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Abstract: The purpose of this research is to understand sponge species, micro symbiont, performance, mechanisms, and types of compounds resulting from biodegradation. The analytical method is applied by selecting the model, results from characterization and morphological identification, phenotype, genotype. Selected of micro symbiont are made as suspensions, interacted with modified naphthalene contaminated waste. The biodegradation process using the *Bacillus Sp* (BS) isolates sponge *Neopetrosia Sp* and *Acinetobacter Calcoaceticus* (AC) isolated from sponge *Callyspongia Aerizusa*, was carried out by interacting a microscopic suspension of 5,000 mg/L naphthalene waste for 25 days. Micro symbiont biodegradation results by determining the concentration of contaminants and biodegradation products, in the form of pure organic compounds using GC-MS, and the analysis of functional groups of natural components using IR. Destruction of the naphthalene molecule occurs through an enzymatic reaction mechanism, observed based on visible parameters. The performance of BS symbiont biodegradation on naphthalene is in the range of 7.34% - 51.37%, while the AC range is 5.84% -37.26% in w/v, achieved within 5-25 days interaction. Biodegradation products based on functional groups were identified as compounds of aldehyde, ketone, carboxylic acids, esters, alkanes. Observation of biodegradation parameters confirmed pH changes, increased optical density values, gas bubbles formed and the smell of fermentation.

1. Introduction

Sea activities are very potential as a source of pollution, especially oil by ship transportation, the petroleum processing industry, spills due to ship accident, loading and unloading and washing of tanker vessels which often dispose of oil sludge in the form of ballasts [1]. The pollution characteristics of this type of petroleum contain many toxic components which are dominated by

carcinogenic and mutagenic aliphatic and polycyclic kinds of hydrocarbons (PAHs) and a small portion of heavy metal content with high toxicity [2-4]. Carcinogenic and mutagenic properties of PAH, until now it has become a worldwide concern because it threatens the life of all marine biota, it is even known, there are several types of PAHs seen from the number of aromatic rings making them up them, including naphthalene, anthracene, phenanthrene, pyrene, all carcinogenic and mutagenic categories are very height [5,6]. The living system of live things, recognizing the food chain cycle, makes humans very vulnerable to exposure to these carcinogenic substances, both directly and indirectly through the path of fish consumption and other activities [7,8].

One of the marine biota sponge always symbiotic mutualism with a variety of microorganisms, mainly bacteria to maintain and continue their lives, turned out to have the function of biodegradation, bio-destruction, bio-detoxification, bio-reduction and bio-adsorption of various components of pollutants in the ocean, one of which can degrade PAH components by destroying the structure of aromatic molecules through Enzymatic reactions produce par components of pure organic compounds [7,9]. The ability of sponges and symbiotic bacteria to break down the aromatic structure of hydrocarbons is the effort of bacteria and sponges to obtain nutrients and make the carbon element as an energy source.

Meeting the expectations of various objectives in the study of biodegradation of PAH using micro sponge symbiont, several research focuses were explicitly made to discuss in this study, including the type of sponge symbionts bacteria that are potential as PAH biodegraded, performance and degree of biodegradation of micro sponge symbiont, models and reaction mechanisms change the structure of PAH molecules, types of organic compounds of biodegradation products based on visible functional groups and some parameters of biodegradation that can be observed include pH, optical density, gas bubble formation and odour fermentation of biodegradation media in the test reactor [4,10].

2. Method of research

2.1. Materials and equipment

Bacillus Sp (BS) micro symbionts *Neopetrosia Sp*; *Acinetobacter Calcoaceticus* (AC) micro symbionts *Callyspongia Aerizusa* sponge isolate; Naphthalene; dichloromethane for GC; HCl p.a; Physiological NaCl p.a 0.9%; sterile seawater; Nutrient Agar; alcohol; glycerol 2%; cotton; plastic wrap; aluminum foil; gauze; tissue; filter paper. GC-MS [Agilent 7890], Nicolet IS 10 FT-IR Zhimadsu; Filter of 0.2 µ m [Millex-LH]; Shaker incubator [Enviro-Genie]; Spectrometer 20 D * [Thermo E. Corp] Shimadzu a wavelength of 600 nm; micropipette [Dragon Onemed]; Ultrasonic; Analytic sheet set [Mettler PM-200].

2.2. Microbial sponge as a biodegraded of PAH (Naphthalene)

Potential micro sponge symbiont as PAH degrading biomaterial was isolated from selected sea sponges whose body surface was covered in mucus. Mucus on the surface of the body of the Sponge in the form of enzymes behaving enzymes produced by the symbionts bacteria in response to the Sponge to defend itself to survive the predator attack in the aquatic environment where the sponge breed. One of the sponge predators is the presence of PAH contaminants in the sponge habitat environment. Isolates from selected sponges must meet the results of characterization criteria in the form of phenotype analysis (Gram staining and biochemical tests) and genotypic analysis [11,12].

2.3. Experiment of biodegradation

Selected isolates were propagated by culture method on agar nutrient media, then isolate culture was changed to suspension by adding 2 ml of physiological 0.9% NaCl, shaken. The suspension was put into a 100 mL Erlenmeyer volume was sufficient by using 0.9% physiological NaCl. Pipette every 5 ml of suspension into six degradation reactors, then incubated for 1 x 24 hours. Interaction between bacterial symbionts suspension with PAH was done by entering 5 ml of 5,000 mg/L naphthalene solution. The reactor was put into the incubator shaker with each contact time (5; 10; 15; 20; 25) days.

After reaching the specified contact time, isolates that have been contaminated with naphthalene as modified waste, each added a 5 mL dichloromethyl solution through a separating funnel, then allowed to stand for 1 minute until there are two layers of polar and non-polar fluids. Taken PAH (non-polar) solution which has been separated from isolate (polar) suspension. Na_2SO_4 solid was added as much as + 0.01 grams into the non-polar extract containing PAH, to remove the water content in it so that each sample could be measured using GC-MS and IR [2,12,13].

3. Results and discussion

3.1. Biodegradation performance of Naphthalene PAH compounds by symbionts sponge

The decrease in naphthalene PAH compound concentration after contact with micro sponge symbionts suspension was shown by a reduction decrease in the height of the chromatogram peak GC-MS measurement results, with a tendency to the longer contact time, the percentage decrease in naphthalene concentration was also higher. The decrease in PAH contaminant concentration is effective, and the maximum occurs during the contact period of 10-20 days. The decrease in naphthalene concentration means a reduction in the level of toxicity and carcinogenic properties of PAH as a result of the action of sponge symbiont bacteria. The increase in the level of biodegradation of naphthalene as indicated by the performance of BS symbiont bacteria significantly occurred during the contact period of 15-20 days with a value of 47.18% (w/v) or a difference of 20.42% (w/v). In contrast, whereas for AC of bacteria the percentage of biodegradation the highest occurred during the interaction period of 10-15 days with a value of 29.62% (w/v) or a difference of 18.26% (w/v) at each contact time for five days.

Data of the GC-MS analysis showed a decrease in peak height of the naphthalene component as the contact period increased between the micro symbiont sponge of *Bacillus sp* and *Acinetobacter calcoaceticus* to components naphthalene. Chromatograms measured by GC-MS also showed a number of new peaks, showing components of organic compounds that were thought to be the result of biodegradation of symbionts bacteria, as shown in Figures 1 and 2.

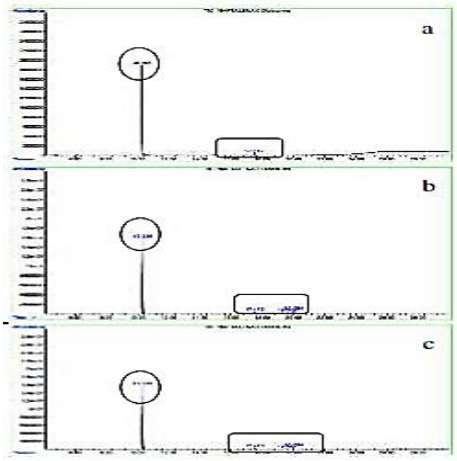

Figure 1. Chromatogram of naphthalene GC-MS and components of organic compounds from the bacterial biodegradation of BS; (a) 5 days contact; (b) 15-day contact, and (c) contact of 25-day.
◯ = Chromatogram of the naphthalene comp.

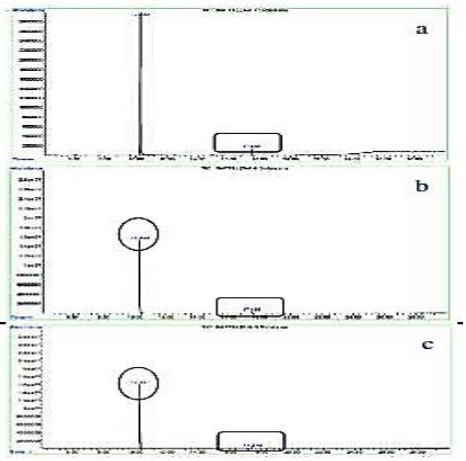

Figure 2. Chromatogram of naphthalene GC-MS and components of organic compounds from the bacterial biodegradation of AC; (a) 5 days contact; (b) 15-day contact, and (c) contact 25-day.
▭ = Simple organic compound products

The level of biodegradation of naphthalene shown by both micro sponge symbionts in the first 5 and 10 days of contact period looks insignificant. This is because the growth of bacterial cells is in the adaptation phase which lasts about 2-5 days, then enters the second growth stage namely the

exponential phase is marked with increasing size and number of bacterial cell splitting it is predicted to last for the next 10-15 days. This phase until entering the stationary phase it is estimated that there are very many active bacterial cells and work optimally to degrade the naphthalene molecule or to destroy the structure of naphthalene, finally, from the third phase to the death phase the next 5-10 days is the mass of dead bacterial cells. The performance and level of biodegradation of sponge symbiotic bacteria (BS and AC), as shown in Table 2, above can be concluded that the naphthalene biodegradation process follows the general pattern of bacterial growth [4,14,15].

The naphthalene chromatogram appears to have decreased peak height as the contact period between the suspension of bacterial isolates BS and AC to naphthalene as shown in Figure 1a and c, and Figure 2a, and 2c decreased peak of the naphthalene component, showing that the aromatic concentration decreased due to biodegradation by sponge symbionts bacteria. Further analysis of the chromatogram measured by GC-MS found new peaks that were thought to be simple organic compounds as biodegradation products. The organic compounds shown in the box (Figures 1 and 2), are alcohol, a small portion of ether and ketone, aldehydes and carboxylic acids as well as esters and aromatic components, observed from two types of measurements, namely GC-MS by knowing the types of organic compounds of biodegradation products and IR data wave numbers to determine the functional groups of organic compounds [1,4].

In a detailed search, it was found that in contact 5-10 days, the components of biodegradation products in the form of alcohol, aldehyde and ketone compounds, then the contact period of the next 15-25 days, found more varied compounds in the presence of organic components of carboxylic acid groups and esters. According to the theory, it is said that the biodegradation of hydrocarbon components by microorganisms begins with the destruction of the molecular structure into alcohol, then becomes an aldehyde and the biodegradation product simultaneously becomes carboxylic acids, esters and other simple organic compound products [2,6,16]. The above data can be said that the work of biodegradation of naphthalene by BS and AC bacteria in marine sponge isolates in accordance with the pattern of bacteria in-phase and follows the mechanism of enzymatic reaction or fermentation.

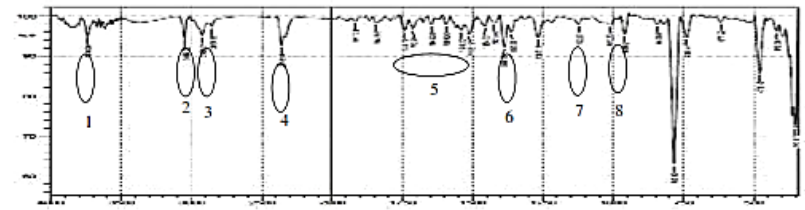

Figure 3. Chromatogram of IR measurement against naphthalene biodegradation media using BS bacteria isolate symbiont *Neopetrosia sp* sponge, contact period of 25 days.

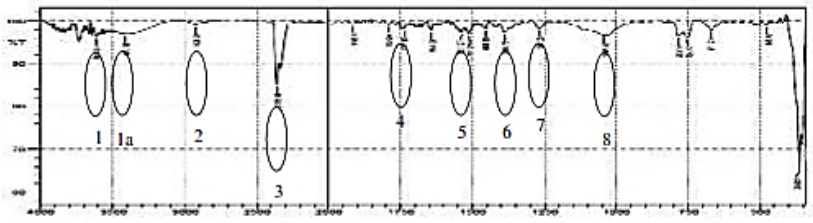

Figure 4. Chromatogram results of IR measurements on naphthalene biodegradation media using AC bacteria which is an isolate *Callyspongia aerizusa* symbiont of Sponge, contact period of 25 days.

Data from the GC-MS analysis, regarding the components of biodegradation results by BS and AC bacteria, were strengthened by analysis of data using IR, (Figures 3 and 4), that the chromatogram with peaks number 1, 2 and 3, showed the existence of functional groups on the wavenumber values, consecutive ὺ: 3200-3600 cm^{-1} (OH) alcohol; ὺ: 3050-3000 cm^{-1} (C-H) aromatic; ὺ: 3000-2850 cm^{-1} (C-H) alkanes and ὺ: 2500-2200 cm^{-1} (O-H) from carboxylic acids. Subsequent chromatograms number 4, 5 and 6 respectively showed functional groups, ὺ: 1735-1500 cm^{-1} (C = O) ketones and esters, aldehydes and carboxylic acids; ὺ: 1680-1650 cm^{-1} (C = C) aromatic and alkenes; ὺ: 1300-1120 cm^{-1} (C-O) ether and ester. Numbers 7 and 8 are functional groups ὺ: 1100-1040 cm^{-1} (C-O) alcohol and ὺ: 1120-1100 cm^{-1} (C-O) esters. This IR data illustrates that organic compounds of alcohol,

aldehyde, ether, ketone, esters and carboxylic acids are present in the media as a result of naphthalene biodegradation which also matches the GC-MS measurement results [5,8].

The level of decrease in naphthalene concentration by the action of biodegradation of BS and AC bacteria according to the contact period in days is shown in Figure 5, as follows:

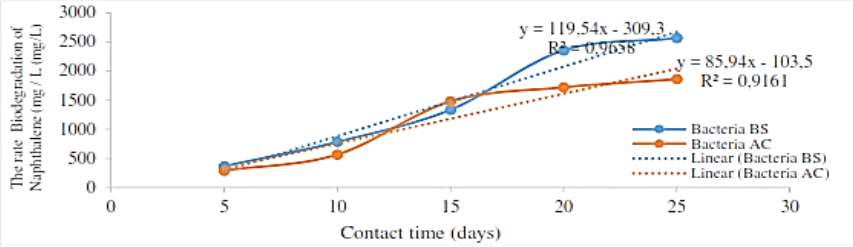

Figure 5. The level of biodegradation of naphthalene by BS bacteria symbionts sponges *Neopetrosia sp,* and AC bacteria symbionts sponge *Callyspongia aerizusa* based on contact time.

The decrease in PAH concentration after interaction between BS and AC bacterial suspensions with naphthalene translates as the level of biodegradation which is the result of the work of sea sponge symbiotic microorganisms. The biodegradation performance of the two types of bacteria on naphthalene showed a significant difference (Figure 5). The performance of BS bacteria is superior to AC, characterized by the achievement of BS performance in naphthalene biodegradation reaching 51.37% while AC is only 37.28% with a contact period of 25 days, and there seems to be a downward trend when interactions continue for the next few days [3,7,9]. There is an exciting side to AC performance, which appears dominant compared to BS occurring during the ten-day contact period. This situation is understood, that differences in the production of biodegradation of microorganisms (certain bacteria) can happen given that the growth and development of bacteria undergo several phases and is highly dependent on the growth environment. Value of $R^2 = 0.9658$ and $R^2 = 0.9161$, respectively biodegradation performance by BS and AC bacteria, shows that there are no other factors that contribute to naphthalene biodegradation other than BS and AC test bacteria [1,2]. The results of biodegradation in the form of carboxylic acids and organic components that are acidic and other components contribute to increasing the acidity of the biodegradation media, which has an effect on weakening the performance and the ability of bacteria to keep growing. This condition is interpreted as a limiting factor for biodegradation.

4. Conclusion

The *Bacillus sp* AB353F partial micro symbiont (BS), isolate from sea sponge *Neopetrosia* sp. able to reduce naphthalene PAH at maximum biodegradation rate of 51.37% (w/v), higher than the performance of the bacterium *Acinetobacter calcoaceticus* strain PHCDB14 (AC), isolate sponge *Callyspongia aerizusa* with a biodegradation performance of 37.28% (w/v), both were achieved during the contact period of 25 days. Naphthalene biodegradation process follows the pattern and stages of bacterial action. The main types of organic compounds for biodegradation include alcohols, aldehydes, ketones and ethers, carboxylic acids and esters. Biodegradation of naphthalene PAH is running, following the mechanism of enzymatic reaction characterized by gas bubble parameters, odour fermentation, increase in acidity (pH) and increased optical density of degradation media. The R^2 value, indicating that BS and AC bacteria are the only components that cause biodegradation. The level of biodegradation achieved by BS and AC bacteria to naphthalene is low but can be increased by using a consortium of bacteria (carbon clastic bacteria) accompanied by the provision of nutrients to the proportional method biodegradation by bacteria.

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