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To: nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id>
Cc: asg17845@gmail.com

Thu, Nov 28, 2019 at 2:40 PM

Dear author: Ni'matuzahroh

We glad to inform you that your manuscript with following details :

ID : 1119

Title : Isolation and Characterization of Cockroach Endosymbiont Bacteria Potential to Produce Hydrolysis Enzyme of Organic Material

has been recommended to be published in **"Ecology, Environment and Conservation"** indexed by scopus as Q4. In this email we have attached the review result of your manuscript, please revise it accordingly. While revising your manuscript, you need to consider following informations :

1. Manuscript should be written within 5 pages, or there will be an extra fee for every extra page.

2. We have attached the Sample or Author guideline for your reference. Please pay more attention on how the reference format of the journal.

3. Please set your manuscript to single column, to simplify the editing process.

Please complete the revision by December 23rd 2019 . Thank you very much

Icobiodiv Editorial Team

3 attachments

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[Reviewed] 1199_Corn cob hydrolizate from Penicillium citrinum H9 as an Alternative Substrate for Biosurfactant Production by Hydrocarbonoclastic Bacteria.docx
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EEC Sample.pdf
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Revised articles

1 message

nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id> Fri, Dec 27, 2019 at 12:06 AM
To: IcoBiodiv FST Universitas Airlangga <icobiodiv@fst.unair.ac.id>

Dear committee Icobiodiv

I hereby submit a revision of 2 articles that have been adapted to the EEC journal template. Titles of the article are as follows
1. Isolation and Characterization of Cockroach Endosymbiont Bacteria with Potential to Produce Hydrolytic Enzyme of Organic Material (Article code 1119)
2. Corn Cob Hydrolyzate from Penicillium citrinum H9 as an Alternative Substrate for Biosurfactant Production by Hydrocarbonoclastic Bacteria (Article code 1199)

Thank you very much for your help and attention.

Best regards

Ni'matuzahroh and team

2 attachments

26_DES_[Reviuwed]_1119_Isolation and Characterization of cockroach endosimbion bacteria_ sesuai template Jurnal EEC.docx
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26_DES_[Reviewed] 1199_Corn cobs hydrolysate form Penicillium citrinum_sesuai template jurnal EEC.docx
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please correct your manuscript

Enviro Unair <enviro.unair@gmail.com> Sat, Mar 7, 2020 at 6:50 PM
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Dear Author,

Please find attached the PDF of your paper. Please let us know corrections by sticky notes on PDF.
However, only minor corrections and/or typo errors are allowed.
And send it back to me in 48 hours.

Best regards.
Guest Editor
Special Issue
Prof. Agoes Soegianto
Department of Biology
Faculty of Science and Technology
Universitas Airlangga
Surabaya Indonesia

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EEC 28 1199_revision

4 messages

nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id> Mon, Mar 9, 2020 at 5:50 PM
To: enviro.unair@gmail.com

Dear Guest Editor,

The following attachment is our paper. There are some mistakes in the paper and we have given notes for correction. Thank you for your help.

Best regards,
Dr. Ni'matuzahroh

EEC 28 1199_Corn cobs hydrolysate form Penicillium citrinum_rev 1.pdf
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Enviro Unair <enviro.unair@gmail.com> Mon, Apr 6, 2020 at 8:28 PM
To: nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id>

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Prof. Agoes Soegianto
Department of Biology
Faculty of Science and Technology
Universitas Airlangga
Surabaya Indonesia

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nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id> Tue, Apr 7, 2020 at 4:51 PM
To: Enviro Unair <enviro.unair@gmail.com>

Thank you for your information.

Best regards

Dr. Ni'matuzahroh
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nimatuzahroh nimatuzahroh <nimatuzahroh@fst.unair.ac.id> Wed, Apr 8, 2020 at 1:56 PM
To: Enviro Unair <enviro.unair@gmail.com>

Dear Guest Editor

Herewith, I send proof of bank transfer for publication fee of our article that will be published in Ecology, Environment and Conservation, entitled **Corn Cob Hydrolyzate from *Penicillium citrinum***

H9 as an Alternative Substrate for Biosurfactant Production by Hydrocarbonoclastic Bacteria.

Thank you for your attention

Best regards

Dr. Ni’matuzahroh

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Corn Cob Hydrolyzate from *Penicillium citrinum* H9 as an Alternative Substrate for Biosurfactant Production by Hydrocarbonoclastic Bacteria

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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 wasted 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 have a lowering surface tension value 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 that was got the lowering surface tension 23.93±3.56 mN/m, 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, was increased in each 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 [illegible] xylose and glucose (Oktaviani et al., 2019). [illegible]so has been reported that, agricultural wastes, such as corn cob, is promising substrate for biosurfactant production due to their low cost (Ni'matuzahroh et al., 20[illegible]).

The world's interest in biosurfactants has increased considerably in recent years due to their potential application in various industrial fields. In 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. [illegible]biosurfactants high demand is due to their low toxicity, biodegradability, and stable in temperature, pH and salinity with broad

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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 on the world market due to 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 bacteria with a reduction in surface tension of 12.55 ± ± 3,95 mN/m (Ni'matuzahroh et al., 2018). This paper explored the potency of corn cob hydrolysate 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., 2019) 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 Bojonegoro area, 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°C. The mixture was filtered and washed to adjust the pH into 7 then dried in an oven for 24 hours at 60°C. 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), $ZnSO_4.7H_2O$ (0.4 mg/L), and $CoCl_2$ (2 mg/L) and arranged at pH 5 using citrate buffer. The media which was cooled to room temperature then added 4% (v/v) of spore suspension of *Penicillium citrinum* H9 and 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 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 for potential isolates for 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. These factors were chosen as an aim 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/50ml), K_2HPO_4 (2.6207 g/50mL), and Fe_3O_4 (0.0006 g/50mL). 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 its 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:

$$r = r_o \frac{\theta}{\theta o} \qquad (1)$$

where r_o was water surface tension at t °C, θ was the sample values shown on the Tensiometer, and θo 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:

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

Result 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. Corn cob 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 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 also being able to avoid hemicellulose fragmentation of polymer, thus making the biomass increasing on 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%, also reduce the lignin up to 7.07%.

From that result, it is known that delignification process plays an important role in decreasing lignin content and increasing cellulose and hemicellulose content, so it can be easy 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. So 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., 2019). 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., 2009 Moldes et al., 2007).

Besides being used as a 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 then 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

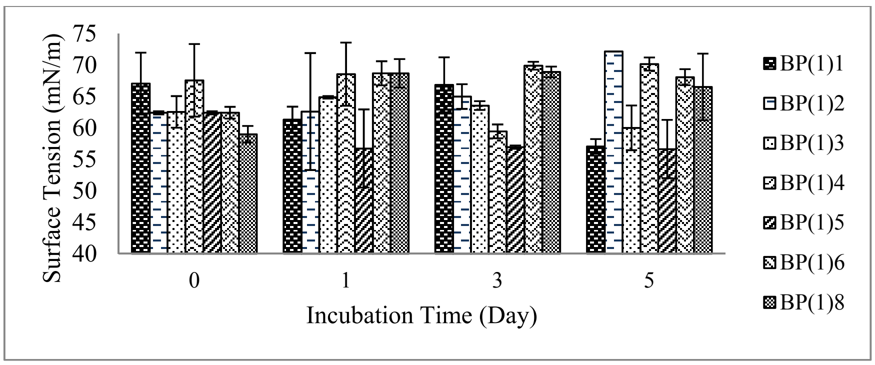

Fig 1. Surface tension value of a BP(1)5 in various incubation time

Optimization of biosurfactan production by BP(1)5

In this research, it was proven that sugar from corn cob hydrolysis can be used in biosurfactant production. Based on this research, 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_{600}) was 0.137. Concentration of sugar 200 ppm also could decrease the surface tension from 62.996 mN/m at day 0 to 48.866 mN/m at day 5. The lowest surface tension value compared to culture with the others sugar concentrations, in the amount of 56.728 mN/m dan 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 death phase.

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

Acknowledgement

We gratefully thank to Faculty of Science and Technology, Universitas Airlangga for funding this research through Penelitian Unggulan Fakultas (PUF) 2019.

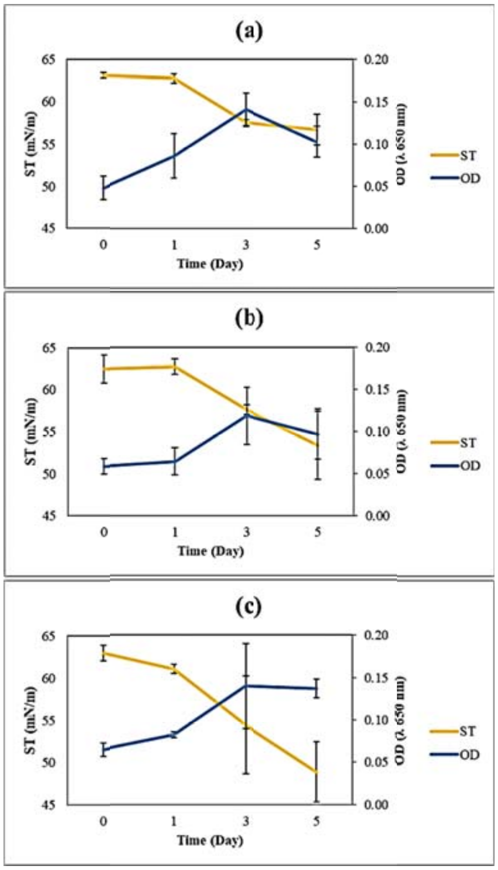

Fig. 2. Optical density and surface tension of the BP(1)5 on CCH strain with various sugar concentration (a) 0ppm, (b) 100ppm, (c) 200ppm

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