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The Kinetics both of Growth and Metabolite Production of *X.campestris* Using of 4% Liquid Sugar Substrate from Cassava Hydrolisate

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Abstract

X. campestris is aerobic bacteria producing extracellular biopolymers (EPS, extracellular polysaccharide) known as xanthan gum. To determine the technology and the process conditions appropriate to the formation of this product, therefore the understanding of both the kinetics of growth and metabolite production of bacteria is needed. In this research, to assess the growth of *X. campestris* using the method of calculation of dry cell weight. For determining the kinetics of production of metabolite used substrates of 4% liquid sugar from cassava starch hydrolysate. From this research was showed that *X. campestris* maximum growth in NB medium obtained in about 58thhour, at the growth rate of about 0.04 g / hour, stationary phase obtained at the 60th hour with a maximum dry cell weight of 2.7688 g/L and specific growth rate (μ) of *X. campestris* amounted to 0.043 hour$^{-1.}$ Based on the kinetic curves both on growth and its metabolite production, *X.campestris* has non-growth associated product pattern. In this case the production of xanthan gum occured after cell growth stopped then its product is a secondary metabolite with highest amount of 3.73 g / L at 102nd hour, ie the 4th day of fermentation. Overall of this research indicated that Nutrient Broth (NB) may be used for the growth of *X. campestris*. But based on the value of μ above, the rate of cell reproduction was still low. Liquid sugar can be used as a substrate to produce xanthan gum. However to increase its productivity, there should be an addition of other carbon or energy and nitrogen sources.

INTRODUCTION

X. campestris belongs to the member of aerobic bacteria from the family of *Xanthomonadaceae.* Its secondary metabolite product is known as xanthan gum. Xanthan gum is chemically known as one of carbohydrate polymer (carbohydrate biopolymer) which is widely used in different field of industry such as foods, pharmacy, cosmetics, farming, paints, inks, textiles, ceramics even medics and mining field (Freitas et al., 2011; Petri, 2015).

Up to now, various studies about *X. campestris* is only focusing on the optimization of metabolite production using several substrates and the macro nutrients variation such as carbon and nitrogen sources.

Common problem encountered in batch fermentation is related to the maximum growth of *X. campestris*. It is due to, some substrates have been transformed into products and intermediates. These things are important since it involves processing time. Therefore, kinetic of the process is an important information required in this study. Understanding of the kinetics both of *X. campestris* and its metabolite production is necessary to determine the right technology and process condition strategy

To assess the growth of *X. campestris*, it is necessary to evaluate its cell population wth

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appropriate techniques. Commonly used method is calculation of cell dry weight. Furthermore, the addition and growth of cells in batch can be described in the form of growth curve which shows the phase of growth gradually from the beginning to stop holding activity. From the exponential grwoth model $dX/dt = \mu X$ and specific growth rate (μ) we can obtain rate of both on growth and reproduction of *X. Campestris*.

In bioprocess, in addition to cell monitoring (biomass) simultaneously during the process also measurements of the resulting product and the substrate used. The relationship of growth kinetics and the production of metabolites depends on the role of products in cell metabolism. According to Mangunwidjaya et al. (1994), three known patterns of association are known, ie growth patterns associated with product formation, product formation patterns not associated with growth and mixed patterns of associated growth and unrelated. Based on the above, the results of the experiment which are the amount of biomass, the resulting product and the character *X.campestris* can be determined either qualitatively or quantitatively. Overall from the above understanding will be used to achieve the ultimate goal of maximizing production and product concentration.

In this research, liquid sugar syrup from cassava flour processing is used as a substrate because it can shorten the xanthan gum production process chain. In this case there is no need for hydrolysis as occurs when using a source of raw material from long chain carbohydrates such as starch flour or bagasse. In addition, the use of liquid sugar as a substrate is one of the efforts to diversify and expand the utilization of liquid sugar because liquid sugar is only used in the food and beverage industry with the main function as a sweetener.

METHODS

X. campestris Culture Preparation

At this stage, X. campestris was regenerated on a slant nutrient agar (NA) + glucose aseptically. Regeneration was done once a week continuously. Cultures were stored in incubators at 28-30 ° C for 3-4 days, then stored in microbial cabinets at room temperature.

X.campestris Inoculum Preparation in Nutrient Broth (NB)

At this stage *X. campestris* was inoculated into 100 ml of sterile NB (nutrient broth) medium containing 0.3 g beef extract, 1 g of peptone, 0.5 g NaCl, and 0.5 g glucose, incubated at 25 ° C, 150 rpm for 24 hours. Furthermore, this inoculum was used to determine the growth kinetics and metabolite production of *X.campestris*.

Making *X.campestris* Growth Curve Using Dry Cell Method (g / L)

At this stage first prepared and weighed each empty microcentrifuge tube using the analytical balance. For the measurements, 1.5 ml of liquid culture from the microbial growth medium were put into centrifuge tubes and centrifuged at 12,000 rpm. After separated between the liquid and its precipitate, the precipitate-filled tube was dried in an oven at a temperature of 55-60 ºC and obtained dry cell. Then it weighed until the weight of the dry cell (X, g / L) was stable. This sampling was done every 2 hours until obtained curve and reached stationary phase. Next plot all the dry cell weight data (X, g / L) above to the sampling (hour to-) to obtain the growth phases. To determine the rate of growth was then made a graph between X to the sampling (hour to-). To determine the specific growth rate of *X. campestris* was to convert the value of X to ln X. Then made a graph between the ln X of the sampling (hour-) to obtain tg α = μ, the specific growth rate μ can be determined.

Making Metabolite Production Curve of *X. campestris*

At this step 85% liquid sugar diluted to 4% as much as 250 mL.the use of 4% liquid sugar was based on the research Djenar et al. (2015). Next to the 4% liquid sugar added 0.1% KH_2PO4 (w/v). The pH of the solution was adjusted to 7, and sterilized at 121^0C, 1.4 atm for 15 minutes, then this solution is referred as a production medium. Into the production medium was added *X. campestris* inoculum as much as 10% of its total volume (Stanbury et al., 1984). Batch fermentation process carried out at a temperature of 28 ± 20 C, 250 rpm for 120 hours. During the fermentation the sampling done every 2 hours to calculate the biomass (g / L) and the product of *X.campestris* (g / L). From the obtained data a curve between product (P, g/L) as a function of sampling time (hour-)was made. Furthermore, the relationship pattern between growth rate and the metabolite production curve of *X.campestris* can be determined.

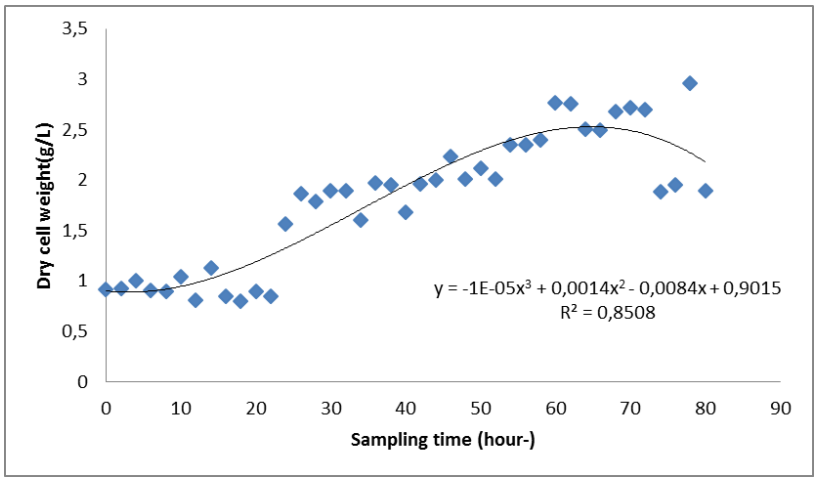

Figure 1. Exponential growth curve of *X. campestris.*

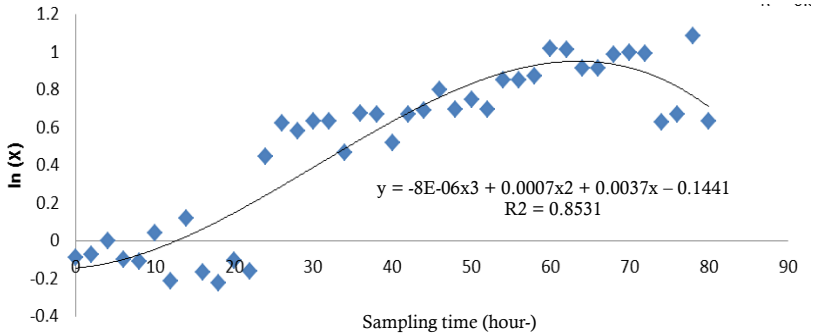

Figure 2. Relationship curve between ln (x) as a function of sampling time

RESULTS AND DISCUSSIONS

Based on the research method above, the first step in this research was *X.campestris* inoculation in NB (Nutrient Broth) at 25°C for 24 hours. Then proceed with determining the maximum growth rate by sampling every two hours. Therefore, the exponential growth curve of *X.campestris* as shown in Figure 1.

Based on Figure 1 it can be seen that *X.campestris* requires adaptation time to growth medium, in this case nutrient broth (NB) about 22 hours (11 th sampling) at 28^{0}C. According to Gilani et al (2011) *X. campestris* requires adaptation to the environment around 24-48 hours, using YMB (yeast malt broth) medium. Furthermore, *X. campestris* has begun to adapt to its environment, which can be observed from a significant increase in cellular or biomass weight. Maximum cell growth was obtained at about 58th hour, with a growth rate of about 0.04 g / hr. In this condition the growth rate reached the maximum and there was a logarithmic or exponential growth.

X.campestris dry cell weight reached a maximum of 2.7668 g / L at about 60th hour, in this stationary phase there is generally a decrease in nutrient content and modification of cell biochemical structure causing its growth rate to stop. The decreasing phase is characterized by a decrease in the number of living cells (viable) in the medium due to death (mortality) followed by autolysis by cellular enzymes. In this condition also the number of living cells equal to the number of dead cells (Stanbury et al, 1994). Based on reached dry cell weight can be mentioned that the increase in the the dry cell weight has met the average value of the generally yield, ie 1- 10 g / L dry cell (Ochoa et al. 2000). It showed that nutrient broth (NB) can be used as growth medium of *X. campestris.*

Cell reproduction rate can be obtained by determining the specific growth rate (μ) of *X.campestris*, ie changing the value of X to lnX as shown in Figure 2.

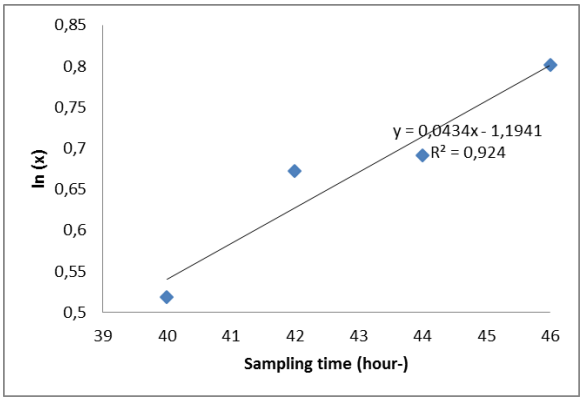

Figure 3. Specific growth rate curve of *X. campestris.*

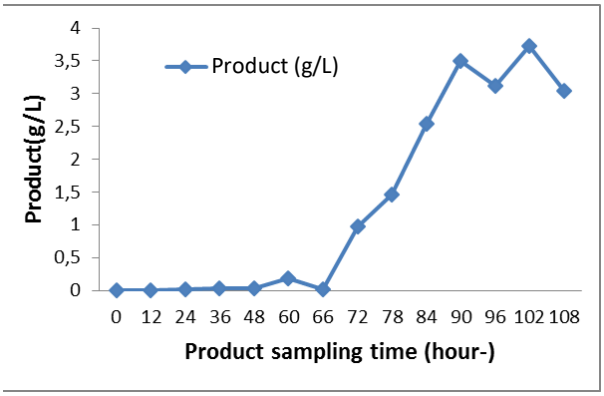

Figure 4. Production curve of *X. campestris* product.

From Figure 2 we obtained a straight line relationship on the exponential phase. Thus obtained a relationship curve between the ln X to t,where the specific growth rate (μ) is the slope as shown in Figure 3.

Based on Figure 3 showed that a linear regression line was Y = 0.043x - 1,194, with R^2 = 0,924, tangent value indicating a specific growth rate (μ) = *X. campestris* of 0.043 $hour^{-1}$. Gilani et al (2011) reported that using YME medium, obtained growth rate specific of *X. campestris* between 0,087-0,089 $hour^{-1.}$ The value of μ from this study was still relatively low, it showed that cell growth rate was still slow, ie the amount of reproduction of cells *X. campestris* was 0.043 grams per hour.

The kinetic relationship between growth and metabolite production depends on the product role in cell metabolism. Two commonly kinetics used are kinetics that describe product synthesis during growth and kinetics that describes product synthesis after growth stops. From this study between the growth kinetics of *X. campestris* with its product kinetics ie xanthan gum has non-growth associated product pattern with its cell growth as shown in Figure 1 and Figure 4.

As shown in Figure 1 the growth of *X.campestris* was decreased after 58 hours and finally stopped. Meanwhile in Figure 4 showed that the bacteria started to produce the product after the 66th hour with its maximum product achievement at 102nd hour, ie the 4th day of fermentation. Based on Fig. 1 and 4, the pattern of product formation in *X.campestris* fermentation occured in the end phase of growth and its rate tends to be proportional to cell concentration rather than with growth rate. Based on these observations it can be mentioned that the xanthan gum product is a secondary metabolite, ie the metabolite produced after cell growth stopped (Freitas, et al., (2011).

As shown Figure 4 *X. campestris* can produce a maximum xanthan gum of 3.73 g / L. Ochoa (2000) in Research Review Paper stated that *X.campestris* fermentation broth contains 10-30 g/L xanthan gum. This showed that not all liquid sugars are converted to xanthan gum but some are used to support its cell growth.

To produce a quality product, the characteristic of *X. campestris* inoculum is very important. In this case the growth medium composition greatly determines the quality of bacterial cells, such as carbon sources, nitrogen, organic acid, minerals, buffers and others. When viewed from the composition of Nutrient Broth (NB) medium consists of beef extract, NaCl and glucose, in this case carbon and energy sources obtained only from glucose. This will affect the quality and productivity of these bacterial cells. Thus it can be mentioned that the medium of NB is good enough for the growth of *X. campestris* as shown in Fig. 1. However to increase its productivity, there should be an addition of other carbon or energy and nitrogen sources such as maltose, starch, peptone, etc.

CONCLUSION

From the result of the research, it can be concluded that the maximum growth of *X. campestris* in the NB medium was obtained at about 58th hour, with its rate of about 0.04 g/h.The stationary phase was obtained at the 60th hour with maximum weight of dry cell of 2.7688 g / L. The value of μ from *X. campestris* was 0.043 $hour^{-1,}$ it showed that kinetic both on the growth of

X.campestris and its metabolite production in NB media was still low.

Based on the kinetic curve both of growth and its metabolite production, *X.campestris* has non-growth associated product pattern. The xanthan gum product is a secondary metabolite of 3.73 g / L at 102nd hour, ie the 4th day of fermentation.

The Nutrient Broth (NB) medium may be used for the growth of *X. campestris*. However to increase its productivity, there should be an addition of other carbon or energy and nitrogen sources.

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