Corn Cob Hydrolyzate from Penicillium citrinum H9 as an alternative substrate for Biosurfactant production by Hydrocarbonoclastic Bacteria

by Ni'matuzahroh Ni'matuzahroh

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Corn Cob Hydrolyzate from *Penicillium citrinum* H9 as an alternative substrate for Biosurfactant production by Hydrocarbonoclastic Bacteria

Fatimah[12], Silvia Kurnia Sari[12], Nastiti Trikurniadewi[12], Syahriar Nur Maulana Malik Ibrahim[12], Ana Mariatul Khiftiyah[12], Khudrotul Nisa Indriyasari[1], Tri Nurhariyati[12], Tini Surtiningsih[12] and Hanif Yuliani[3], Ni'matuzahroh[12*]

[1]Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia
[2]Research Center for Bio-Molecule Engineering, Universitas Airlangga, Surabaya, Indonesia
[3]Agency for the Assessment and Application of Technology, BPPT, South Tangerang, Indonesia

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ABSTRACT

Corn cob is a potential agricultural waste that can be utilized as raw materials in bioindustries. Commonly, corn cob is regarded as agricultural waste and would be eliminated by burning, however this elimination method would produce a toxic fume that polluted the atmosphere. So, new utilization methods of corn cob need to be developed. This research aimed to reveal the potency of corn cob hydrolyzate (CCH) that produced enzymatically using *Penicillium citrinum* H9 as substrate for biosurfactant production. The CCH was used as growth substrate for biosurfactant production by seven indigenous bacteria of Balongan oil sludge. All isolates were tested the growth and the capability of biosurfactant production through surface tension and emulsification activity measurement. One potential isolate was obtained from this observation. BP1(5) isolate has a lowering surface tension at 56.64±4.63 mN/m in five days incubation and the growth rate was 0.52 "cell/hour. The best biosurfactant product was obtained at concentration 200 ppm of CCH in five days incubation by 23.93±3.56 mN/m in surface tension reductions but the emulsification activity was not detected. This research showed that the CCH be utilized as substrate for biosurfactant production by hydrocarbonoclastic bacteria.

Key words: *Biosurfactant, Corn Cob Hydrolyzate, Hydrocarbonoclastic Bacteria*

Introduction

Agricultural waste especially corn cob in Indonesia, are increasing very year, and to reduce their accumulation, most of them are eliminated by burning. Some Indonesian researchers have observed their utilization to decrease the waste and increase the value by conversion, for instance, to feedstock with high protein (Marhaeni, 2002), as well as, to xylose and glucose (Oktaviani *et al.*, 2019). Furthermore, corn cob has been reported that it is promising substrate for biosurfactant production due to their low cost (Ni'matuzahroh *et al.*, 2019a).

The world's interest in biosurfactants has increased considerably in recent years due to their potential application in various industrial fields. In

Corresponding author's email: nimatuzahroh@fst.unair.ac.id

the pharmaceutical sector biosurfactants were used due to their anti-bacterial properties, anti-biofilm and anti-cancer activities (Rangarajan and Sen, 2013; Karlapudi *et al.*, 2018). In the food industry, biosurfactants were used as emulsifiers of the bread making process. Furthermore, biosurfactants can also be employed for bioremediation of environments polluted by petroleum and heavy metals (Banat *et al.*, 2000; Mulligan and Wang, 2006).

Based on global market insights, the biosurfactant market in 2017 reached USD 1.8 billion and predicted to be more than USD 2.7 billion in 2024. The high biosurfactants demand is caused by their low toxicity, biodegradability, and stable in temperature, pH and salinity with broad range compared to synthetic surfactants (Zambry *et al.*, 2017; Varjani *et al.*, 2017). Despite people's interest in biosurfactants, the molecules are still unable to compete with synthetic surfactants in the world market because of high production cost, inefficient production methods, and expensive substrates requirement (Makkar and Cameotra, 2002). The solution for this problem is to explore the potential of isolates, optimize production, and use inexpensive raw materials and substrates from waste. Several previous studies have published the ability of organic waste such as rice straw, wheat straw, and sugar cane-bagasse to hydrolyze to produce sugar as a substrate for biosurfactant production (Joy *et al.*, 2019; Ni'matuzahroh *et al.*, 2019b).

This study used corn cob as a substrate for biosurfactant production by hydrocarbonoclastic bacteria. Corn cob consist of mainly three types of polymers cellulose, hemicellulose, and lignin, so that it is potentially hydrolyzed (Ni'matuzahroh *et al.*, 2018; Saha, 2004). Previous studies have shown that corn cob can be hydrolyzed into simple sugars and used for the production of biosurfactants by *Bacillus subtilis* 3KP with a reduction in surface tension of 12.55 ± ± 3,95mN/m (Ni'matuzahroh *et al.*, 2018). This paper explored the potency of corn cobhydrolysate as a substrate for biosurfactant production by hydrocarbonoclastic bacteria isolates with variations in substrate concentration and incubation time.

Materials and Methods

Microorganism

Hydrocarbonoclastic bacteria, which were isolated from Balongan oil sludge in the previous study (Ni'matuzahroh *et al.*, 2019a) and collection of microbiology laboratory of Universitas Airlangga, were used in this study. The seven phenotypically different colonies obtained, namely BP(1)1, BP(1)2, BP(1)3, BP(1)4, BP(1)5, BP(1)6, and BP(1)8, were initially transferred onto Nutrient Agar (NA) slant for re-culture before used as an inoculum for screening biosurfactant production using corn cob hydrolyzate (CCH) as main substrate.

Enzymatic hydrolysis of corn cob by *Penicillium* citrinum. H9

Corn cob was collected from plantation in the Bojonegoroarea, East Java. Sample was dried and ground roughly into 1-5 mm pieces. Chemical delignification process was carried out by mixing corn cob powder with NaOH 1% solution with a ratio of 1:10 (w/v) for an hour at 100 ºC. The mixture was filtered and washed to adjust the pH into 7 then dried in an oven for 24 hours at 60 ºC. The hydrolysis process was carried out enzymatically by *Penicillium citrinum* H9. Two grams of pretreatment corn cob as the sole carbon source was added to 100 mL of Mandel-Sternberg's medium which consisted of KH_2PO_4 (2 g/L), $(NH_4)_2SO_4$ (1.4 g/L), $CaCl_2$ (0.3 g/L), $MgSO_4.7H_2O$ (0.3 mg/L), $FeSO_4.7H_2O$ (5 mg/L), $MnSO_4.H_2O$ (1.6 mg/L), $ZnSO_4.7H_2O$ (1.4 mg/L), and $CoCl_2$ (2 mg/L) and arranged at pH 5 using citrate buffer. The media was cooled to room temperature then it was add 4% (v/v) of spore suspension of *Penicillium citrinum* H9 and was incubated in shaker incubator for 6 days. The concentration of corn cob hydrolysate sugar was measured by Somogyi-Nelson's method after the glucose standard equation was made by using the same method.

Screening for an efficient isolate for biosurfactant production

A loopful of each purified isolate was separately inoculated into 100 mL Erlenmeyer flasks containing 20 mL nutrient broth and incubated for 24 hours. This culture was used for starter. Screening which was taken for potentials isolates in biosurfactant production was carried out on mineral water synthetic medium with a total volume of 20 mL. The media was added with corn con hydrolysate (CCH) with a concentration of 209.3 ppm as much as 7.5% (v/v) and bacterial starter 2% (v/v) OD_{650}0.5. The cultures were agitated at 120 rpm, at 30 °C for 0,1,3, and 5 days. Potential isolate was screened on the basis of emulsification, reduction of

surface tensions of fermented broth, and total plate count. The potential isolate then was used on the optimization of biosurfactant production process.

Optimization of biosurfactant production

The optimization of biosurfactant production was conducted by varying the concentration of corn cob hydrolysate sugar and the incubation time. The concentration of corn cob hydrolysate sugar was varied (0, 100, and 200 ppm) with 0, 1, 3, and 5 incubation time. Become these factors were chosen to obtain higher productivity of the biosurfactant. Mineral water synthetic medium utilized for biosurfactant production in this study was modified from Pruthi and Cameotra (1997) with the composition of: $(NH_4)_2SO_4$ (3 g/L), NaCl (10 g/L), $MgSO_4.7H_2O$ (0.2 g/L), $CaCl_2$ (0.01 g/L), $MnSO_4.H_2O$ (0.001 g/L), H_3BO_3 (0.001 g/L), $ZnSO_4.7H_2O$ (0.001 g/L), $CuSO_4.5H_2O$ (0.001 g/L), $CoCl_2.6H_2O$ (0.005 g/L), and $Na_2MoO_4.2H_2O$ (0.001 g/L). Mineral water synthetic medium's buffer consisted of KH_2PO_4 (5 g/50 mL), K_2HPO_4 (2.6207 g/50 mL), and Fe_3O_4 (0.0006 g/50 mL). The total culture volume was 20 mL which consisted of mineral water synthetic medium, 7.5 % (v/v) of corn cob hydrolysate sugar, and 2% (v/v) of bacteria suspension. Microbial culture was incubated in a shaker at room temperature with the agitation rate of 120 rpm.

Biosurfactant productivity test

Before the bacteria cultures were tested for their surface tension (ST) and emulsification activity (EA), the optical density was measured by spectrophotometry. Samples of the culture medium were centrifuged for 15 minutes with an agitation rate of 3000 rpm. As many as 10 mL of supernatant was taken for surface tension analysis using Tensiometer Du-Nuoy ring method. The value of its surface tension was stated in mN/m or equivalent to dynes/cm. Its surface tension was calculated as follows:

$$r = r_o \frac{\theta}{\theta_o} \quad (1)$$

where r_o was water surface tension at t°C, θ was the sample values shown on the Tensiometer, and θ_o was distilled water values shown on the Tensiometer Du-Nouy. The emulsification activity was obtained by mixing 1 mL of supernatant and 1 mL of kerosene then homogenized with vortex for 2 minutes. The emulsification activity was observed after 1 hour and 24 hours and calculated using the following formula:

$$EA(\%) = \frac{\text{Total height of the emulsified layer}}{\text{Total height of the liquid layer}} x100\% \quad (2)$$

Results and Discussion

Enzymatic hydrolysis of corn cob

Corn cob was composed by heterogeneous complexes of carbohydrate polymers such as lignin, cellulose, and hemicellulose that can be hydrolyzed into simple sugar. However cellulose and hemicellulose are coated with lignin, which protects them against enzymatic hydrolysis. Physical and chemical pretreatment (delignification) were performed to break the lignin seal to expose cellulose and hemicellulose for enzymatic action. Corncob was physically treated by grinding and milling to reduce particle size and increase the surface area that will be accessed by *Penicillium citrinum* H9. In addition to further optimizing the hydrolysis process, chemical delignification is also carried out by using NaOH. Alkali delignification using NaOH has been proven to be more effective in breaking ester bonds between hemicellulose, cellulose, and lignin compared to acidic or oxidative pretreatment, while it also being able to avoid hemicellulose fragmentation polymer, thus making the biomass increasing in the porosity (Tarkow and Feist, 1969; Gaspar *et al.*, 2007).

Zhang and Cai (2008) stated that delignification of rice straw using 2% NaOH for 1 hour at 85 °C was able to reduce lignin content up to 36%. While in this research, it was showed that corn cob delignification using 1% NaOH at 100 °C for 1 hour was able to increase the hemicellulose content until 10.48% and cellulose 17.22%, and reduce the lignin up to 7.07%.

According to this result, it indicates that delignification process plays an important role in decreasing lignin content and increasing cellulose and hemicellulose content, so it is easy to be hydrolyzed enzymatically. Enzymatic hydrolysis is the second step in the production of biosurfactants from lignocellulosic waste. It involves the breakdown of compound cellulose and hemicellulose using enzymes. The hemicellulose contains several sugars such as xylan, mannan, glucan, arabinan, and galactan, while cellulose is only composed of glucans. Therefore, the main hydrolysis product of the hemicellulose was pentoses and hexoses while cellulose was glucose (Taherzadeh and Niklasson, 2004).

Screening for an efficient bacteria using CCH as carbon source

Seven indigenous bacteria of Balongan oil sludge had been successfully isolated in previous studies (Ni'matuzahroh *et al.*, 2019a). All isolates were tested for growth and their ability to produce biosurfactants on the CCH substrate. The results of the test are shown in the Table 1.

Based on the Table 1, it can be seen that bacteria can grow in the substrate. Based on the growth rate and generation time of all bacterial isolates, show that all isolates are able to use CCH as a carbon source for their growth. This is due to the hydrolysis of corn cob contains xylose, glucose, glycerol, arabinose and acetic acid (Chen *et al.*, 2019 Moldes *et al.*, 2007).

In addition to be used for growth substrate, CCH has also been proven capable of being used as a substrate for biosurfactant production. This is consistent with the study of Moldes *et al.*, (2007) which states that *Lactobacillus pentosus* grown on reducing sugar of corn cob and able to reduce surface tension by 18 mN/m. Biosurfactant production were indicated by presence of surface tension and emulsification activity (El-Sheshtawy *et al.*, 2015). Based on this research, all of the isolates didn't show emulsification activity, while the lowest surface tension were shown by BP(1)5 after 5 days incubation (Table 1). Surface tension of BP(1)5 was also lower than uninoculated media, as many as 72.69 mN/m. Furthermore BP(1)5 isolate showed surface tension reduction after 1, 3, and 5 days of incubation, in contrast to other isolates which showed fluctuated value of surface tension reduction (Fig. 1). From this screening proses we obtained the potential isolate for biosurfactant production using CCH which is BP(1)5. BP(1)5 is used in the optimization of biosurfactant production with variations in substrate concentration and incubation time.

Table 1. The growth rate, generation time, and surface tension of BP(1)5 bacteria on CCH substrate

Isolates	Log TPC at 5th day (CFU/mL)	Growth Rate (cell/hour)	Generation Time (hour)	Surface Tension (mN/m) 5th day
BP(1)1	19.57±0.07	0.42	2.38	57.06±1.17
BP(1)2	14.83±0.00	0.24	4.18	72.17±0.00
BP(1)3	17.80±2.91	0.36	2.82	60.01±3.55
BP(1)4	19.89±0.05	0.42	2.40	70.16±1.03
BP(1)5	19.82±0.09	0.52	1.94	56.64±4.63
BP(1)6	19.85±0.47	0.42	2.40	68.06±1.27
BP(1)8	18.89±0.41	0.40	2.47	66.50±5.29

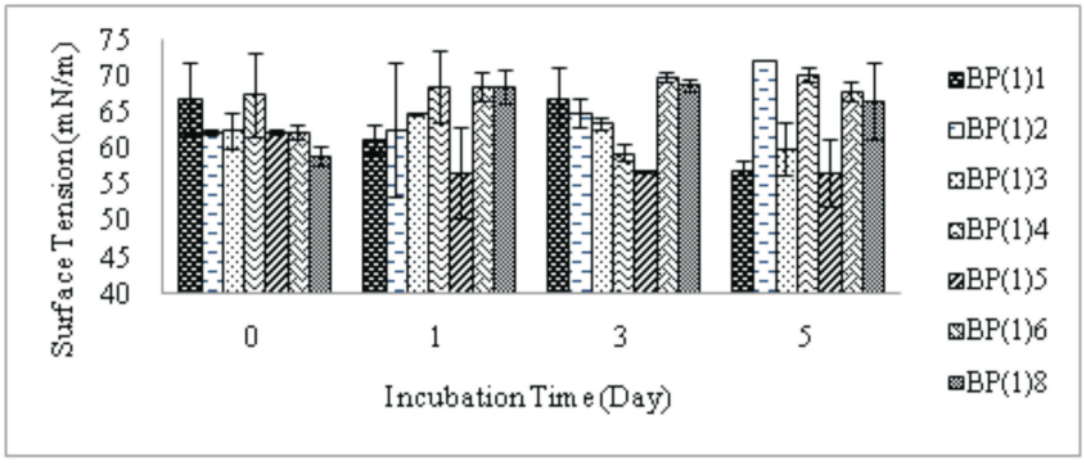

Fig. 1. Surface tension value of a BP(1)5 in various incubation time

Optimization of biosurfactan production by BP(1)5

The research was proven that sugar from corn cob hydrolysis can be used in biosurfactant production. Moreover, the optimal concentration of sugar that gave the best yield of biosurfactant was 200 ppm in 5 days incubation (Fig. 2). The addition of 200 ppm CCH on the culture of BP(1)5 could hold stationary phase longer than the others concentrations. In the culture with 200 ppm of CCH, in 5 days incubation, the density of microbes (OD_{650}) was 0.137. On the other hand concentration of sugar 200 ppm could decrease the surface tension from 62.996 mN/m at day 0 to 48.866 mN/m at day 5, it was the lowest surface tension compared to other cultures in some sugar concentrations which were 56.728 mN/m and 53.351 mN/m for 0 and 100 ppm sugar respectively. Microbial ability to decrease surface tension is an indicator of biosurfactant production by bacteria (El-Sheshtawy *et al.*, 2015). Based on the Picture 2C, the lowest surface tensions were showed at stationary phase, so biosurfactant was mostly produced at stationary phase. The same results were also shown in the research conducted by El-Sheshtawy *et al.* (2015) and Thavasi *et al.*(2011). Patowary *et al.* (2017) stated that biosurfactant production was started at stationary phase until deathphase.

Conclusion

Based on the research, CCH can be used as substrate for growth of bacteria and biosurfactant production by hydrocarbonoclastic bacteria BP(1)5. The optimum concentration of CCH that could be used as substrate for growth and production of biosurfactant was 200 ppm in five days incubation. By using 200 ppm of the hydrolyzate, the surface tension could be decreased to 48.866 mN/m and the bacterial optical density was 0.137 (OD_{650}) after five days incubation

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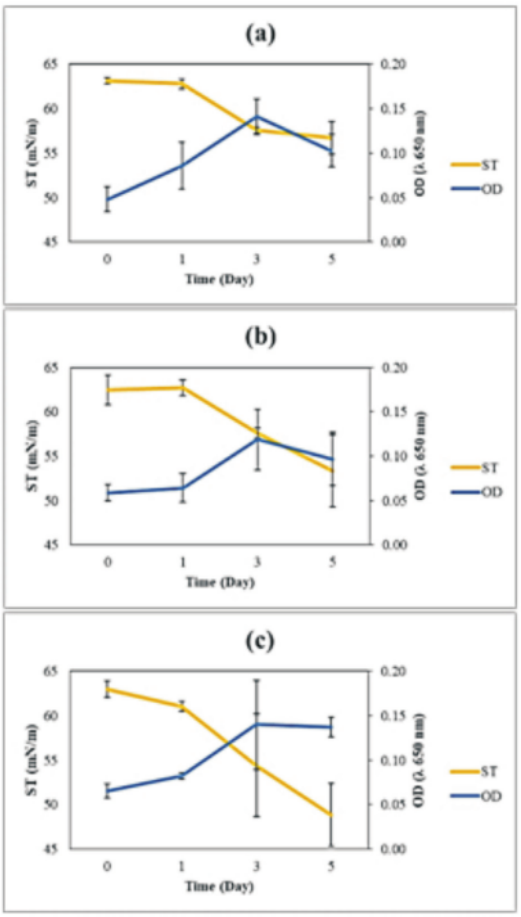

Fig. 2. Optical density and surface tension of the BP(1)5 on CCH strain with various sugar concentration (a) 0 ppm, (b) 100 ppm, (c) 200 ppm

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