

## Isolation and Potential Testing of North Sumatera Berastagi Agricultural Soil In Degrading Marshal Insecticide With Carbosulfan Active Material

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**Abstract.** The isolation of bacteria from Berastagi agricultural soil North Sumatera has been done. The aim is to evaluate their ability in degrading carbosulfan. Sixteen bacterial isolates were obtained using selective media Bushnell Hass Agar (BHA) containing 12 ppm of carbosulfan. The parameters observed were the growth of isolates, biosurfactant activity, biosurfactant concentration, and the residue of carbosulfan after 21 days of incubation. The result showed that all isolates were able to degrade carbosulfan as the sole carbon source. Two isolates namely JBM 3 (isolate from citrus agricultural soil Berastagi) and KBM 1 (isolate from cabbage agricultural soil Berastagi) were selected for further test to determine their ability to degrade carbosulfan. The results showed that both of the isolates were able to degrade carbosulfan. Compare to control, isolate JBM 3 was able to decrease the concentration of carbosulfan by 33.33%, while isolate KBM 1 was able to reduce carbosulfan concentration up to 40.47%.

**Keyword:** Carbosulfan, biodegradation of pesticide, bioremediation, biosurfactant

Received 11 June, 2020 | Revised 25 July, 2020 | Accepted 27 August, 2020

### 1 Introduction

Conventional agricultural products cannot be separated from the use of chemical pesticides. In agriculture, pesticides are a means to kill corpses of plant pests [1]. The use of pesticides has helped a lot to increase agricultural and plantation production. However, the use of these pesticides also has a negative impact on humans, biota and the environment [2].

Strengthening contamination of pesticides in the environment appear as continuous use of pesticides, ignore compliance in the use of dose, and the use of pesticides whose use outside official oversight [3]. Based on the type of pesticide, pesticides can undergo a biodegradation process, biologically changed in soil and water. However, there are some pesticides that cannot

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be biodegradable, these types are called persistent pesticides that can pollute the environment in very broad dimensions [4].

Chemical pesticides can be divided into several groups, namely organophosphates, carbamates, synthetic pyrethroids, and neonicotinoids which are broad-spectrum insecticides. Carbamate is an insecticide that has been widely used to control plant pests [5]. All carbamate insecticides have a carbamic acid base building block. In the decomposition environment, some carbamates produce metabolites that are still toxic [6], one of the active ingredients including carbamates is carbosulfan which works as a stomach poison. One of the insecticides that uses the active ingredient carbosulfan is Marshal 200 EC. This insecticide is very often used by farmers to kill insects.

Berastagi is the largest horticultural production center in North Sumatra, where its production cannot be separated from the use of various types of pesticides. The use of pesticides has been done by farmers for a long time, so that the microbes in the soil are able to use pesticide residues as a carbon source for their metabolic activities [3].

The decomposition of pesticides in the environment can occur through microbial activity, chemical reactions, and sunlight which can occur at any time [2]. To overcome residual contamination from insecticides, the use of the role of degrading microbes can be a promising alternative for further study [3]. This paper reports the potential of the Berastagi agricultural soil bacterial isolates in degrading carbosulfan.

## **2 Materials and Methods**

### **Tools and Materials**

The tools used in this study include a centrifuge, shaker, a spectrophotometer and a ZB-1 CP-3800 variant Gas Chromatography (30m x 0.25mm x 0.25 $\mu$ m) with an ECD detector. The materials used include samples of Berastagi agricultural land, insecticide with active ingredient carbosulfan with the trademark Marshal (200 EC), bushnell media - haas broth (BHB), bushnell - haas agar (BHA) (consisting of  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{NH}_4\text{NO}_3$ ,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ ,  $\text{FeCl}_3$ ,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , agar),  $\text{H}_2\text{SO}_4$ , o rsinol, rhamnose, diethyleter, tripton soy agar (TSA), plate count agar (PCA), Mc-Farland standard solution, distilled water, N-hexane, 0.05 M sodium bicarbonate ( $\text{NaHCO}_3$ ), dichloromethane, isooctane, toluene and 70% alcohol.

### **Isolation and Characterization of Agricultural Soil Bacteria in Berastagi, North Sumatra**

Soil samples were taken as much as 1 kg from Berastagi agricultural land which contained pesticides. Soil sampling was carried out at four agricultural locations (chilies, oranges, cabbage and tomatoes). Sample the sample is weighed with as much as 1 gram and diluted in 10 ml of

aquades sterile until 10<sup>-2</sup>, inoculated 0.1 ml to the BHA media which has to contain 12 ppm carbosulfan with trademark Marshal 200 EC. The samples that have been inoculated onto the media are incubated at 30°C for 10-15 days. Colonies of growing isolates were purified on TSA media. Then characterization colony morphology, cell shape, Gram staining, and a biochemical test consisting of citric test, gelatin, catalase, hydrogen sulfide, citric and starch.

### Isolate Growth Measurement

Growth isolates during the incubation period is done by growing the bacteria on BHB mineral medium containing 12 ppm carbosulfan. A total of 2 ml in okulum liquid bacterial isolates s Etara by turbidity of the solution Mc-Farland inoculated into 100 ml of media in aseptic, incubated on shaker (150 rpm) in dark conditions at a temperature of 30°C for 21 days. The growth of isolates was observed every 7 days, namely on the 0<sup>th</sup>, 7<sup>th</sup>, 14<sup>th</sup> and 21<sup>st</sup> day. The measurement of the number of cells was carried out using the Standard Plate Count method. A total of 1 ml of culture media was diluted to a concentration of 10<sup>-7</sup>, then inoculated into PCA media aseptically using the scatter plate method and incubated for 24 hours. The number of colonies that grow calculated by colony counter. To calculate the estimated number of cells, it can be calculated with the formula:

$$\text{Estimation of cell count} = \text{colony count} \times \frac{1}{\text{the dilution factor}} (\text{CFU/ml})$$

### Biosurfactant Activity Screening

Activities biosurfactant produced by bacteria used method Drop Test Collapsing modified [7]. Bacterial isolates were grown on BHB media plus 2% dextros. A total of 2 ml of bacterial isolate liquid inoculum equivalent to the turbidity of the Mc-Farland solution ( $\approx 10^8$  cells/ml) was inoculated into 100 ml of media aseptically, incubated in a shaker (150 rpm) under dark conditions at 30°C for 15 days. After 15 days of incubation, each culture medium was filtered and the filtrate was taken. A total of 4 ml of media filtrate was added to the test tube, plus 4 ml of N-hexane and 2 ml of distilled water. It was vortexed for 10 seconds, let stand for 1 minute. The thickness of the emulsion formed is measured with a caliper. Then converted to volume.

### Determination of the Biosurfactant Standard Curve

Biosurfactant standard curve determination was performed using pure rhamnose from Sigma Aldrich Company, United States. The solution rhamnose made with different concentrations (0 (blank), 10, 50, 100 and 200 ppm) dissolved in a solution of sodium bicarbonate (NaHCO<sub>3</sub>) 0,05M, then each inserted into a test tube 2 ml. The solution of rhamnose was added with 3.6 ml of orsinol solution, heated to a boil, cooled at room temperature for 15 minutes and the absorbance was measured using a spectrophotometer with a wavelength of 421 nm. The regression equation of the standard rhamnosa curve was determined by the Least Square method.

## **Biosurfactant Production**

The bacteria were grown on BHB media containing carbosulfan as a carbon source. Liquid inoculum bacteria solution which is equivalent to Mc-Farland much as 2 ml was inoculated into 100 ml of media, incubated on a shaker (150 rpm) with a dark conditions at a temperature of 30°C for 21 days. The resulting biosurfactant production was analyzed using the modified orsinol method [8]. Bacterial culture was centrifuged (6000 rpm) for 10 minutes to separate bacteria from the culture medium. The supernatant was taken 4 ml and extracted with 2 ml of diethyl ether for 5 minutes, the extraction was repeated 3 times. The ether layer was taken, dried and redissolved in 2 ml of 0.05 M sodium bicarbonate (NaHCO<sub>3</sub>) solution. Then the sample solution was vortexed and added with 3.6 ml of orsinol solution, heated to boiling, cooled at room temperature for 15 minutes and analyzed for its absorbance. using a spectrophotometer with a wavelength of 421 nm.

## **Potential Test of Biosurfactant Producing Bacteria in Degrading Carbosulfan**

Potential test of biosurfactant-producing bacteria in degrading carbosulfan during the incubation period was carried out with bacterial isolates grown on BHB mineral media containing 12 ppm carbosulfan. A total of 20 ml of bacterial isolate liquid inoculum which is equivalent to the turbidity of the Mc-Farland solution was inoculated into 980 ml of media aseptically, incubated in a shaker (150 rpm ) under dark conditions at 30° C for 21 days. A sample of 300 ml was put in a separating funnel, 100 ml of dichloromethane was added. The sample and dichloromethane were extracted to form two phases. The organic phase is collected in a round flask. To water, 50 ml of dichloromethane was added again. This cycle takes place twice. Next, evaporation is done until it is almost dry. Then isoocan: toluene (9:1) is added until the volume reaches 3 ml. Analyzed by using Gas Chromatography Variant CP-3800 ZB-1 column (30m x 0.25mm x 0.25µm) with ECD detector , column temperature 200-270°C, detector temperature 300 ° C, injector temperature 280°C. The sample is injected as much as 1 µl, the area is obtained, converted into mg/kg.

## **3 Result and Discussion**

### **Agricultural Soil Bacteria in Berastagi, North Sumatra**

The isolation results obtained were 16 isolates of biosurfactant-producing bacteria grown on Bushnell - Hass Agar (BHA) medium containing 12 ppm carbosulfan (Marshal 200 EC) as a carbon source. The isolates obtained were 4 isolates from chili farming land (CBM 1, CBM 2, CBM 3 and CBM 4), 3 isolates from citrus farming land (JBM 1, JBM 2, and JBM 3) , 5 isolates from cabbage farming (KBM). 1, KBM 2, KBM 3, KBM 4 and KBM 5) , and 4 isolates

from tomato farming (TBM 1, TBM 2, TBM 3, TBM 4 and TBM 5) . These bacterial isolates have varied morphological and Gram characteristics.

The isolates obtained showed a circular colony of 6 isolates, 6 isolates irregular (irregular) and 4 isolates, where the edges of the colony were entire (flat) as many as 11 isolates, lobate (split) as many as 4 isolates and undulate (choppy) 1 isolate. Meanwhile, the colony elevation is flat. Colonies of bacteria are white, milky white, fluorescent and transparent. Bacteria acquired shaped cocci by 7 isolates, cocobacil by 5 isolates and bacil by 4 isolates. The bacteria obtained were dominated by Gram negative bacteria as many as 15 isolates and 1 Gram positive isolate. Biochemical characterization showed that all isolates had different characteristics .

### Isolate Growth

The bacterial isolates obtained from the isolates were regrown on BHA media and 10 isolates were selected based on their growth ability. Each isolate has a different growth (Figure 1) .

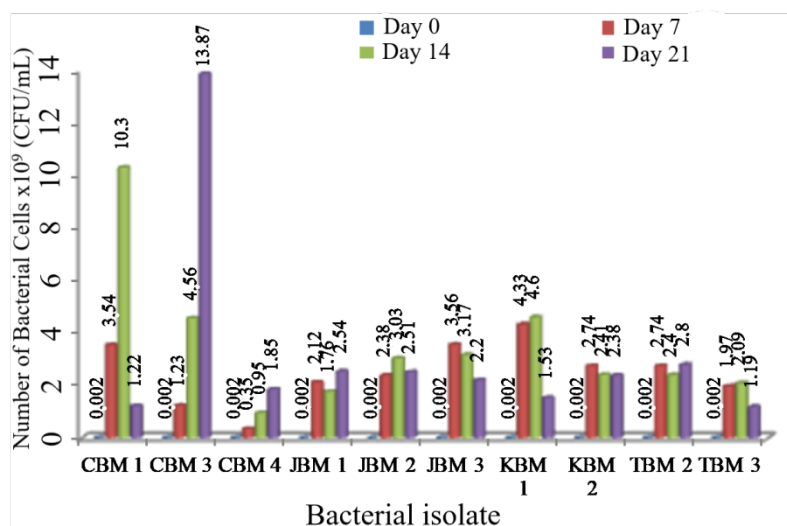


Figure 1. Growth of bacterial isolates during the culture period on BHB media containing carbosulfan

The growth rate of each bacterial isolate reached its optimum on day 14 and the growth of cell numbers decreased on day 21, however, some bacteria such as CBM 3, CBM 4, JBM 1 and TBM 2 experienced an increase in the number of cells on day 21st. This is probably because each isolate has different growth phases, namely the adaptation period of each isolate to the available nutrients and the ability of each isolate to use carbosulfan or other compounds such as synthetic surfactants contained in the pesticide Marshal (200 EC) as carbon sources are also different, causing different growth rates in each bacterial isolate .

Lay [9], states that the difference in the growth rate of bacteria is caused by many factors, including the type and type of bacteria itself and the ability of these bacteria to use the nutrients available in the media for their metabolic processes. Hayatsu et al. [10] stated that bacteria

isolated from soils contaminated with carbaryl (1-naphthyl N- methylcarbamate) were able to utilize carbaryl as the only carbon source. Carbaryl and carbosulfan are insecticides in the carbamate class which have the basic structure of carbamic acid in their structure.

Nasution [11] states that bacteria originating from the Tanjung Balai (TJB) are able to grow on media containing herbicides with active ingredients of glyphosate and use it as a source of carbon and energy. The growth of bacterial isolates reached  $6.2 \times 10^{12}$  CFU/ml on the 6th day. The level of carbosulfan toxicity based on its half-life is longer than glyphosate, which is likely to cause growth in media containing lower carbosulfan.

### Biosurfactant Activity Screening

The biosurfactant activity was observed based on the volume of emulsion formed between the N-hexane layer and the liquid media. The biosurfactant activity showed varied results as shown in Figure 2.

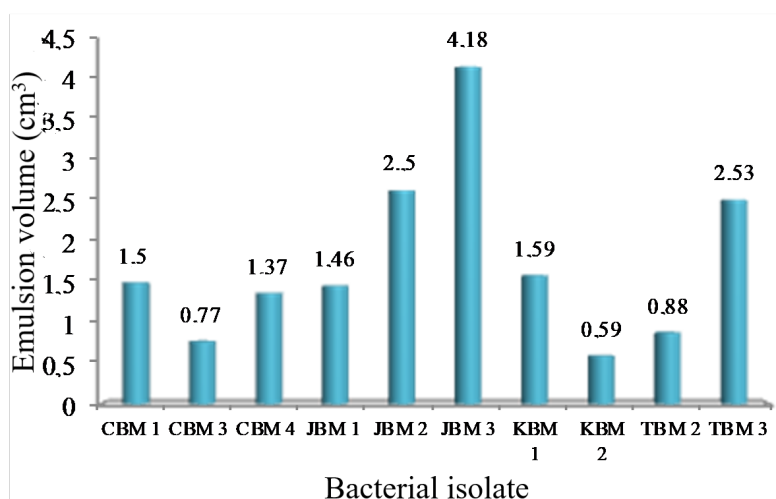


Figure 2. Biosurfactant activity of the Berastagi agricultural soil bacteria isolate

I solat JBM 3 had the highest emulsion activity with an activity value of 4.18 cm<sup>3</sup> followed by isolates JBM 2, TBM 3 and KBM 1 with activity values of 2.65, 2.53 and 1.59 respectively. Meanwhile, the lowest emulsion activity was shown by the KBM 2 isolate, and it was not much different from the CBM 3 isolate with activity values respectively 0.59 and 0.77 cm<sup>3</sup>. I CBM 3 isolate had the highest growth rate, while its biosurfactant activity had the lowest value compared to all tested isolates. This is probably due to the different components and types of biosurfactants produced.

Each isolate has a different biosurfactant activity which is indicated by the formation of an emulsion. According to Rosenberg et al., [12] the different types and components of biosurfactants produced by each isolate will affect the emulsion activity that occurs on the surface of the liquid. Warsito [13] states that the highest value of biosurfactant activity is shown by bacteria originating from the Tanjung Balai sea with a value of 13.50 mm grown on BHB

media containing naphthalene (a hydrocarbon compound). The biosurfactant activity of the Berastagi agricultural soil bacteria in this study was higher when compared to bacteria originating from the Tanjung Balai sea.

According to Pacwa-Płociniczak et al . [14] the main characteristic of biosurfactants is that they have hydrophilic and hydrophobic parts, with these parts making it easier for bacteria to dissolve substances that are insoluble in water. The surfactant activity makes the surfactant a good emulsifying agent. Kosaric [15] states that emulsification of biosurfactants can occur due to several factors. Among them are the presence of hydrophobic and hydrophilic compounds, water conditions (salinity and pH), temperature and components or the biosurfactant molecule itself.

### Biosurfactant Production

The production of biosurfactants from each bacterial isolate has varying amounts as can be seen in Figure 3.

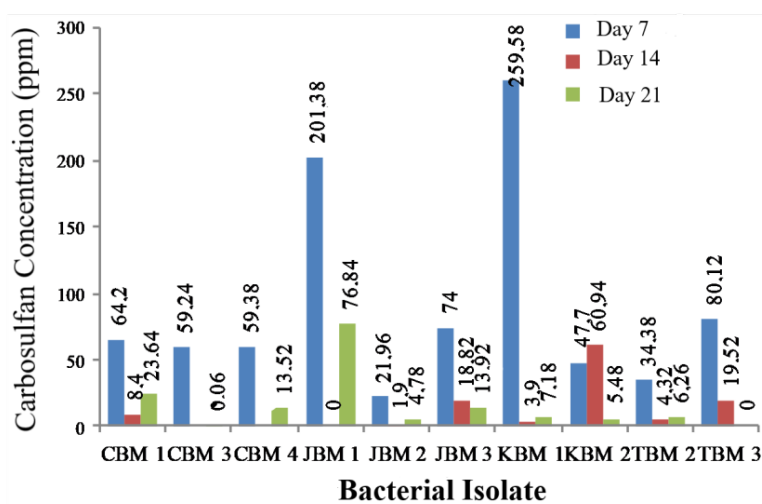


Figure 3. Production of bacterial isolate biosurfactants

The highest biosurfactant production of each isolate occurred on day 7 and biosurfactant production decreased on day 14 and day 21. The highest concentration of biosurfactant was produced by bacterial isolates of KBM 1, JBM 1, TBM 3 and JBM 3 respectively. From the results of cell growth measurements (Figure 1) and screening of biosurfactant activity (Figure 2) the KBM 1 isolate had a high enough cell number and biosurfactant activity, JBM 1 had a lower number of cells and biosurfactant activity than KBM 1, TBM 3 had biosurfactant production which was higher than JBM 3 but the cell growth and biosurfactant activity of TBM 3 was lower, while JBM 3 had a fairly high number of cells and the highest biosurfactant activity among all isolates. The high growth of bacterial isolates at the beginning of growth (day 7) can be associated with high biosurfactant production, where the high production of biosurfactants will help in the process of degradation of carboxulfan which is used as a carbon source. The presence of biosurfactants will increase the solubility of carboxulfan thereby increasing its

availability to be degraded by bacteria. Likewise, the high biosurfactant activity of bacterial isolates can be associated with high biosurfactant production as well. On the 14th day the production of biosurfactants decreased significantly, but the growth had increased and also decreased, this was probably due to bacterial isolates preferring to use other carbon sources such as synthetic surfactants found in pesticides.

The difference in bacterial growth is thought to occur due to differences in the production of biosurfactants produced by bacteria. Biosurfactants play a role in emulsion formation so that hydrocarbon compounds become available as a carbon source in multiplying cell density in the test media. Li & Chen [16] positive effect of surfactants on the degradation of hydrocarbons is associated with increased solubility and dissolution of hydrocarbon compounds. According to Batubara [17], the amount of biosurfactant produced also depends on how these microorganisms use the available nutrients. The difference in nutrition will affect the production of biosurfactants.

### Potential of Biosurfactant Producing Bacteria in Degrading Carbosulfan

Test potential biosurfactant producing bacteria in degrading carbosulfan conducted by selecting two isolates based on the pattern of growth, activity and concentration biosurfactant biosurfactant is JBM 3 and KBM 1. Both isolates had the ability to lower the concentration of tar carbosulfan as seen in Figure 4 below.

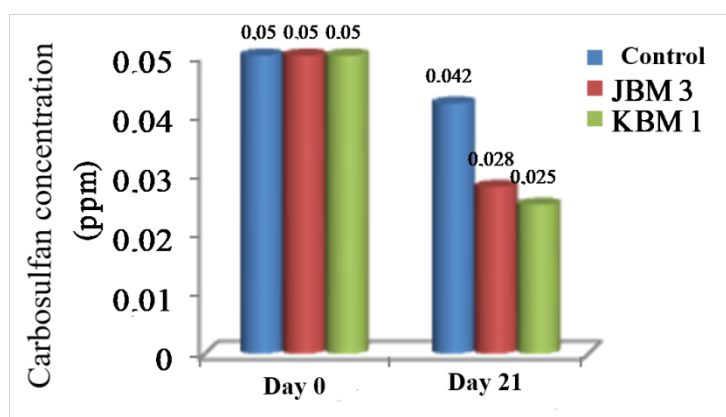


Figure 4. The remaining carbosulfan concentration of degradation on the 21<sup>st</sup> day

Isolat bacteria JBM 3 and KBM 1 were able to reduce the carbosulfan concentration on the 21<sup>st</sup> day. This shows that the two isolates are able to use carbosulfan as a carbon source which is indicated by a decrease in the concentration of carbosulfan. The decrease in carbosulfan concentration also occurred in the control treatment and bacterial isolate treatment. When compared with the control treatment, isolate JBM 3 was able to reduce the carbosulfan concentration by 33.33% (residual carbosulfan 0.028 ppm), while the KBM 1 bacterial isolate was able to reduce the carbosulfan concentration by 40.47% (residual carbosulfan 0.025 ppm). The decrease in the concentration of carbosulfan in the control treatment was probably due to



the fact that carbosulfan could be degraded naturally, such as through light. Katagi [18] states that photodegradation by sunlight is one of the destroyers of pesticides after being applied to the environment. Degradation due to sunlight is influenced by the intensity, spectrum of sunlight, duration of exposure and the nature of pesticides.

The ability of the JBM 3 and KBM 1 bacterial isolates to reduce the carbosulfan concentration after 21 days of incubation showed that the bacterial isolates were able to use carbosulfan as a carbon source. Suherman [19], bioremediation of diazinon organophosphate pesticides using indigenous microbes from rice fields showed a decrease in the concentration of diazinon after incubation for 27 hours, namely 55.52 % initial concentration of 50 ppm. Based on its half-life, carbosulfan is longer when compared to diazinon, this is likely to cause a lower carbosulfan reduction. Andreu & Pico [20], the degradation time of carbofuran (carbamate group) reaches 117 days, while diazinon (organophosphate group) reaches 40 days. The ability of bacteria to use carbosulfan in this study was also supported by cell growth (Figure 1), surfactant activity (Figure 2) and high biosurfactant production (Figure 4) from bacterial isolates.

## Conclusion

Isolate JBM 3 was able to decrease the concentration of carbosulfan by 33.33%, while isolate KBM 1 was able to reduce carbosulfan concentration up to 40.47%.

## Acknowledgement

The authors are thankful to the ministry of education for providing fund through Lembaga Penelitian, Universitas Sumatera Utara 2011/2012.

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