

ENHANCEMENT OF BIOCELLULOSE PRODUCTION IN MIXED MEDIUM CULTURE

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Abstract

In this research, the results for biocellulose production by *Acetobacter xylinum* in mixed medium culture were reported. Biocellulose production was determined by utilizing different feedstocks of single sugars and sugar mixtures which were applied according to certain glucose to fructose ratio. Data for pH changes and the biocellulose production from every medium culture were thoroughly analyzed. In this experiment, it was ensured that all the samples had the initial temperature and pH of 30°C and pH 5.5 respectively. The temperature was kept constant throughout the whole experiment while the changed pH value was taken as final pH at the end of the experiment for every sample. The highest production using sugar mixtures of 1:9 glucose to fructose ratio was 1.57g/l. The final pH values recorded in the different sugar mixtures were in the range of 4.0–5.5. The lowest final pH of 4.56 was determined in the medium that contained a single carbon source of glucose, as most of the glucose was converted into gluconic acid and lead to lowest biocellulose production of 0.69g/l. In contrast, the highest pH value of 5.3 was determined in the medium that contained a single carbon source of fructose and lead to the higher biocellulose production of 0.9g/l. Analyzing profiles for final pH and biocellulose production for the medium with higher glucose concentration showed that the glucose was preferable to be converted to gluconic acid rather than biocellulose synthesise. Besides, it was also determined that biocellulose production rate in mixed culture medium was higher than in culture medium that only consist of a single carbon source and this had proved that the experiment of enhancing biocellulose production with mixed medium culture was applicable. Results reported in this study demonstrated that the production of biocellulose can be enhanced by using carbon sources mixture with a suitable ratio.

Keywords: *Acetobacter xylinum*, carbon source, fructose, glucose, ratio

INTRODUCTION

Payen was the French Chemist who found out the existence of cellulose in 1838. Samples are taken from the plant matters and the chemical formula of cellulose was designed (Klemm *et al.*, 2001). Biocellulose is a form of cellulose product, produced by a specified bacterium. It is also called microbial cellulose and it was first recognized as cellulose in 1886. The bacteria which can produce cellulose are from the genera *Aerobacter*, *Acetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azotobacter*, *Pseudomonas*, *Rhizobium* and *Sarcina* (Ross *et al.*, 1991). However, *Acetobacter xylinum* is the only species which can synthesis enough cellulose for commercial purposes. In a survey, it shows that the industry produces 200000 tons of biocellulose in 2006 and is targeted to be reaching 5000000 tons during 2015 align with the increasing world demand for biocellulose products as is estimated that the world market will face the problem of inadequate fossil feedstock in the next 10 to 20 years. Many of the synthetic polymers nowadays are manufactured from petrochemicals and are non-biodegradable (Gautam 2009). It gives negative impacts towards our mother nature.

Moreover, fossil carbon source is limited and will out of stock one day. Hence, cellulose which is biodegradable is the next polymer that will replace the non-biodegradable polymer. However, cellulose is gained from plants and will damage the mother nature if trees are cut off to obtain cellulose. Therefore, biodegradable biocellulose that is produced by bacteria (*A. xylinum*) is preferred to reduce the consumption

of trees.

Normally, single sugar is used in preparing the medium for biocellulose production. For example, biocellulose is produced from medium culture containing glucose with *Acetobacter xylinum* (Masaoko *et al.*, 1992). In this research, the medium culture is prepared using fructose glucose mixture in different ratio. The ratio of the two components in the mixture that will produce the highest amount of biocellulose is determined in the end of the research and will be recommended for the usage in the real polymer industry. It will be a new era for polymer industry.

In the previous research which was done by Yaser Dahman, Kithsiri E. Jayasuriya and Magdalina Kalis, the biocellulose production rate in agitated mixed medium culture was higher than the biocellulose production rate in single culture medium (Dahman *et al.*, 2010). This is very helpful information for the effort to enhance the biocellulose production in this era. This research is a further on project as the suitable ratio of two carbon sources in the mixed culture medium which will produce the highest amount of biocellulose among the samples is the main target to be determined.

MATERIALS & METHODS

Material and Apparatus

The apparatus list in the research includes incubator, cone flask, autoclave, FTIR, SEM, and Electronic Weighing Machine. On the other hand, the material used consists of carbon sources (glucose and fructose), 5g/l yeast extract, 5g/l peptone, 2.7g/l dibasic sodium phosphate (buffer) and 1.15g/l citric acid.

Measurement and Analysis

Biocellulose in every different medium is weighed with electronic weighing machine and is compared and the production efficiency of every culture medium containing different ratio of mixed carbon sources is determined. The properties of biocellulose are determined using Fourier Transform Infrared Spectroscopy (FTIR). Besides, the surface and cross section properties of biocellulose produced are observed by Scanning Electron Microscope (SEM).

Water Absorption Capacity (WAC)

In order to determine the WAC, the dried biocellulose samples are immersed in DI water at room temperature till it reaches equilibrium. Then, the samples are taken out from the water and excess water left on the biocellulose surface is blotted out with Kimwipes paper. After that, the weights of the hydrated biocellulose samples are measured, and repeat the procedure till no observation of further weight change. The water content is calculated using the following formula:

$$\text{WAC (\%)} = [(W_h - W_d)/W_d] \times 100\% \quad (1)$$

W_h and W_d indicate the weight of the hydrated and dried biocellulose samples respectively.

Biocellulose Production

Carbon sources (glucose and fructose) which are the experiment's main materials are consumed from Sigma & Aldrich. Figure 1 shows overall method for biocellulose production.

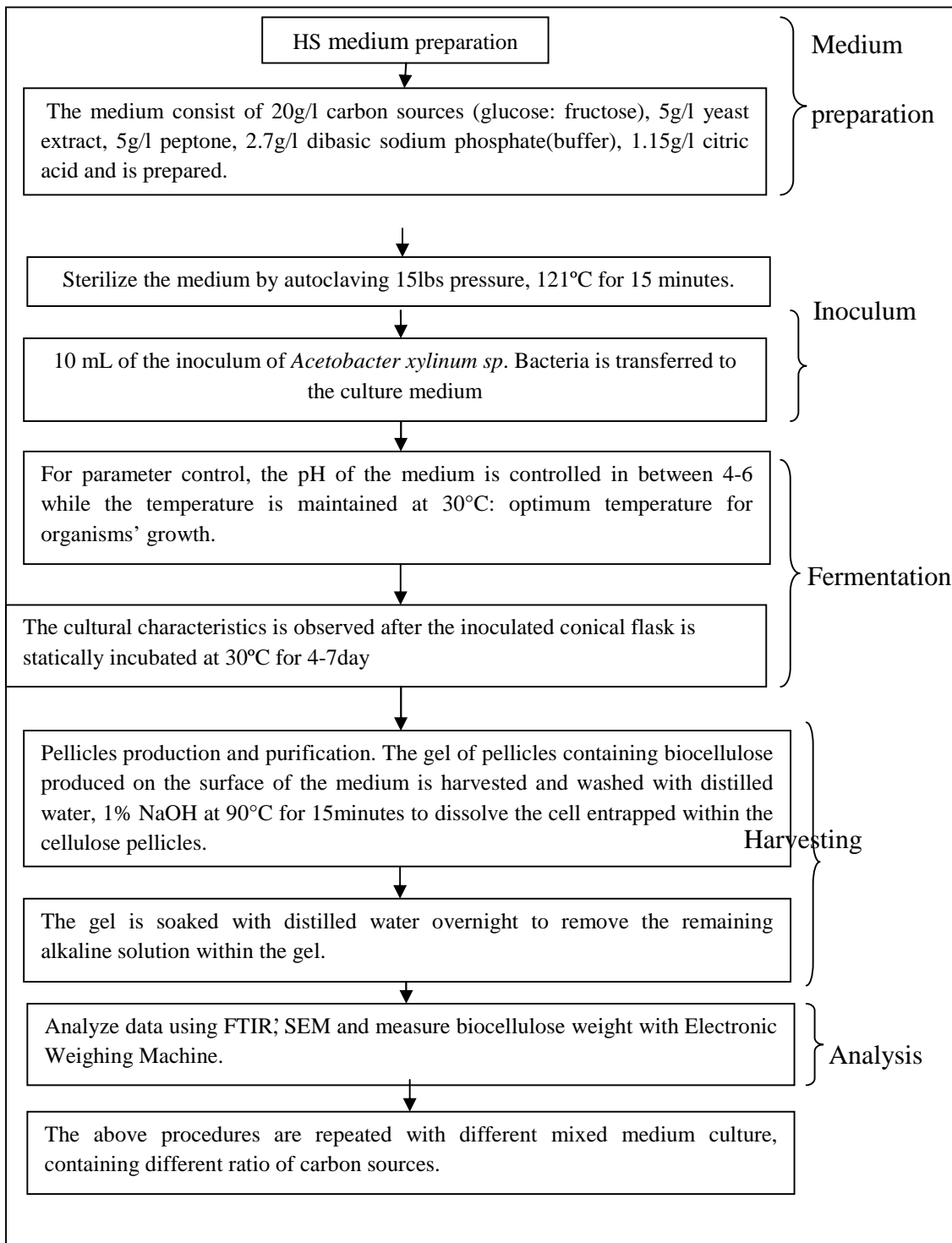


Figure 1: Overall methods for biocellulose production

RESULTS & DISCUSSIONS

Relationship between Carbon Source and Biocellulose Production

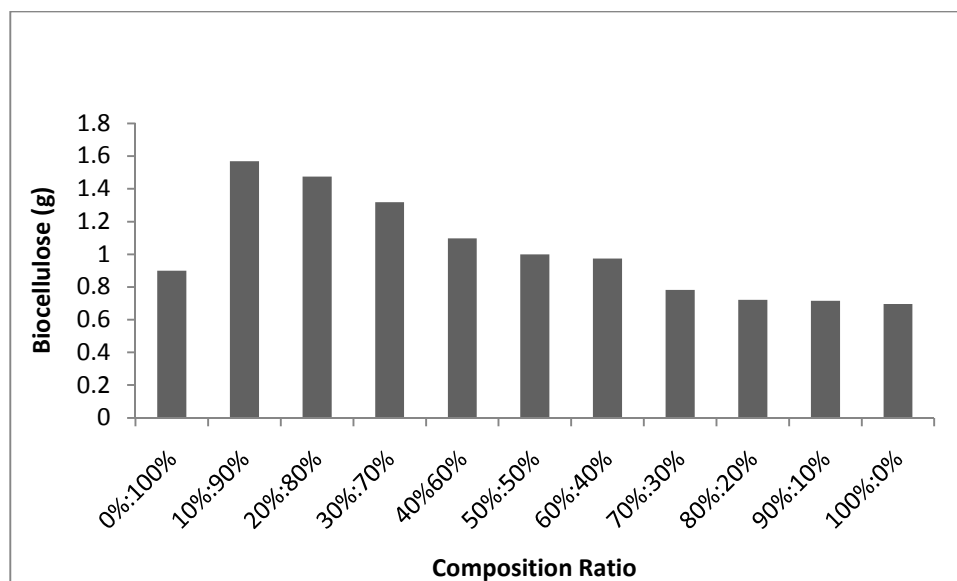


Figure 2: Graph of cellulose dried weight versus composition ratio of carbon sources in mixed culture medium

According to the graph in Figure 2, it shows that the biocellulose production is decreasing when the glucose concentration in the medium culture is increasing. The experiment is brought out with 11 conical flasks (250ml) that contain Hestrin and Schramm medium, also referred as HS medium. However, each of them comprises of different carbon source composition ratio (Glucose: Fructose) and will lead to different biocellulose production. Two of those conical flasks are prepared with medium culture that contain single carbon source that is glucose alone (100% glucose) and fructose alone (100% fructose) respectively while the other nine conical flasks are prepared mixed medium culture. Each of the nine conical flasks which are prepared with mixed medium culture contains different composition ratio of glucose and fructose concentration which varies from 10% to 90% glucose composition.

From the result, it shows that the conical flask with culture medium that contains the mixture of 10% glucose and 90% fructose lead to the highest biocellulose yield of 1.57g/l. Meanwhile comparing both of the conical flasks which are with different single carbon source each, the conical flask with culture medium that contains fructose alone lead to a higher biocellulose production than the conical flask that contain glucose alone. The biocellulose yield of both conical flasks which contains glucose alone and fructose alone are 0.696 g/l and 0.9 g/l respectively. Anyway, this has proved that the conical flasks with culture medium that contain mixed carbon sources bring about higher biocellulose yield than culture medium that contains only single carbon source. The biocellulose yield in the culture medium that contains mixed carbon sources of glucose and fructose is higher compared to the culture medium that contains only a single carbon source.

However, this statement is only accurate to be used to compare between single carbon source of glucose and mixed carbon sources medium. This is due to the fact that the biocellulose yield in the mixed medium culture is decreasing with the increasing of glucose composition and finally it comes to point that the biocellulose production in mixed medium is less than the biocellulose production from the culture medium that contain single carbon source of fructose alone. This turning point is at the mixed culture medium that contain 70% glucose and above. From the graph and data in the table above, it shows that the production of 70%, 80% and 90% glucose is 0.782 g/l, 0.722 g/l and 0.715g/l respectively while culture medium with single carbon source of fructose lead to the biocellulose productivity of 0.9 g/l. This obviously shows that the productivity from the 3 mixed culture mediums that contain 70%, 80% and 90% glucose respectively are lower than the medium that only with fructose alone. Anyway, the lowest biocellulose yield belongs to culture medium that contains glucose alone that is 0.696 g/l.

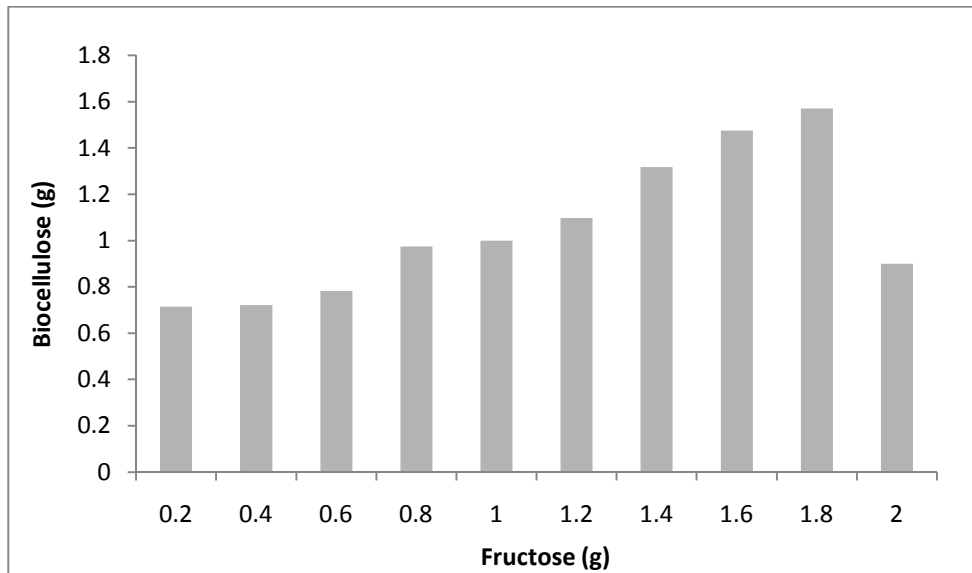


Figure 3: Graph of biocellulose weight versus fructose weight

The graph in Figure 3 is extracted from the previous result. It gives a clearer image in how the increasing concentration of fructose in the mixed culture medium leads to the increasing of biocellulose yield. Moreover, it also presents that the biocellulose yield is mainly depends to fructose. Their relationship is proportional to each other. It shows the highest biocellulose yield of 1.57 g/l when the fructose is in the amount of 1.8g, means 90% of the mixed carbon sources with 10% glucose. However, the graph shows a drop to 0.9 g/l although the amounts of fructose supplied in the medium culture is the highest, which is 2g because it indicates the 100% fructose as the carbon source in the culture medium. In other words, it is a single source culture medium.

Relationship between pH and Composition Ratio of Carbon Sources in Mixed Culture Medium

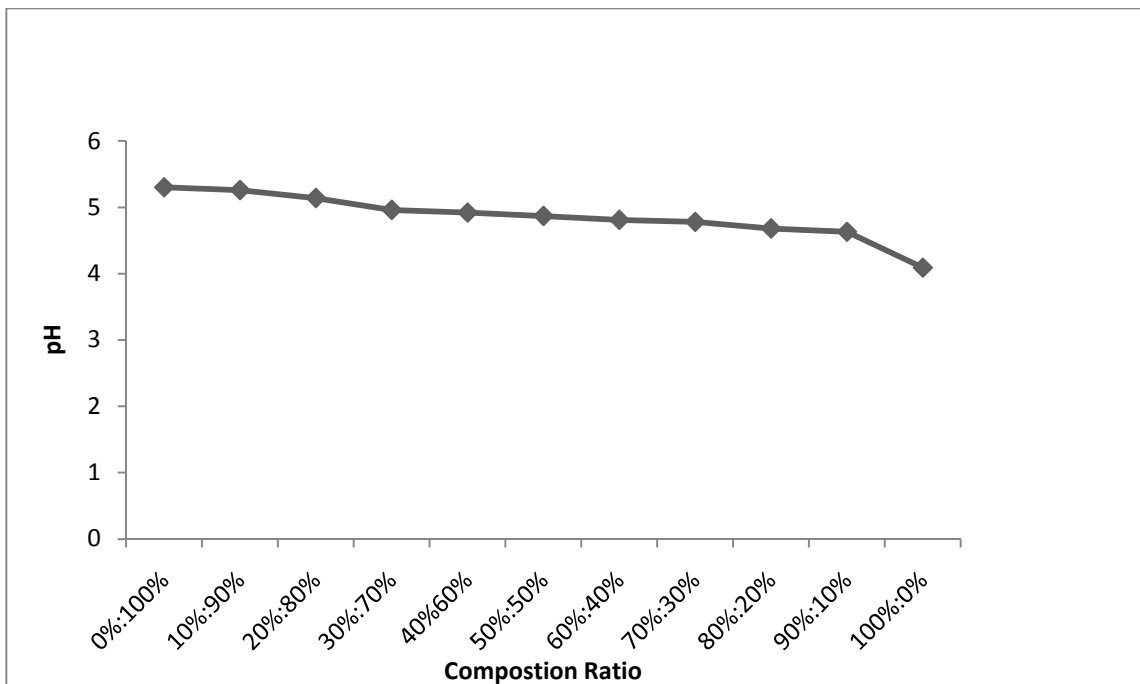


Figure 4: Graph of biocellulose production versus pH

Initially, the pH of the 11 culture mediums in each conical flask is all adjusted to 5.5 with acetic acid

and pH meter. According to previous researches, the optimum pH for the growth and activity of *Acetobacter xylinum* is in the range of 4 to 7 while pH 6.5 leads to the best biocellulose yield. However, the chance of contamination will increase when the pH approach 7 as many other organism can live under this medium condition (Shazia *et al.*, 2010). Hence, the pH is adjusted to pH 5.5 and so, fewer organisms can adapt to such acidic condition and reduce the chance of contamination. Meanwhile, the culture medium condition cannot be lower than 4 or it will lead to the deactivation of *Acetobacter xylinum* itself.

The graph and data above are the result of the final pH for the project. According to the graph, the pH of the medium culture is decreasing when the glucose concentration is increasing. The medium culture with glucose alone lead to the lowest final pH, that is pH 4.09 while there is only a slightly pH change for the medium culture with fructose alone, that is 5.3.

This is due to the production of glucosidic acid from glucose by *Acetobacter xylinum* that decreases the pH of the culture medium while fructose is not metabolized to be acid. *Acetobacter xylinum* synthesizes glucose to glucosidic acid rather than biocellulose synthesis when the glucose concentration increases in the medium. In other words, higher glucose concentration in culture medium lead to higher production of glucosidic acid but brings about lower biocellulose production as most of the glucose is already used up to be converted to glucosidic acid. *Acetobacter xylinum* will preferentially synthesize glucosidic acid from glucose when the glucose concentration increases and this is the interesting characteristic of the *Acetobacter xylinum* itself. When glucose coexists with fructose, glucose is preferred to be converted into glucosidic acid while producing cellulose mainly by fructose (Kamide *et al.*, 1990).

In addition to that, the condition of the culture medium is becoming more and more unsuitable for the growth of *Acetobacter xylinum* when the pH is decreasing and approaching to pH 4 because the bacteria will be totally deactivated when the condition of the culture medium is below pH 4. Therefore from graph figure 3.3, it can be observed that the biocellulose production from the medium with fructose alone is higher (0.9 g/l) than the biocellulose production from the medium which is with the glucose alone (0.696 g/l) when comparing cellulose yields of both culture mediums which only contain a single carbon source. However when comparing with the culture mediums that contain mixed carbon sources, the mixed culture medium of 10% glucose and 90% fructose gives highest biocellulose yield although its final pH shows that it has a lower figure than culture medium with fructose alone, that is 5.26. The accumulated glucosidic acid decreases the pH of the culture medium and inhibits biocellulose production.

This is because the *Acetobacter xylinum* performs better in mixed culture medium and the low glucose concentration of 10% does not affect its biocellulose production as most of the biocellulose is synthesized from the 90% fructose in the culture medium. Overall, the lowest final pH and the lowest biocellulose yield are the results from the culture medium with glucose alone. In other words, almost all of the glucose is metabolized by the *Acetobacter xylinum* to be glucosidic acid and only a little amount of glucose left for biocellulose synthesize. This is the reason why the increasing amount of the single carbon source of glucose fails to increase biocellulose yield but in fact, deceases the biocellulose yield.

FTIR Testing

The following Figures of 5, 6 and 7 are the results from the FTIR testing for the 3 biocellulose samples which are produced from mixed medium cultures of 100% glucose, 100% fructose as well as mixture of 90% fructose and 10% glucose respectively.

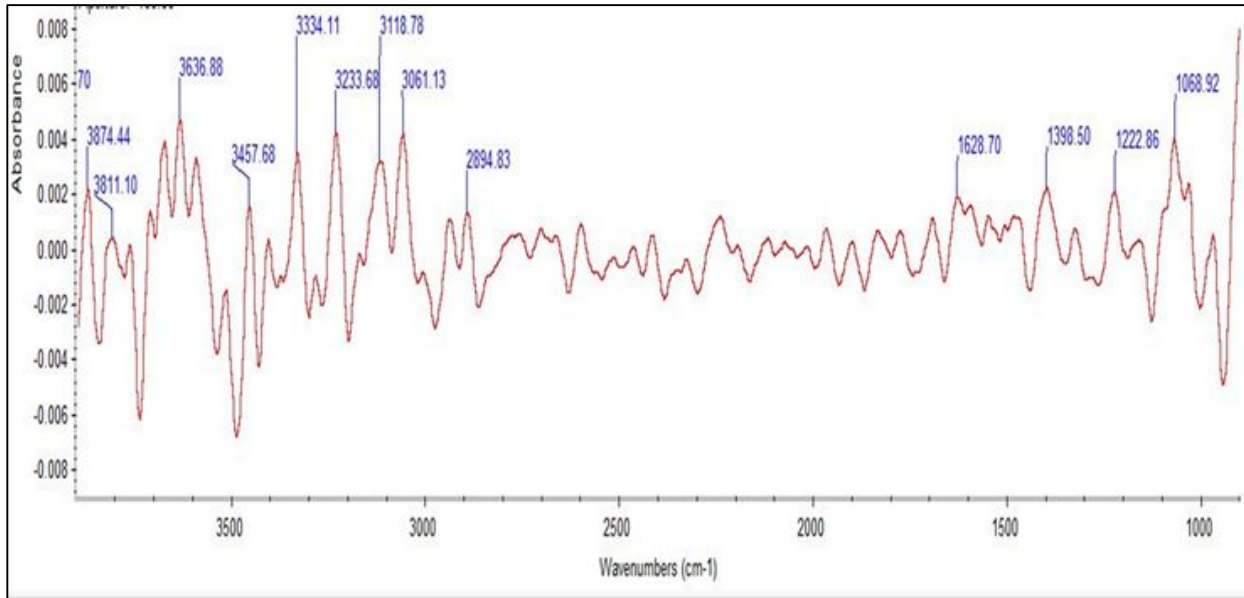


Figure 5: FTIR image of biocellulose produced from culture medium with 100% glucose

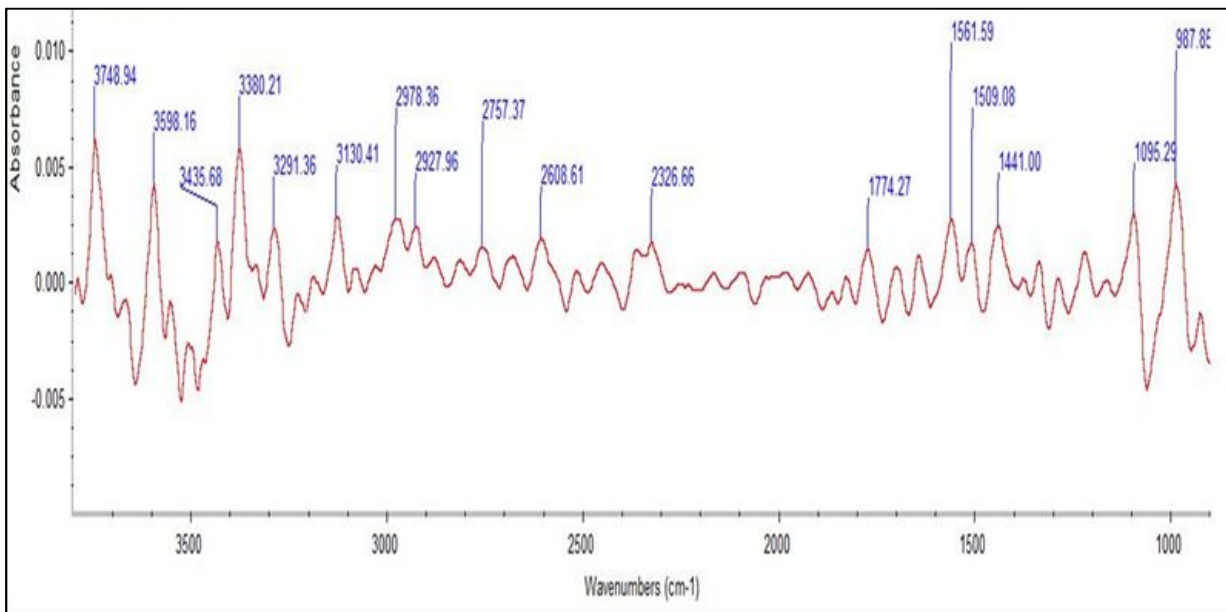


Figure 6: FTIR image of biocellulose produced from culture medium with 100% fructose

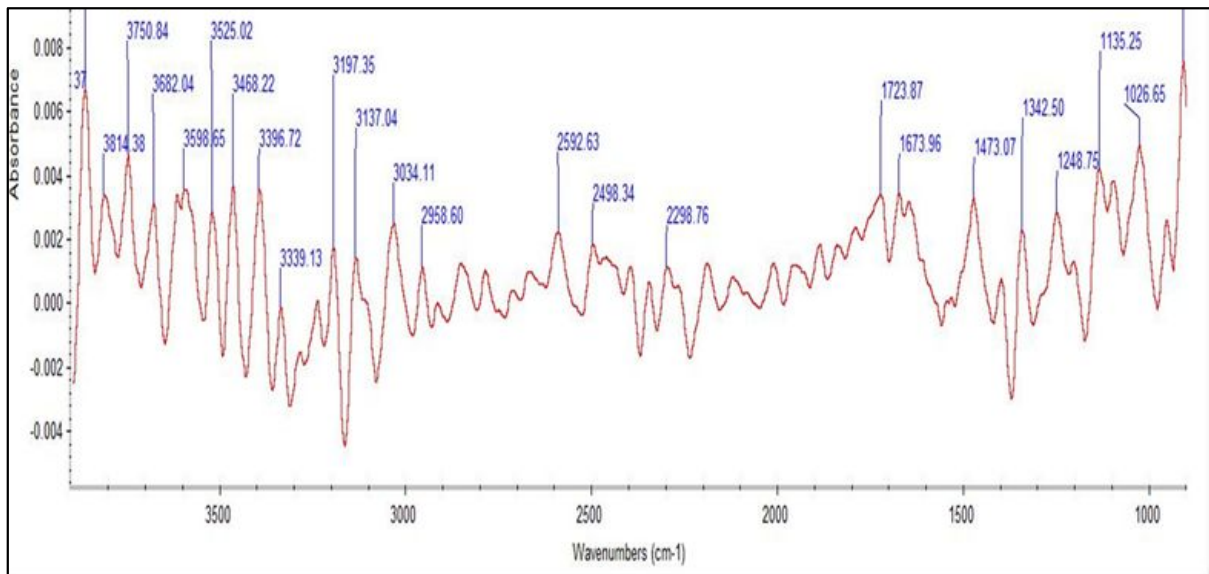


Figure 7: FTIR image of biocellulose produced from culture medium with 10% glucose and 90% fructose

There are three biocellulose samples from this project chosen to carry out the FTIR testing. They are the biocellulose sample from the 100% glucose culture medium, biocellulose sample from 100% fructose culture medium and the biocellulose sample from the mixed culture medium of 10% glucose and 90% fructose. This testing is carried out to ensure that the components produced in those culture mediums are cellulose.

Figure 5 shows the FT-IR spectra of the biocellulose from the 100% glucose culture medium. It shows a band at 3334.1/cm which can be associated to the intermolecular and intramolecular hydrogen bonds. Then the spectra also consists of a band at 2894.83/cm that can be related to the C-H stretching and it also has a band at 1068.92/cm which can be associated to the ether C-O-C functionalities. Moreover, it also has a region of 3233.68-3457.68/cm which can be indicated as the intermolecular and intramolecular hydrogen bonds.

Figure 6 represents the FT-IR spectra of the biocellulose from the 100% fructose culture medium. It shows a band at 3380.21/cm which can be associated to the intermolecular and intramolecular hydrogen bonds. Then the spectra also consists of a band at 2757.37/cm that can be related to the C-H stretching and it also has a band at 1095.29/cm which can be associated to the ether C-O-C functionalities. Furthermore, it also has a region of 3291.36-3435.68/cm which can be indicated as the intermolecular and intramolecular hydrogen bonds.

Figure 7 represents the FT-IR spectra of the biocellulose from the mixed culture medium of 10% glucose and 90% fructose. It shows a band at 3339.13/cm which can be associated to the intermolecular and intramolecular hydrogen bonds. Then the spectra also consists of a band at 2958.6/cm that can be related to the C-H stretching and it also has a band at 1136.25/cm which can be associated to the ether C-O-C functionalities. In addition to that, it also has a region of 3339.13-3468.22/cm which can be indicated as the intermolecular and intramolecular hydrogen bonds.

According to a previous biocellulose research, a band at 3300/cm can be associated to the hydroxyl bonds while the spectra also consists of a band at 2880/cm that can be related to the C-H stretching and it also has a band at 1100/cm which can be associated to the ether C-O-C functionalities (Saied et al., 2008). Then, the FTIR spectra region of 3230 -3455/cm can be referred to as the intramolecular and intermolecular hydrogen bonds of the cellulose. Hence the FT-IR spectra of the three samples consist of bands that similar to the strong bands that appear in a biocellulose. Therefore, it is proved that the component produced from the culture mediums of the project is 100% biocellulose.

Water Adsorption Capacity of Biocellulose

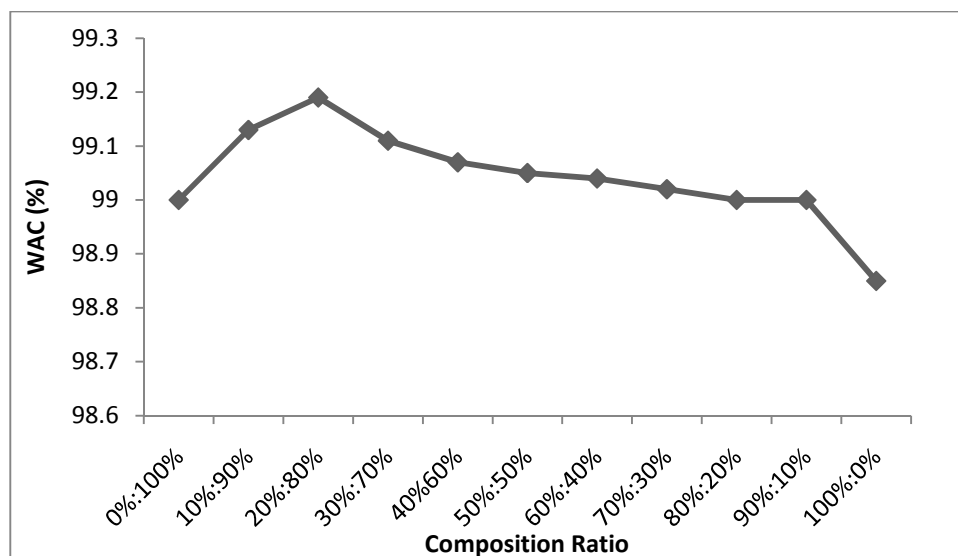


Figure 8: Graph of water adsorption capacity versus composition ratio

Figure 8 shows the water absorption capacity for each of the biocelluloses produced from the culture mediums in the project. According to the graph, the highest water absorption capacity is determined from the biocellulose that is synthesized from the culture medium that contains the mixed carbon source of 20% glucose and 80% fructose that is with 122.22. The fibrils size of the biocellulose are 100 times smaller than the plant cellulose and this provides the biocellulose with a special nano morphology results that lead to the remarkable water absorbing ability of the biocellulose (Shirai *et al.*, 1997). This indicates the mixture of fructose and glucose at a certain ratio can produce biocellulose that possesses strong structures and fine distribution of microbial fibrils which lead to a wider surface area for the biocellulose to hold the big amount of water. The water absorption capacity of biocellulose is analogous to its mechanical strength (Saibuatong *et al.*, 2010).

The biocellulose which is the highest yield from the mixture of 10% glucose and 90% fructose leads to the second largest value of the water absorption capacity that is with 111.46. It also can be observed that the biocellulose produced from the culture medium that contains fructose alone has a higher water absorption capacity than the biocellulose produced from the culture medium which contains glucose alone. The biocellulose from the medium with fructose alone has a water absorption capacity of 107.15. Meanwhile, the biocellulose which is produced from the glucose alone has the lowest water absorption capacity that is 85.7. The graph also shows that the water absorption capacity is decreasing when the glucose concentration is increasing. In other words, the developed biocellulose structure becomes miscellaneous and weak. Hence the ability to hold the water also becomes weaker. The water absorption capacity value is important for the biocellulose to be commercialized as the wound dressing.

Scanning Electron Microscope

Figure 9 is the SEM image of the biocellulose surface at magnification of 300X. It is the surface of the enhanced biocellulose which is produced from the mixed medium culture of 10% glucose and 90% fructose. It can be observed that the biocellulose surface is unsmooth and possess non-porous morphology. Pores can not be observed on the surface of the biocellulose. Figure 10 is the SEM image of the biocellulose cross section at a magnification of 2K X. According to the image, it can be observed that the cross section of the biocellulose is in layered form. It has been reported earlier that the biocellulose is a layer formation (Klemm, 2001).

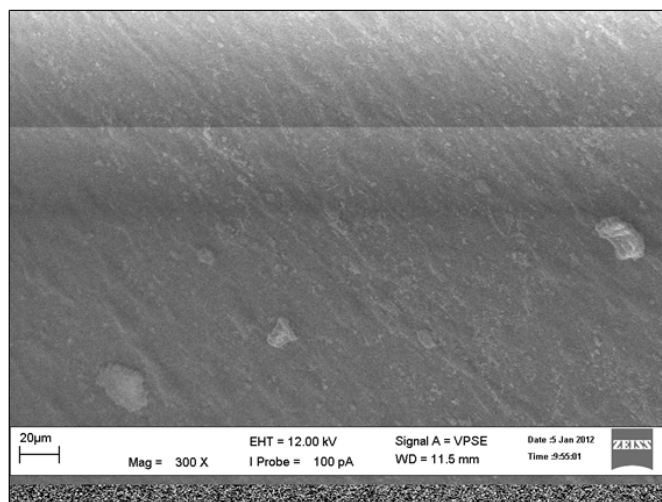


Figure 9: SEM image of biocellulose surface

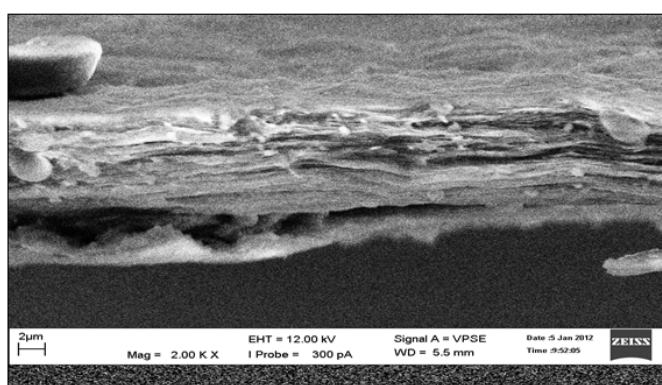


Figure 10: SEM image of biocellulose cross section

CONCLUSION & RECOMMENDATIONS

As a conclusion, the application of mixed medium culture for the enhancement of biocellulose production is a success. A suitable composition ratio of the fructose and glucose in the culture medium as the carbon sources for *Acetobacter xylinum* can lead to a high yield of biocellulose. The mixed medium culture that contains 10% glucose and 90% fructose produce the highest biocellulose yield in the project that is with 1.57 g/l. In other words, it is the optimum medium culture for the enhancement of biocellulose production. Increasing the initial concentration of glucose as the carbon source for the *Acetobacter xylinum* in the culture medium fails to increase the biocellulose productivity because glucose is preferred by the bacteria to be converted to glucosidic acid. It is an interesting characteristic of *Acetobacter xylinum* that it can maximize the metabolism of the carbon sources to biocellulose in the mixed culture medium and the cellulose is synthesized by the bacteria mainly by consuming fructose. Therefore, it also means that biocellulose still can be synthesized when the glucose carbon source in a culture medium is limited.

The characterization of the biocellulose produced from the culture mediums in the project has been done by using FTIR, SEM and water absorption capacity. The FTIR spectra has confirmed that the components produced by the *Acetobacter xylinum* in medium cultures are cellulose with the existing bands that represents the strong bonds of hydrogen bonding, carbonyl groups and C-H stretching that represents the cellulose properties. Then, SEM also shows that biocellulose has unsmooth, non-porous and is the formation of layered sheets. Moreover, all of the biocelluloses produced in the project possess high water absorption capacity and this is also one of the main characteristic of biocellulose. The highest water absorption capacity value belongs to the biocellulose produced from the culture medium with carbon source of fructose alone. Hence, the biocellulose synthesized from the fructose alone possess of stronger structures and contain more microbial fibrils that provides it to have a bigger surface area to hold a higher quantity of water within it. The biocellulose produced from the mixture of 10% glucose and 90% fructose lead to a lower water absorption capacity than the biocellulose synthesized by the fructose alone but it is still acceptable to be commercialized as there is only a slightly difference.

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