# Kinetics study of bacterial cellulose production by *Acetobacter xylinum* FNCC 0001 with variation of carbon sources

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**Abstract.** Glucose and fructose (2.0% (w/v)) were used as the carbon sources and peptone was used as nitrogen source (1.5% (w/v)) in order to determine the kinetics study and yield of bacterial cellulose production. Coconut water was prepared as the fermentation medium. Data of the fermentation rate was observed at 19 points of certain time during 7 days of fermentation process. The number of cell biomass was calculated using cell dried weight method, total plate count method and optical density method which was observed at 660 nm. The remaining media substrate was analyzed using the DNS method, while the BC yield was determined by the gravimetric method. The results showed that the best values of μ, Td, Rx, Rs, Rp, Yp/s, Yp/x were achieved by fructose, while the best n and Yx/s value were achieved by glucose. The corresponding values were 0,1141/h, 6,0737 h, 0,1141 g/L/h, 0,0635 g/L/h, 0,3015 g/L/h, 0,317 g/g, 0,5927 g/g 1,3631 times and 0,0983 g/g respectively. The highest BC yield at the end of fermentation process was achieved by glucose carbon at 5.83 g/L, followed by fructose at 4.91 g/L.

**Keywords.** Fermentation kinetics, bacterial cellulose, Acetobacter xylinum FNCC 0001, glucose, fructose

## 1 Introduction

Bacterial cellulose (BC) is an extracellular polysaccharide, i.e., cellulose, produced by several genera of bacteria such as Gluconacetobacter (formerly *Acetobacter*), *Aerobacter*, *Agrobacterium*, *Azotobacter*, *Rhizobium*, *Sarcina*, *Salmonella*, *Enterobacter* and *Escherichia*. The gram-negative *Acetobacter xylinum* is claimed to be an effective bacterium in producing BC [1]. BC has several different characteristics from plant cellulose. One of the fundamental differences is the absence of hemicellulose and lignin in BC, making it a highly pure source of cellulose [2]. Another characteristic that distinguishes the two is that BC fibrils have a thickness of 100 times thinner than plant cellulose [3]. In addition, BC also has a higher water holding capacity, hydrophilicity, degree of polymerization, mechanical strength, crystallinity index, and porosity compared to plant cellulose [4]. Due to its unique

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characteristics, BC has been known for its multitude applications in paper, textile, food industries, pharmaceutical, prosthetic applications and other biomedical materials [5-6].

In Indonesia, BC is also well-known as nata de coco and is synthesized by *Acetobacter xylinum* using coconut water and other organic compounds as substrates. This bacterium can grow at pH level between 3.5-7.5 with an optimum pH at 4-5 [1] and can produce BC at temperature of 30-40°C in a static state with an optimum temperature between 28-31°C [7]. In common, nata de coco is produced by Small and Medium Enterprises (SMEs). Some problems have occurred during the production of nata de coco in SMEs, e.g., unstable production rate, yield, and quality of BC. The resulted BC is expected to have good physical qualities, viz. to produce high *yield*, thickness, and water content as well as a chewy texture. A good quality BC should have more than 85% water content to maintain the chewy texture [8].

The availability of nutrients is one of the main factors in controlling the rate of bacterial growth. *Acetobacter xylinum* requires a source of carbon, hydrogen, and nitrogen as well as minerals for its growth. These bacteria can oxidize sugars, sugar alcohols, and ethanol into acetic acid as primary metabolites and produce cellulose as secondary metabolites [1, 6]. Coconut water as the fermentation contained some of the nutrients needed, but to produce the optimal BC rate, macro substrates such as carbon and nitrogen sources must be added [6, 9].

Carbon and nitrogen as the main sources of nutrients have a significant influence on cell proliferation and metabolite biosynthesis [10]. Previous studies reported that peptone is one of the best nitrogen sources as it contains abundant nitrogen components and many growth factors [10-11], whereas glucose and fructose are favorable carbon sources [12-14]. In addition, 15 g/L peptone concentration and 2 g/l carbon concentration were found to produce the maximal BC yield [10, 13].

Each bacterial culture has a specific growth rate and nutrient concentration limit that can be measured based on the entire cycle of the growth curve and the relationship between them is called growth kinetics [15-17]. Determination of fermentation kinetics was carried out to determine the efficiency of substrate utilization in the fermentation process and thus can be used to characterize the growth of microorganisms [18-19]. This study aims to determine the kinetics of BC production in the two variations of carbon sources for *Acetobacter xylinum* FNCC 0001 to obtain optimal BC production. The results of this study are expected to help SMEs to minimize production problems, viz. the instability of the production rate and quality of BC produced by providing information that can be used as a reference in the production process.

## 2 Materials and methods

### 2.1 Microorganism and cultures condition

*Acetobacter xylinum* FNCC 0001 agar slant was obtained from Food and Nutrition Culture Collection (FNCC) Division, Gadjah Mada University. Hestrin-Schramm (HS) medium containing 2.0% D-glucose, 0.5% peptone; 0.5% yeast extract; 0.27% Na2HPO4 and 0.115% citric acid in distilled water with pH adjusted to 5 [20] and sterilized at 121°C for 15 minutes [21] was used as the culture growth medium. Stock culture was inoculated into 50 ml of sterile HS medium in a 250 ml conical flask and incubated for 48 h at 30°C under static conditions [12]. This culture was then used as the seed culture.

## 2.2 Fermentation in an artificial medium.

Coconut water as a fermentation medium went under a sorting process based on the total dissolved solids. The total dissolved solids of coconut water were tested using a hand refractometer and referred to 4.23±0.25 °Brix [22]. Glucose (F1) and fructose (F2) (2.0% (w/v)) were added as the carbon sources and peptone (1.5% (w/v)) as the nitrogen source was added to each formulation. The starting pH in each formulation was adjusted to 5 and sterilized at 121°C for 15 minutes. After it was cooled to 28±1°C, the formulated medium was transferred to a sterilized 100 ml plastic containers (each contained 40 ml medium) aseptically in a laminar flow followed by the inoculation of 5% (v/v) seed culture which contained at least $\pm 2 \times 10^7$ cells/ml [12]. Fermentation was carried out for 168 h at 30°C under static conditions and was sampled for analysis at 19 observation points with different time intervals, namely every 2 hours during the first 12 hours, then every 3 hours at the $12^{th}$-$24^{th}$ hour, every 8 hours at the $24^{th}$-$48^{th}$ hour, and every 24 hours at the $48^{th}$-$168^{th}$ hour.

## 2.3 Analytical methods

### *2.3.1 Biomass*

Calculation of the cell biomass was carried out in order to observe the growth curve by combining the results of the optical density method, total plate count (TPC) method and the cell dried weight (CDW) method. The cell concentration was estimated by measuring the optical density at 660 nm [23] which was then compared to the TPC result by a standard curve to get a total cell/ml. The TPC method was conducted in HS-agar medium using spread plate method [24] and the CDW method was carried out to determine the dry weight of cells using centrifugation and gravimetric methods [21]. The CDW data combined with total cell/ml were used to make a standard curve to get g/L biomass results at every observation point.

### *2.3.2 Sugar concentration in media*

Substrate residue or reducing sugar concentration in media was analyzed using 3,5-*dinitrosalicylic acid* (DNS) method. This test was conducted by adding 1 ml DNS reagent to a 1 ml sample (fermentation medium) in the test tube. The mixtures were heated in boiling water for 5 minutes and then cooled under running tap water to ambient temperature. The color intensities were measured by spectrophotometer at 575 nm [25].

### *2.3.3 Bacterial cellulose (BC) yield.*

BC produced during the fermentation process was purified by boiling in 0.1 M NaOH at 80°C for 20 minutes to remove residual microorganisms followed by filtration using filter paper and rinsing with distilled water until the pH of the water becomes neutral [13]. The purified BC was then dried overnight to constant weight at 105°C [12].

### *2.3.4 Fermentation Kinetics.*

Determination of fermentation kinetics was carried out to determine the efficiency of substrate utilization in the fermentation process. The parameters used are X which is the weight of cell biomass (g/L), S is the remaining substrate (g/L) and P is the BC *yield* (g/L). The calculated parameters and their formulas as follow [18] :

Growth rate or biomass formation (Rx) = (g/L/h) = (dx/dt) (1)
Substrate use rate (Rs) = (g/L/h) = (ds/s) (2)
Growth rate BC (Rp) = (g/L/h) = (dp/s) (3)
Ratio of biomass to substrate (Yx/s) = (g/g) = (X-Xo)/(So-S) (4)
Ratio of BC yield to substrate (Yp/s) = (g/g) = (P-Po)/(So-S) (5)
Ratio of BC yield to biomass (Yp/x) = (g/g) = (P-Po)/(Xo-X) (6)
Maximum growth rate per hour (μ max) = slope of ln X = f(t) (7)
Specific rate of formation of BC per hour (σ) = slope of ln P = f(t) (8)

# 3 Results and discussion

## 3.1 Biomass

Changes in the growth phase of *Acetobacter xylinum* FNCC 0001 in both variations of carbon sources can be seen in **Fig. 1**. A typical bacterial growth curve during batch culture shows distinct phases of growth: lag phase, exponential or logarithmic (log) phase, stationary phase and death phase [26]. **Fig. 1** showed the lag phase, log phase and stationary phase of *Acetobacter xylinum* FNCC 0001, while the death phase has not been seen. The initial phase is the lag phase. This phase begins shortly after the inoculation of seed culture into the fermentation medium. In this phase, growth does not occur because new cells are adapting to environmental conditions, but the cell size increases and begins to prepare for the growth and reproduction process so that the amount of cell biomass will tend to remain [26-27].

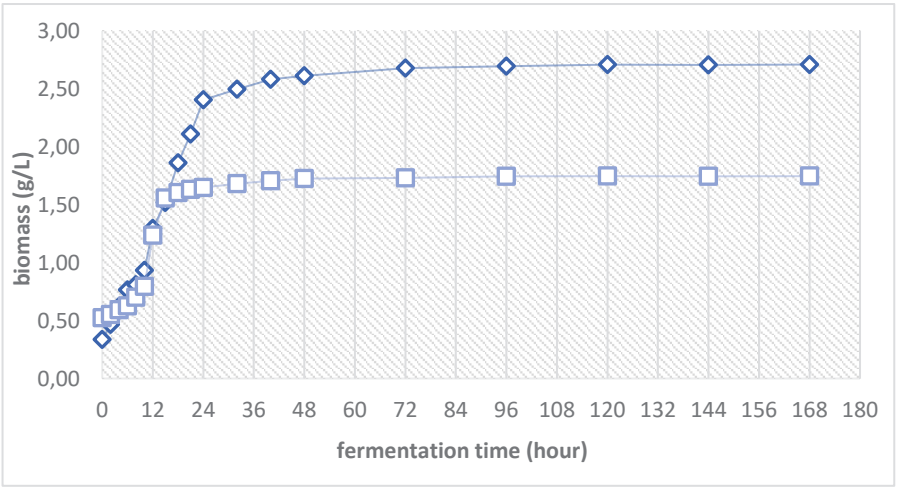


**Fig. 1.** The Growth Curve of *Acetobacter xylinum* FNCC 0001 in Two Variation of Carbon Sources. (Glucose (□) and Fructose (△))

After the lag phase is completed, *A. xylinum* will enter the log phase. This log phase is defined as a balanced growth stage because bacterial biomass increases linearly with time, with the number of bacterial cells doubling per unit time [27]. The lag phase for both formulations (F1 and F2) occurred at the first 10 hours, followed by the log phase starting from the $10^{th}$ h - $24^{th}$ h for glucose carbon (F1), whereas the length of the log phase for fructose carbon (F2) was quite short from $10^{th}$ h - $15^{th}$ h.

The log phase length obtained in this study was shorter than the reported previous studies. The log phase of A*cetobacter xylinum* with Hassid-Barker medium ended at 48 hours [18]

while the log phase of *A. xylinum 0416* with HS medium started after the 3rd day [28]. In this phase, the growth medium is consumed maximally. All nutrients in the fermentation medium will be used by cells as the population grows. This resulted in the accumulation of toxic metabolites and waste materials in the media, therefore the growth rate of bacteria will experience a slowdown and enter a stationary phase [28-29].

The stationary phase of F1 started from 24th h and F2 started from 15th h until the fermentation was completed at 168th h. The stationary phase is the phase with the highest number of cell populations where the number of bacteria that die and grow is balanced so that there is no growth rate [30]. In this phase, bacteria will produce more secondary metabolites than primary metabolites so that BC will be produced optimally in this phase [31]. After passing a certain period time, bacteria will enter a death phase where the number of living cells will undergo lysis due to accumulation of inhibitors of metabolic products, depletion of cell energy and changes in pH [27].

**Fig. 1.** showed that the death phase did not occur until the fermentation time is completed. The death phase usually occurs from the 8th day to the 15th day [32]. A reported study showed that the death phase occurred starting on the 16th day of BC production with *A. xylinum 0416* grown in HS medium [28]. Overall, the two formulations growth curves showed an increase in the total dry cell biomass. From the results of the research conducted, it can be seen that the highest amount of cell biomass was achieved by glucose carbon at 2.7093 g/L followed by fructose at 1.7462 g/L).

### 3.2 pH analysis on the fermentation medium

The initial pH value of the fermentation medium was adjusted until the pH value reached 5. The pH value was chosen referring to several studies that stated the optimum pH for producing BC in a static state is 5 [13, 33]. It can be seen that for the first 12 hours, the pH of the fermentation medium on both formulations did not change significantly. This is because the bacterium *Acetobacter xylinum* FNCC 0001 has just entered the lag phase where the bacteria are still in the adaptation stage to their environment [27].

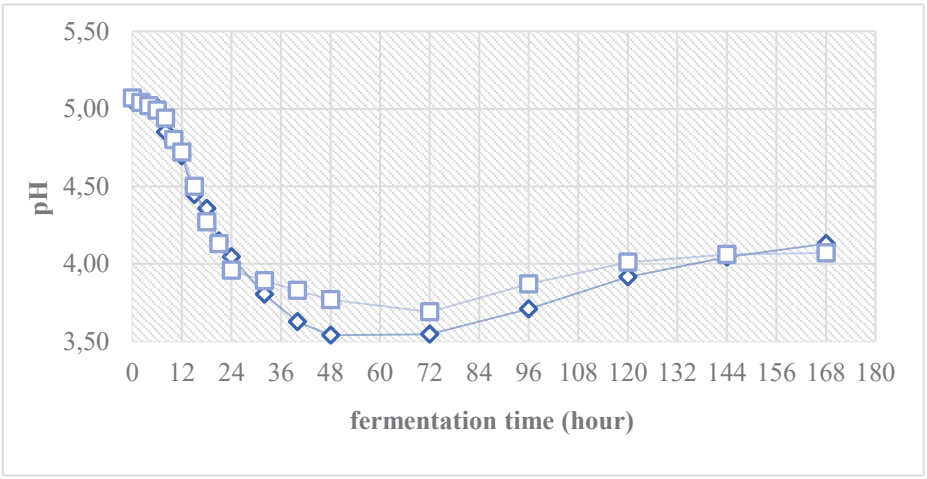


**Fig. 2.** Changes in The Fermentation Medium's pH during Incubation (Glucose (□) and Fructose (△))

The pH of the medium then decreased until 72nd h for both formulations followed by an increase in pH until the end of the incubation period (168 hours). The decrease in pH was because bacteria starting to enter the log phase. In this phase, there is an increase in the rate of bacterial growth and the production of primary metabolites, namely (keto) gluconic acid

from the breakdown of carbon sources by bacteria with the help of enzyme phosphoglucoisomerase which is characterized by a decreasing pH value [34-35], while the increase in pH was due to the oxidative decarboxylation of gluconic acid. Glucose metabolism will result in the accumulation of gluconic acid along with a decrease in pH. When all the glucose in the medium has been oxidized, bacteria begin to metabolize gluconic acid to obtain an energy source and a gradual increase in pH can be seen [36].

### 3.3 BC yield

During the fermentation process, bacteria *A. xylinum* will oxidize carbon in the media and produce primary metabolites in the form of (keto) gluconic acid and acetic acid as well as secondary metabolites in the form of cellulose or called *bacterial cellulose* (BC) [18]. Glucose and fructose carbon can be directly entered into the carbon metabolism pathway by *A. xylinum*. The process begins with the catalysis of glucose by *glucokinase* into glucose-6-phosphate. Glucose-6-phosphate is then isomerized to glucose-1-phosphate by the enzyme *phosphoglucomutase*, then converted to UDPG-glucose by UDPGlc *pyrophosphorylase* and ends with the formation of BC by *cellulose synthase* [37].

Each cell of *A. xylinum* can polymerize up to 200,000 glucose molecules per second into 1,4-glucan chains. The 1,4-glucan chain will be released into the media through the cell membrane and form a series of cellulose microfibrils that will bind to each other to form a cellulose layer on the surface of the medium [38]. BC usually starts to appear on the surface after 2 days of incubation and the number will continue to increase until the 8th day [34]. Cellulose formed on the surface of the medium will help *A. xylinum* in the metabolic process and glucose synthesis by supplying the oxygen need.

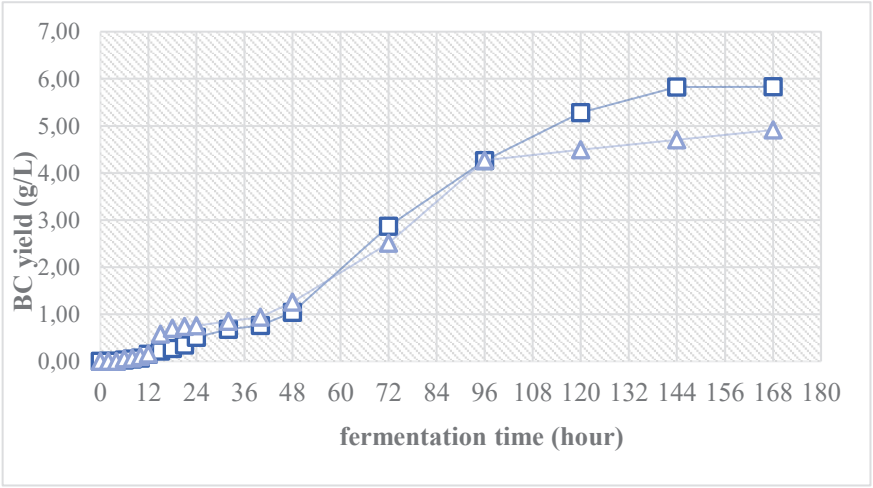


**Fig. 3.** BC Yield during Fermentation Time (Glucose (□) and Fructose (△))

BC yield in this study began to form at 6 hours in both formulations. Based on **Fig. 3.,** it can be seen that the amount of BC yield continued to increase until the end of the fermentation. At 168$^{th}$ h, the highest BC was produced by glucose carbon at 5.8292 g/L, followed by fructose at 4.9125 g /L. The BC yield obtained in this study is higher than previous studies [13-14, 34].

### 3.4 Relationship between Total Cell Biomass, pH and BC Yield

The bacterial growth curve is a graph of the relationship between the number of living cells and the unit of time. The normal bacteria growth curve has four stages: the lag phase, log phase (exponential), stationary phase and death phase [27]. These phases usually occur in batch culture in a closed environment, where needed nutrients are depleted over time and metabolic products begin to accumulate due to bacterial growth. In each phase of bacterial growth, different biochemical reactions will occur for the synthesis of cellular components needed for bacterial cell growth and division [27]. The difference of these reactions can be seen through the relationship between cell biomass data, pH and BC yield [18].

The lag phase is the initial phase of bacterial growth, where new bacteria adapt to environmental conditions. In this phase, bacteria will prepare themselves for the process of growth and reproduction by synthesizing needed cellular components such as DNA and cellular enzymes [26-27]. The lag phase in the study occurred at the first 10 hours for both carbons. It can be seen in **Fig. 1**, **Fig. 2** and **Fig. 3** that the values of cell biomass, pH and BC yield at these hours tend to be constant. This is consistent with the theory that in the lag phase bacterial growth has not yet occurred, but the cell size will increase so that the amount of cell biomass will tend to remain and bacteria have not yet produced primary metabolites, gluconic acid and secondary metabolites, BC [26, 34].

After the lag phase is completed, the bacteria will enter the log phase which is a balanced growth stage because the average cell composition remains constant with the bacterial culture properties (protein and DNA) increasing at the same rate [39]. The lag phase in the study occurred from $10^{th}$ - $24^{th}$ h for glucose and $10^{th}$ – $15^{th}$ h for fructose. A clear increase in biomass at the same points can be seen in **Fig. 1** and **Fig. 2**. pH value decreased rapidly starting from the $10^{th}$ h for glucose and fructose while at the same points, the increase in BC yield has not been seen (**Fig. 3**). This is in following the theory, that in the log phase, bacteria will produce more primary metabolites (gluconic acid) than secondary metabolites (BC) so that the decrease in pH can be seen [27, 35].

After the log phase, the growth rate of bacteria will experience a slowdown and enter a stationary phase. The stationary phase in the study started from $24^{th}$ h for glucose carbon, from $15^{th}$ h for fructose until the incubation time was complete (168 h). The amount of cell biomass at the same hour (**Fig. 1**) showed a value that tends to be constant and simultaneously there was an increase in BC yield (**Fig. 3**). Then in **Fig. 2,** it can be seen that the pH began to decrease which was followed by an increase in the value of pH until the end of the fermentation time.

Carbon metabolism will result in the accumulation of gluconic acid along with a decrease in pH. When all of the glucose in the medium has been oxidized, bacteria begin to metabolize gluconic acid to obtain an energy source through the oxidative decarboxylation process, where the breakdown products can also be used for BC formation and an increase in the pH value can be seen [36]. The results obtained are by the theory that the number of bacteria that dies and grows is balanced in the stationary phase so that there is no growth rate [30]. In addition, in this phase, the bacteria will produce more secondary metabolites than primary metabolites hence BC will be produced optimally [31].

After passing a certain period, the bacteria will enter the death phase. In this study, the death phase of the bacteria *Acetobacter xylinum* FNCC 0001 has not been seen. At the death phase, the number of living cells will undergo lysis due to the accumulation of inhibitors resulting from bacterial metabolism, depletion of cell energy and changes in pH [27]. The amount of cell biomass in this phase will decrease so that the growth rate will show a negative value [30] and the amount of BC yield will tend to remain because carbon metabolism will stop [34] whereas pH value tends to decrease because in the death phase of acetic acid bacteria, cell wall lysis will occur therefore intracellular acid is going to be released into the media and increasing acidity [40].

### 3.5 Fermentations Kinetics

The calculation of the fermentation kinetics of *Acetobacter xylinum* FNCC 0001 was used to determine the cell growth rate, the rate of substrate utilization and the rate of product formation/BC yield. These three data will provide information on how the fermentation process can be carried out efficiently. Parameters observed in the calculation of fermentation kinetics include specific growth rate (μ), doubling time (Td), degree of multiplication (n), rate of biomass formation (Rx), rate of substrate use (Rs), rate of BC yield formation (Rp), ratio of biomass to substrate (Yx/s), ratio of biomass to BC yield (Yx/p), ratio of BC yield to substrate (Yp/s), maximum specific growth rate (μ max), and rate of formation of specific BC yield (σ). In calculating the parameters above, data are needed in the form of residual substrate/sugar residue in the fermentation medium, the amount of biomass contained in the media, and the amount of BC yield produced. The graph of the relationship between the three data can be seen in Fig. 4 and Fig. 5.

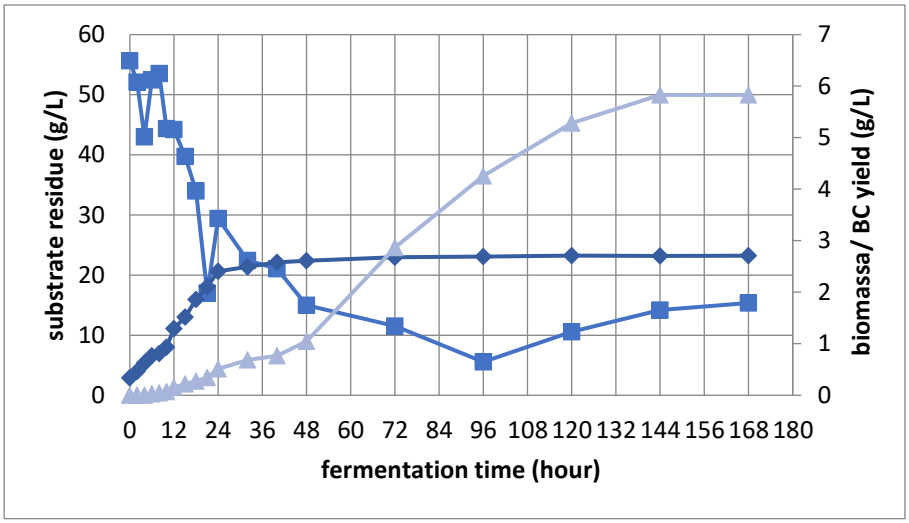


**Fig. 4.** Relationship of Cell Biomass, Substrate Residue and BC Yield during the Fermentation Time for Glucose Carbon (Substrate Residue (□), Cell Biomass (◊) and BC Yield (△)).

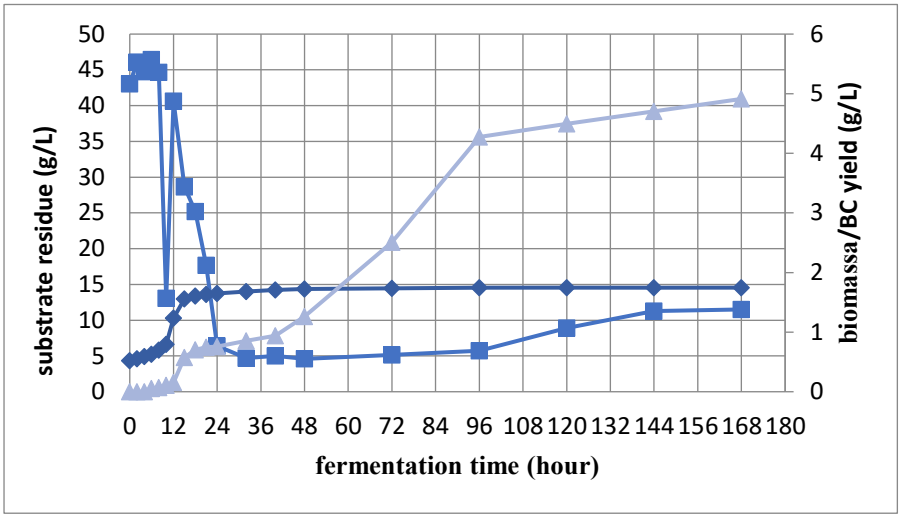


**Fig. 5.** Relationship of Cell Biomass, Substrate Residue and BC Yield during the Fermentation Time for Fructose Carbon (Substrate Residue (□), Cell Biomass (◊) and BC Yield (△))

Based on Fig. 5, it can be seen that the biomass data and BC yield tend to increase during fermentation process and are inversely proportional to the remaining reducing sugar data. The data for the remaining reducing sugars or substrate residue inclined to decrease. This is because the sugar in the media will be increasingly used for metabolism by *Acetobacter xylinum* as an energy source for cell growth and as a substrate for BC formation [8].

**Table 1.** Fermentation Kinetic Parameters of *Acetobacter xylinum* FNCC 0001

| Kinetics Parameter | Formulation | | Unit |
|---|---|---|---|
| | Glucose (F1) | Fructose (F2) | |
| μ | 0,0675 | 0,1141 | Per h |
| Td | 10,2648 | 6,0737 | h |
| | 615,8894 | 364,4241 | minutes |
| n | 1,3631 | 1,1518 | Time |
| Rx | 0,0675 | 0,1141 | g/L/h |
| Rs | 0,0294 | 0,0635 | g/L/h |
| Rp | 0,1409 | 0,3015 | g/L/h |
| Yx/s | 0,0983 | 0,0534 | g biomass/g sugar |
| Yp/s | 0,0296 | 0,0317 | g BC/g sugar |
| Yp/x | 0,3016 | 0,5927 | g BC/g biomass |
| μ max | 0,0631 | 0,1220 | Per h |
| Σ | 0,1232 | 0,2985 | Per h |

In the parameters of specific growth rate (μ) and maximum specific growth rate (μ max) F2 obtained the highest results at 0.1141/h and μ max at 0.1220/h, followed by F1 at 0.0675/h and μ max at 0.0631/h. The value of the specific growth rate (μ) obtained will be the same or close to the value (μ max). The maximum specific growth rate (μ max) is calculated by finding the slope of the biomass ln with time during the logarithmic phase. Cells will have a maximum growth rate (μ max) if there is an excess of substrate in the medium (Stanbury et al., 2015). The fastest doubling time (Td) was also achieved by F2 at 6.0737 h, followed by F1 at 10.2648 h. Td value is the time required for cells to double the number of cell populations during the logarithmic phase. The greater the value of μ, the lower the Td value obtained so that the required cell doubling time is faster [41].

The highest degree of multiplication or the number of doublings (n) was achieved by F1 at 1.3631 times, followed by F2 at 1.1518 times. n value serves to find the number of cell multiplication during the fermentation process. This value is obtained by dividing the amount of cell biomass at the end of the log phase (x) by the amount of cell biomass at the beginning (xo) [41].

Table 1 showed the best values of Rx, Rs, Rp, Yp/s, Yp/x were achieved by F2, while the best Yx/s value was achieved by F1. The corresponding values were 0,1141/h, 6,0737 h, 0,1141 g/L/h, 0,0635 g/L/h, 0,3015 g/L/h, 0,317 g/g, 0,5927 g/g and 0,0983 g/g respectively. The values of Yx/s and Yp/s showed that in F2, most of the substrate tends to be used for BC formation rather than for biomass growth, while in F1 most of the substrate was used for biomass growth rather than for BC formation.

Based on the results of the research above, it can be seen that the overall treatment of carbon fructose has the best results for all parameters except for parameters n and Yx/s. When viewed from these results, the relationship between the parameters is in accordance with the theory, where a high specific growth rate (μ) value and a low Td value are expected to have high activity in product formation [41]. This can be seen in the Rp value for F2 which also showed high yields.

The Yx/s parameter by F1 was higher at 0.983 g biomass/glucose followed by F2 at 0.0534 g biomass/glucose. This is in accordance with the results of biomass in the study, where the cell biomass produced in F1 was higher than F2. However, there is a difference if we look at the relationship between the Rp and Yp/s parameters. In reference to those kinetic parameters, F2 should have the highest BC yield, whereas in the study the highest BC yield at the end of fermentation process was achieved by glucose carbon at 5.83 g/L, followed by fructose at 4.91 g/L.

The parameters of the fermentation kinetics used data on the logarithmic phase of bacteria. The carbon fructose variation has the best results on most of these parameters because it enters the log phase faster than the glucose and also has the lowest doubling time in that phase. These results are similar to previous study [14] where fermentation using fructose medium, resulted in short log phase and it can be seen that the initial biomass increase in fructose is very rapid compared to glucose. However, at the end of the fermentation, fructose medium produced the least biomass and yield.

There are several possible explanations such as bacteria must use isomerase to convert fructose to glucose before polymerization begins which will lead to greater energy requirements due to enzyme synthesis, resulting in lower BC production. Another possible explanation is that the bacteria initially grew on glucose (HS medium) which was then inoculated on the culture medium with fructose so that it could trigger a negative effect on the activity of the fructokinase (FK) [14].

Many studies on the use of various types and concentrations of carbon in BC production have been carried out. Some of these studies include: *Acetobacter xylinum* KJ-1 was cultured in the medium using 2% carbon concentration for 5 days and produced the highest BC yield using fructose at 2.7 g/L, followed by glucose at 2 g/L [13]. *Komagataeibacter medellinensis* using the same carbon concentration for 8 days fermentation produced the highest BC at 2.8 g/L using glucose, and at 0.38 g/L using fructose [14] and *Acetobacter* sp. A9 was cultured for 7 days produced the highest BC using glucose, followed by fructose at 2.7 g/L and 2.53 g/L respectively [12]. Based on these studies, the optimum BC yields obtained varied even though the carbon concentration used was uniform. The difference in yield was largely due to different bacterial strains, fermentation time, and other environmental factors. This is because, each microorganism has its own preferences and physiological characteristics in selecting the source of the components it needs.

This study used *Acetobacter xylinum* FNCC 0001 obtained from isolated cultures in Indonesia with artificial media of coconut water as it is in line with the most common BC

product in Indonesia which is nata de coco, using coconut water as its substrate added with carbon and nitrogen sources. We are hoping the result of this study could help SMEs as nata de coco supplier to minimize their production problems, viz. the instability of the production rate and quality of BC produced by providing information that can be used as a reference in the production process. In addition, the results of this study on growth curve, specific growth rate, nutrient concentration limit, and other fermentation kinetic parameters can be used as new information that can be developed for further research for this strain. The use of other carbons or combination of carbons should also be further investigated.

## 4 Conclusion

The results showed that the highest cell biomass and BC yield was achieved by F1 at 2.71 g/L and 5.83 g/L, followed by F2 at 1,75 g/L and 4.91 g/L respectively. F2 has the best values of μ, Td, Rx, Rs, Rp, Yp/s, Yp/x, while the best n and Yx/s values were achieved by F1. In reference to cell biomass, BC yield and kinetic parameters results, the F1 formulation with 2 % (w/v) glucose and 1.5% (w/v) peptone was the best for bacterial growth.

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