December 2016, Volume 7, No. 6
International Journal of Chemical and Environmental Engineering

Optimization of Pineapple Waste Fermentation Process in Continuous Reactor System

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Abstract
The aim of the present study is to investigate the influence of variations in substrate flow rate and supply O2 in fermentation process by utilizing *Acetobacter xylinum*. The experiments were carried out on a laboratory scale continuous reactor system operated at room temperature , $25^{O}C$-$27^{O}C$, with a pH of 4.5. The parameters analyzed include BOD5, COD, TPC (sel/mL), ash, weight (GSM), fiber length (mm), fines (%) dan cellulose total content. Data analysis showed that those variations influence the nata layer formation. The best result obtained for nata layer is 3 mm with the characteristic of the pulp from nata is cellulose total content values of 62.34%. The values of fiber length and fines are 0.537 mm and 27.25 %, respectively. While the results obtained for removal efficiency of BOD5 is 49.90% and the values of COD is 50%. Treatment variation that gave the best cellulose was at time detention of 12 days with bacteria growth percentage of 80%. The utilization of pineapple waste study as raw material to produce pulp by utilizing *Acetobacter xylinum* has not yet been established. This preliminary research will contribute to improve the quality of nata de pina layer.

Keywords: Continuous reactor system; *Acetobacter xylinum*; Cellulose total content; Fines; Fiber length

1. Introduction

Pineapple is one of the popular fruits as well as leading commodity crops in West Java [1]. One consequence of this pineapple fruit in abundance throughout the year is the increase in organic waste generation. On the other hand, utilization of waste pineapple related efforts to reduce the amount of organic waste generation is still not widely practiced. So this study was conducted as a preliminary study to investigate the potential of pineapple fruit waste as a raw material for making paper.

The process used is the pineapple substrate fermentation using glucose containing *Acetobacter xylinum* bacteria that produce cellulose as the final product. Actually this process may also occur with the used of pineapple waste, because the components required for the growth of *A. xylinum* such carbohydrats, proteins, and trace elements also available in pineapple waste [2]. Accordingly, they can be used as a substrate for the production of cellulose as a raw material for a pure quality paper.

Fermentation research commonly practiced today is in a batch system. But in this study, the type of fermentation process that was carried out is continuous process, an open system was set up. Nutrient solution or the substrate is added to the bioreactor continuously and an equivalent amount of converted nutrient solution with microorganisms is simultaneously taken out of the system [3]. A fermentation process may not be operated optimally for various reasons. For instance, an inappropriate nutrient feeding policy will result in low production yields, even though the level of feed rate is very high. This study will investigate the effect of the influence of the feed rate to produce the optimum fermentation process. It is generally accepted that cellulose synthesis and secretion require highly aerobic condition, besides *A.xylinum* a gram-negative aerobic bacteria [4,5]. Therefore, in addition to the feed rate, the effect of pure oxygen supply to the fermentation process is another thing or object or parameter to be observed. So with the investigation of the optimization of the fermentation process utilized pineapple waste, it is hoped will be a solution to solve the problem of organic waste generation, on the other hand obtain materials that are renewable raw materials for the paper industry.

2. Materials and Method

The bacterial strain of *A.xylinum* that used in this research was obtained from the Laboratorium Mikrobiologi dan Teknologi Bioproses, ITB, Bandung. Further cultivation of the cell *A.xylinum* performed at temperatures of $27^{0}C$ in 100 mL of nutrient broth for 5 days.

The substrate used in this study is derived from pineapple fruit waste samples taken randomly from the

In general, research results obtained for all treatments in the first reactor showed an increase in the number of bacteria *A.xylinum* during fermentation. It can be seen clearly in Figure 1 , that the value of the percentage increase in bacterial Ax growth is the highest obtained by 80 % in P1, while the P3 and P2 , respectively, by 60 % and 52 %. There are two types of growth patterns of bacteria associated with pure oxygen supply. P1 and P2 indicate the presence of bacterial adaptation phase in the beginning of the fermentation process. This is probably caused by the influence of the conditioning period before bacterial fermentation process to obtain stationary phase. At that time did not take place supply pure oxygen. So after the injection into the first reactor flowed pure oxygen, the *A.xylinum* bacteria would adapt prior to the new environment. The color change process is characterized by turbidity occurs on day 4 during 12 days of fermentation process for P1 and day 3 for 5 days fermentation in P2. This could happen even though it is widely known that the *A.xylinum* bacteria an obligate aerobic [5]. While P3 showed bacterial growth curve that had no adaptation phase, but continues to increase exponentially. This occurs because the bacteria do not need to adapt to the environmental conditions which are not much different from the conditioning period prior to the fermentation process. Discoloration occurs on day 2 for 5 days fermentation process.

In contrast with the growth of bacteria, the BOD_5 value decreased during the fermentation time for all treatments. The value of the highest BOD_5 removal efficiency, obtained on a variation of P3, is 72.6%. While the value of BOD_5 removal efficiency for variation of P2 and P1 respectively 65.3% and 49.9%. This indicates that the growth of the bacteria is to utilize the glucose contained in the substrate. As described in [8] that the first stage of the mechanism of formation of cellulose is preceded by an increase in the number of bacterial populations by taking dissolve oxygen and a higher metabolization of glucose in the substrate for to produce a certain amount of microfibril in the entire liquid phase as observed by the appearance of turbidity.

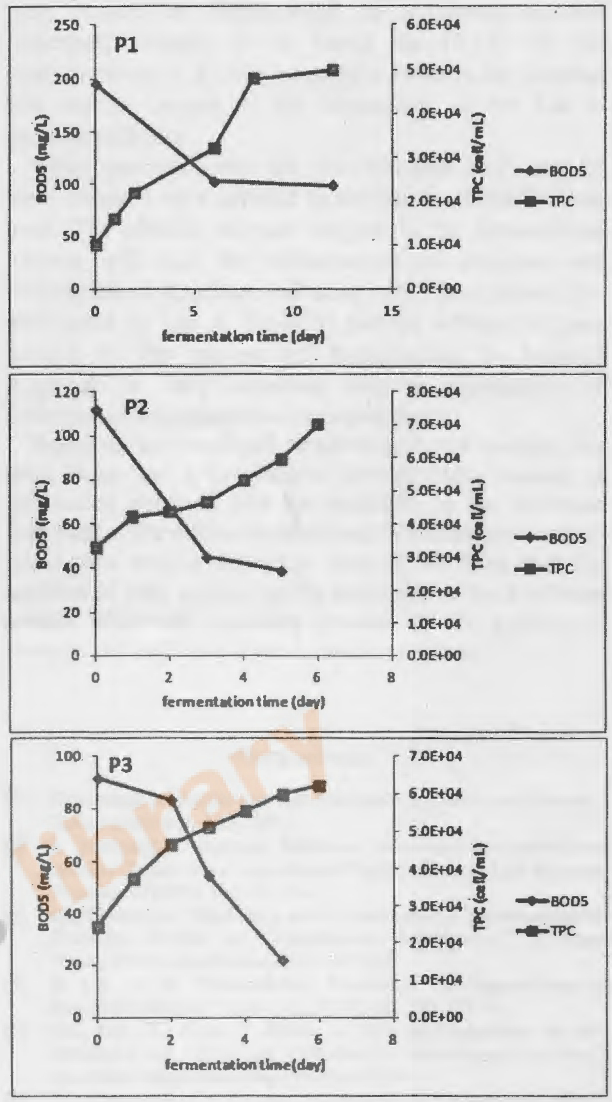

Figure 1. BOD5 and TPC measurement results.

Furthermore microfibril formed in the first reactor will be flowed on the second reactor . It is expected that in calm conditions the second stage process of the formation mechanism of cellulose can occur , namely a white pellicle appears on the surface and its thickness increase steadily with the time a long fermentation time . In the second reactor is not given pure oxygen supply. So that in the process of the growth of aerobic bacteria gel cellulose generated only in the vicinity of surface area and maintain their activity to produce cellulose used by the dissolve oxygen . At this stage, bacterial growth persists despite not increasing exponentially and reached a steady state . Along with fermentation time, there is a portion of bacterial cells that settle to the bottom of the reactor . The bacteria can be re-used as a seed for a new experiment. Based on Figure 2 it can be seen that the process of formation of cellulose began to occur on day 4 of treatment and on days P2 - 5 to treat P1 and P3. In general, the pattern of the cellulose layer formation is the same for all treatments. The first stage is the formation of an initial thin layer of nata and subsequently after a few days nata thickness will increase along with the fermentation process to a point where the thickness of nata is fixed. Nata thickness obtained for P1 and P2 are the same that is 3 mm while P1 is only 1.5 mm. While the quality of the resulting nata further tested at the Center for Pulp and Paper Bandung (BBPK).

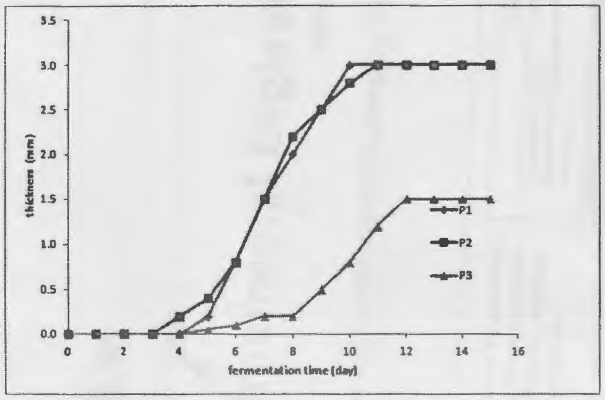

Figure 2. Cellulose thickness measurement results.

Table 3. Parameter experimental result at *Balai Besar Pulp dan Kertas*

Treatment	Experimental result			
	Fibre length (mm)	Moisture content (%)	Ash content (%)	Total cellulose content (%)
Treatment 1	0,537	86,36	8,41	62,34
Treatment 2	Not detected	87,58	8,94	60,51

Source: *Balai Besar Pulp dan Kertas, 2013*

4. Conclusion

Based on the results of the research, the most optimum fermentation processes generated by P1 with the variation of the substrate flow rate (Vc) 0.23 mL/min. Measurement data demonstrate cellulose thickness in P1 is equal to 3 mm and in the terms of its quality nata P1 sample has the most good quality compared to other treatments, both visually and based on test results from BBPK (Center for Pulp and Paper).

In addition to the flow rate, it turns out there is the effect of adding pure oxygen to the process of formation of cellulose. It is shown from the results between P2 and P3. With the same flow rate, the presence of treatment against the pure oxygen supply into the reactor will affect the resulting thickness. The differences are large enough that 50% is shown in P1 which has a thickness of 3mm while nata P2 only 1.5 mm. Then, based on the results of this comparison it can be concluded that the addition of pure oxygen on fermentation by *A.xylinum* is very important, because it is based on [5,14] on the characteristics of *A.xylinum* aerobic bacteria are bacteria that require oxygen in the metabolism of his life to produce cellulose.

When associated with the observed data on P2 and P3 were obtained very suitable to the nature of the bacteria used. The addition of pure oxygen in the fermentation process will help the effectiveness of enzymes and metabolism of *A.xylinum* cellulose in the form layers, it is also stated by Lee & Zhao [5] that the addition of pure oxygen in the process of fermentation by bacteria *A.xylinum* is very important that the production of microbial cellulose obtained more optimal.

Based on the results of measurement and analysis has been done on a continuous fermentation process, a conclusion obtained that the variation of the optimum flow rate of the substrate contained in treatment 1 is 0.23 mL / min with a detention time of 12 days and the addition of pure oxygen on the fermentation by *A.xylinum* greatly affect the breeding process of the growth of bacteria and cellulose layer formation process.

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