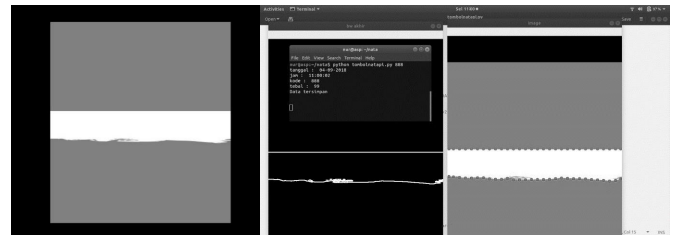


Figure 10. Illustration of BC fermentation final conditions in the transparent field of the fermentor and black and white image display with edge detection and sampling points

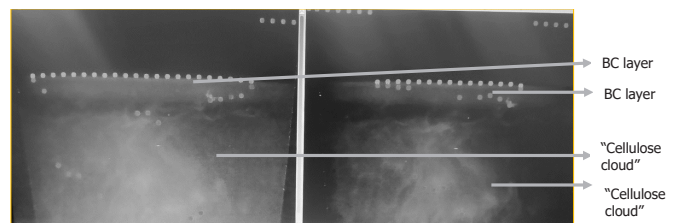


Figure 11. Interference in the thickness detection due to "cellulose cloud"

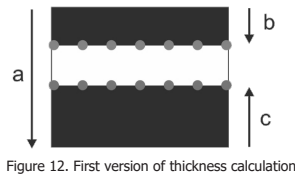


Figure 12. First version of thickness calculation

to form a "cellulose cloud" (CC), which is visible and forms the surface of nata layers. In fact, the "cellulose cloud" can be seen filling the field of observation, such that it interferes with the detection of nata thickness (Figure 11).

The first version of thickness calculation is shown in Figure 12. The direction of detection starts from line a in the downward direction to determine the height of the image. Then, it proceeds with the change in the intensity of the RGB value, which can be detected with a threshold value of 40, from the top (line b). If changes have been detected, detection proceeds from the bottom up (line c). Thickness is expressed by the mean values $a-(b+c)$ at various detection points.

This first version of thickness calculation may have a problem with visible "cellulose clouds," because these clouds are formed from biomass that settles below and slowly rises to the surface. The image of the "cellulose cloud" is white, and hence could be mistaken as finished nata. This may cause the detection results to look as though nata is very thick.

There is an interesting event that occurs during the formation of nata layers of "cellulose clouds," which is accompanied by the appearance of a clear zone between the "cellulose clouds" and the nata that has already formed (Figure 13). This is always observed from several batches of fermentation.

Given this phenomenon, a thickness calculation method will be rearranged, which would negate the observations of "cellulose clouds," as shown in Figure 14. Detection of contrast changes starts from the top until it encounters the first white object (line z). Then, detection continues until it encounters a dark area (line

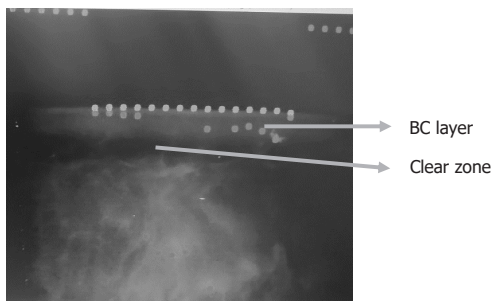


Figure 13. Clear zone on fermentation of BC



Figure 14. New version of thickness calculation

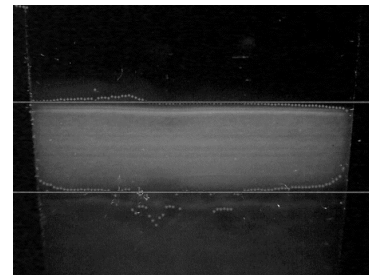


Figure 15. Image processing of thickness calculation that eliminates the "cellulose cloud"

y). Thickness is calculated as the difference between y and z. The result of image processing on a real object by eliminating the "cellulose cloud" is shown in Figure 15.

During the fermentation process, images are captured using a webcam camera at intervals of 15 min for 8 days. BC thickness data are then sent to the MySQL database to be recorded in a table containing the date, hour, fermentation code fields, and nata thickness. The date and time columns will be filled automatically based on server time, whereas the fermentation code is written at the initial time of running the image processing circuit in the console window on Raspberry Pi. Meanwhile, the

nomor	tanggal	jam	kode	tebal
1105	17-09-2019	01:42:22	50919	174
1106	17-09-2019	01:57:26	50919	176
1107	17-09-2019	02:12:31	50919	175
1108	17-09-2019	02:27:36	50919	174
1109	17-09-2019	02:42:40	50919	175
1110	17-09-2019	02:57:45	50919	174

Figure 16. Table in MySQL database showing 1220 rows of data during 8 days of fermentation

thickness is the thickness measurement data of nata, which results from the calculation of the edge margin of the upper and lower limits. The measurement data were successfully carried out in real time in the MySQL database with a total of 1220 rows of measurement data (Figure 16).

CONCLUSION

The use of digital image processing can be used to monitor the development of BC thickness measurements during the fermentation process in real time. Sending the thickness measurement data into the database can be done in real time during the fermentation process.

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CONFLICT OF INTEREST

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

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