# The power of biodegradation and bio-adsorption of bacteria symbiont sponges sea on waste contaminated of polycyclic aromatic hydrocarbons and heavy metals



# The power of biodegradation and bio-adsorption of bacteria symbiont sponges sea on waste contaminated of polycyclic aromatic hydrocarbons and heavy metals

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**Abstratc:** Sponge is often used as a bio indicator and bio monitoring in determining the level of heavy metal contamination in the sea. The purpose of this research is to analyze the destruction of PAHs and the reduction of heavy metal toxicity. Biodegradation and bio-adsorption occur with a 30-day interaction between bacterial symbiont sponge suspension against modified liquid waste containing a mixture of PAHs (naphthalene) contaminants, heavy metals Cr (VI) and Cd (II). The period of interaction of bacteria with heavy metals for 15 days, bio-adsorption power was determined using AAS, while the interaction with PAHs, the level of biodegradation was measured using GC-MS and Infrared Spectrophotometer. The results achieved bacteria BP and PS, have biodegradation and bio-adsorption activities against extreme waste contaminated with a mixture of naphthalene and heavy metal. The level of biodegradation of BP bacteria to naphthalene is weaker than PS bacteria. The bio-adsorption power of PS bacteria is higher for Cr (VI) and Cd (II) ions. The components of naphthalene in extreme waste inhibit the bio-adsorption performance of BP and PS bacterial, conversely there is an effect of inhibition of bacterial activity on the biodegradation process of naphthalene by the presence of heavy metals.

## 1. Introduction

The mining and petroleum processing industry has been booming since the 1970s until now. Exploration and exploitation of petroleum occurred so massive because of its very high economic value and productivity that provided huge profits, so that in that period petroleum held the title "black gold" [1]. Even so, the conversion of petroleum into various fuel products and their derivative products poses major problems that threaten environmental sustainability because the by-products produced are in the form of hazardous and toxic waste. Every 1,000 barrels of refined petroleum products, produce by-products in the form of petroleum mud in the form of semi-solid paste or sludge reaching ± 6.4 barrels and liquid waste [2,3]. The main component in sludge and petroleum liquid waste is hydrocarbons especially the aromatic polycyclic group (PAHs) and also contains a number of

heavy metals [4,5]. Both types of these components are very dangerous, because they have carcinogenic, mutagenic and very toxic properties to most living things, from creatures with tiny body sizes or microorganisms to large ones (plants, animals) including humans [6,7]

The sea area is the most vulnerable area contaminated with petroleum sludge waste, because the ocean is the lowest area compared to land. The movement of liquid flows from high to low due to pressure difference, so it is ensured that sooner or later the liquid waste and sludge of petroleum byproducts of the petroleum processing industry in the plains, flow and empty and accumulate in the sea [8,9]. Vulnerability to contamination of petroleum waste in the sea is caused by the fact that most of the petroleum processing industries are located in the sea or offshore areas, the distribution of oil is not uncommon through distribution pipes that are planted at sea and at times experiences leakage, sea transportation accidents (tankers), washing the ship produces ballast and also the density of the traffic of the transporting ship all of which are sources of PAHs and heavy metal pollutants, so that the sea is a giant container of various types of waste [10]. This situation is the biggest threat to the life of all types of marine life, even humans as consumers of various products from the sea [11].

The natural balance of the sea is always maintained in normal conditions, but the movement and accumulation of hydrocarbon waste occurs massively as if it is out of control, so that the natural balance of the sea is predicted to not be able to compensate for the movement of contaminant exposure that eventually reaches the saturation point [12]. Such conditions require human intervention to develop and implement certain technologies, so that migration of B3 hydrocarbon waste can be controlled, including remediation of the marine environment that has been contaminated, so that the natural balance function of the sea continues to contribute to minimizing the risk and impact of damage to marine biota and adverse effects can occur to human health [13,14].

Many research results report that several marine biota are known to be able to carry out the function of remediation of growth habitats in order to maintain the existence of life, including mollusks, seagrasses, sponges, mangroves and several types of marine microorganisms such as fungus and bacteria [1,15,16,17]. Various research results state that several types of sea sponges can be used as bio monitoring and bio indicators of heavy metal pollution [18,19]. Other studies also report that sponge biomass and some types of symbiotic bacteria can degrade hydrocarbon components [3]. Based on this, inspiring research was carried out with the theme of the utilization of marine sponge symbionts bacteria that can play two roles of bioremediation at once, namely the role of biodegradation of PAHs components and the function of bio-adsorption of heavy metals [1,20]. The research is very important and interesting to study more deeply, because of the toxicity and carcinogenic properties and mutagenic waste sludge containing PAHs and heavy metal components that have a crucial effect on human health [21]. Another thing that adds to the weight of this study is because the population and distribution of sea sponge is very large and grows in many places in the Indonesian ocean. The achievements of this research are: (1) through screening methods to identify new biomaterials of the type of sponge symbionts bacteria that can be applied in overcoming PAHs and heavy metal contaminants in waste through biodegradation and bio-adsorption methods, and (2) the potential for the preparation of hydrocarbon-metalloclastic bacterial formulations that are easily brought to the site and cultured to remediate areas contaminated with PAHs and heavy metals.

## 2. Materials and methods

*2.1 Materials and equipment.*

Bacteria sponge micro symbiont type *Bacillus pumilus* strain GLB197 (BP) sponge isolate *Niphates Sp, Pseudomonas stutzeri* strain SLG510A3-8 (PS) sponge isolate *Hyrtios erectus*, naphtalena [Sigma-Supelco], dichloromethane for GC, $K_2Cr_2O_7$ pa., $Cd(NO_3)_2$ pa., HCl pa., KOH pa., physiological NaCl 0.9% solution, sterile sea water; Nutrient Agar (NA), Marine Agar (MA), 96% alcohol, glycerol 2%, plastic wrap, aluminum foil, Whatman filter paper No.41, formalin 4%, Aquabides, Atomic Absorption Spectrophotometer (AAS) type AA24OFS Variance, Nicolet IS 10 Zhimadsu-IR Spectrophotometer, GC-MS [Agilent 7890], Shaker incubator [Enviro-Genie], separating funnel, micro pipette [Dragon Onemed], Ultrasonic, Analytic sheet set [Mettler PM-200], Filter of 0.2 μm

[Millex-LH, Universal pH paper, laminary air flow, round ose, ovens, tweezers, spirits lights, mortars, counter colonies, microscopes, glass objects, water baths, digital analytical scales, Erlenmeyers, separating funnels and a set of classroom equipment.

The operating conditions for GC-MS: maximum temperature 350 $^0$C, pressure 18,406 psi, capillary column [Agilent 19019S-436HP-5 MS], dimensions of 60 m x (250 x 0.25) µm, the pressure 18.406 psi, retention time of maximum 30 minutes , separation 26.128 cm/sec and a Helium carrier gas, a speed of 150 mL/min, the increase in temperature of 10 0C every 5 minutes.

*2.2 Sample preparation and preparation of modified extreme wastewater.*

The choice of sponge micro symbiont bacteria was obtained from the culture stock of the results of previous studies, namely the types of BP and PS which had previously been isolated from certain sponge types and were characterized by phenotypic and genotypic analysis [21,22]. Each bacterium was acculturated in NA media. The culture results were converted into bacterial suspensions using NaCl 0.9% physiological solution. BP and PS suspensions made at 500 mL each. Prepared 500 mL of Cr (VI) and Cd (II) contaminated liquid waste each of 250 mg / L. Also prepared naphthalene waste concentration of 1,000 mg/L. Cr (VI), Cd (II) and naphthalene contaminants are mixed together and homogenized to obtain modified extreme waste [12].

*2.3 Biodegradation process of sponge symbiont bacteria against PAHs test in waste.*

Bacterial biodegradation experiments on PAHs were carried out by preparing a 2 x 2 of biodegradation reactor with a capacity of 250 mL and labeled (duplo). Each reactor is loaded with 50 mL BP type symbiotic sponge suspension. The suspension is incubated for 1 x 24 hours to adapt to the environment. After the incubation period is reached, each reactor is added 50 mL of extreme waste, then aerated with a 100 rpm incubator Shaker incubator. Measurement of naphthalene concentration that did not undergo biodegradation was done 2 times, at the beginning of the interaction (0 days) and the end of the interaction (30 days) using GC-MS and the biodegradation product was determined using –IR spectrophotometer instrument [23,24]. Sample preparation for GC-MS measurements and -IR spectrophotometry was carried out by filtering the liquid media in the reactor using Whatman no. 41, the filtrate was extracted to attract the remaining naphthalene in extreme waste on the reactor using dichloromethane. Dichloromethane (non-polar) extracts and polar extracts were separated using separating funnels. Dichloromethane extract is ready to be measured using CG-MS and –IR spectrophotometer. The same process above is done using PS type bacteria [25].

*2.4 Bio-adsorption process of sponge symbiont bacteria to test heavy metals.*

Determination of the bio-adsorption power of sponge symbiont bacteria against Cr (VI) and Cd (II) metals, was carried out by preparing a 2 x 6 bio-adsorption capacity of 250 mL capacity and labeled (duplo). Each reactor was filled with 50 mL BP type symbiotic sponge bacterial suspension. BP bacterial suspension in the reactor was incubated for 1 x 24 hours. After the incubation period is reached with 50 mL of extreme waste is added to each reactor, then, agitated using a Shaker incubator with a rotation of 100 rpm. Determination of bacterial bio-adsorption power by measuring the absorbance of the media using the AAS instrument is done every 3 days for 15 days (0, 3, 6, 9, and 15) days. Absorbance measurement by sample procedure in the reactor was filtered using Whatman filter paper No. 41. The filtrate obtained was acidified at pH 3 using the HCl/KOH sauce homogenized on a magnetic stirrer. The sample is ready to be absorbed. The same procedure is carried out using a suspension of PS bacteria symbiont sea sponge [19,25].

*2.5 Presentation and analysis of data.*

Determination of the biodegradation power of BP and PS bacteria to naphthalene was determined by looking at the chromatogram measured by GC-MS according to the abundance, area and apparent retention time, types of organic compounds formed. Biodegradation products in the form of simple organic compounds are determined based on data from chromatograms or transmittances that appear

to be GC-MS measurements and IR results by matching the wave number (frequency) in the chromatogram against the literature to determine the functional groups of organic compounds in the biodegradation media.

Capacity, efficiency and bio-adsorption power of BP and PS bacteria against heavy metals Cr (VI) and Cd (II) based on absorbance data from AAS measurements. The bio-adsorption capacity and efficiency values are determined using the equation:

$$Q = \frac{C1-C2}{C1} x\ V, \quad (1)$$

$$\%\ E = \frac{C1-C2}{C1} x\ 100\%, \quad (2)$$

*Note:* Q = bio-adsorption capacity (mg/L); $C_1$ = concentration before contact (mg/L); $C_2$ = concentration after contact (mg/L); m = absorbance mass (mg); V = volume of solution (L) and % E = bio-adsorption efficiency [26,27].

## 3. Results and discussion

The types of marine sponges and symbionts bacteria are numerous and have their respective characteristics. The selection of sponge symbionts for utilization of environmental remediation contaminated by PAHs and heavy metal waste should meet the criteria and requirements in order to provide maximum results in reducing the toxic properties of waste [1,20]. Criteria for isolation of sponge symbionts bacteria for the utilization of hydrocarbon biodegradation should be isolated from sponge species whose body surface is covered by mucus or enzymes characterized as a sign that the sponge in its growth environment has successfully responded to survive against predators that threaten its life by forcing the symbiont bacteria to produce substances poison in the form of mucus. Bacteria symbiont sponge for bio-adsorption applications of heavy metals by isolating from sponges included in the bio indicator and bio monitoring categories of heavy metals [4,27]. Criterion is that the selected bacterial isolates are sponge symbionts bacteria that meet the phenotype analysis criteria, namely reacting positively to at least 4-5 reagents in biochemical tests, namely catalase, methyl red (MR), Voges Proskauer (VP), lactase and citrate. This selection is intended so that biodegradation and bio-adsorption of extreme waste have a high success rate and also aims to cut procedures, time and use of various types of test reagents that are many [25].

### *3.1 Biodegradation power of the sponge symbionts bacteria against naphthalene.*

Biodegradation of PAHs by the work of bacteria as biodegrading is intend to reduce the power of waste toxicity by establishing specific biodegradation mechanisms according to the type of bacteria used and the type of PAHs waste. GC-MS measurement results (Figures 1 and 2) show a decrease in naphthalene abundance after 30 days of interaction. A decrease in the abundance of naphthalene is followed by a new peak that arises, presumably organic compounds from biodegradation of BP bacteria. Decrease in naphthalene abundance after 30 days of contact as an indicator that naphthalene concentrations in extreme waste are reduce.

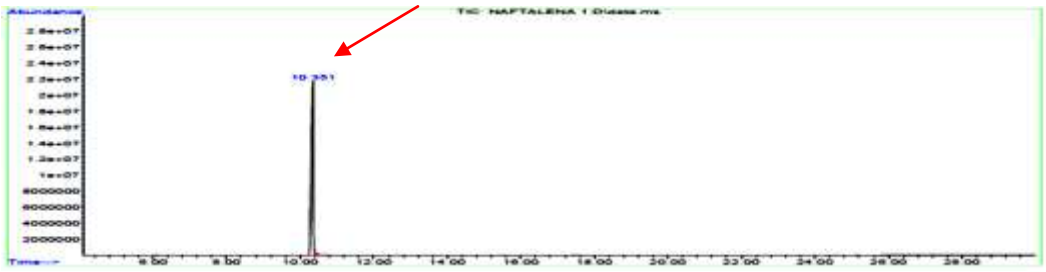

**Figure 1.** Chromatogram of naphthalene GC-MS results from interaction of *Bacillus pumilus* strain GLB197 (BP) sponge *Niphates Sp* isolates to extreme waste containing naphthalene and heavy metals Cr (VI) and Cd (II) contact period 0 days (control)

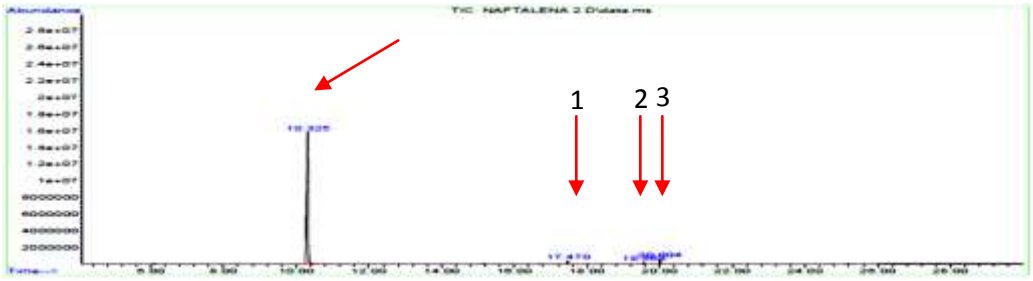


**Figure 2.** Chromatogram of naphthalene GC-MS results from interaction of *Bacillus pumilus* strain GLB197 (BP) sponge *Niphates Sp* isolates to extreme waste containing naphthalene and heavy metals Cr (VI) and Cd (II) with a contact period of 30 days.

The number of new peaks identified on the GC-MS chromatogram (Figure 2) after contact with BP bacteria for 30 days, according to the print text pdf data GC-MS is an organic compound in the form (see number and arrow): (1) Bicyclo [5.3.0 ] decapenta quality 95%, (2) O-1,2,2-trimethylpropyl methyl-phosphonate quality 97% and (3) there are 2 components, namely 2,6-dimethylphenyl and dimethylamino)-henazone quality 98%.

The percentage of biodegradation of BP bacteria to naphthalene after contact for 30 days reached 7.16% or equivalent to 928.4 gm / L. This means that only decreased by 71.6 mg / L, much smaller than the achievements in previous studies with a value of 42.28%, but using a single contaminant naphthalene, without mixing with heavy metals chromium and cadmium [3,23]. This shows that there is an influence of heavy metal activity constraints on the biodegradation performance of BP bacteria.

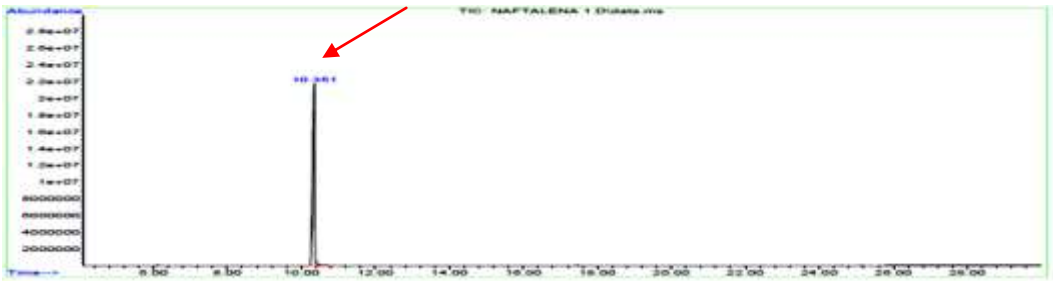

**Figure 3.** Chromatogram of naphthalene GC-MS results of bacterial interaction of *Pseudomonas stutzeri* strain SLG510A3-8 (PS) sponge *Hyrtios erectus* isolate against extreme waste containing naphthalene and heavy metal Cr (VI) and Cd (II) contact period 0 days (control)

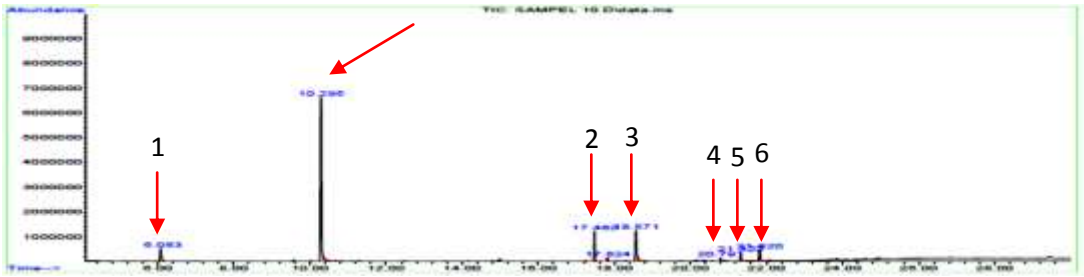


**Figure 4.** Chromatogram of naphthalene GC-MS result of interaction of *Pseudomonas stutzeri* strain SLG510A3-8 (PS) sponge *Hyrtios erectus* isolate against extreme waste containing naphthalene and heavy metal Cr (VI) and Cd (II) with a contact period of 30 days.

Comparing naphthalene according to Figures 3 and 4, there was a decrease in abundance, indicating that the level of naphthalene in extreme waste was reduces due to the biodegradation activity of PS bacteria. The decrease in the abundance of naphthalene is followed by the emergence of about 6 new peaks and 8-9 components of organic compounds of biodegradation products. The components according to GC-MS print text data (Figure 4), arrows 1-6) are: (1) Cyclotetrasiloxane quality 94%, (2) Cyclopenta cyclohe zunamic and Trimethylbicyclo [3.3.0] octan-2,8-dione quality 93 %, (3) 1-ethyldioxyindol and *p* methyl naphthalenol quality 94%, (4) 4,5-Dimethoxyindole-3-carbaldehyde quality 93%, (5) as 9-Octadecen amide and *as* amide (Z) -9-Octadecenoid quality 96% and (6) 2,4-bis (1-methyl-1-phenylethyl) 98% quality. The biodegradation performance of PS bacteria to naphthalene reached 11.24%, slightly higher than BP bacteria, but much smaller than the performance of PS biodegradation against the same PAHs, but without being mix with heavy metal contaminants. This achievement strengthens the suspicion that heavy metals have a strong influence on the biodegradation activity of PS in degrading naphthalene [18].

The organic compounds of biodegradation products of BP and PS bacteria (Figures 2 and 4) are organic compounds of aldehydes, ketones, alcohols and carboxylic acids. These results are consistent with the theory that the bacterial biodegradation product for hydrocarbon components is generally in the form of simple organic compounds, aldehydes, ketones, hydroxyl-alcohols, carboxylic acids and esters. The mechanism of biodegradation of hydrocarbons by bacteria begins with an oxidation reaction on the PAHs molecule, then changes to alcohol in the form of keto-enol, then becomes an aldehyde then carboxylic acid and as an ester and ketone as well as the presence of gas, water and gives off a foul odor such as glucose fermentation reaction. Further explanation of the mechanism and acidic products in the biodegradation reaction of PAHs by bacteria that the process of biodegradation that takes place on the activity of bacteria against the type of PAHs is difficult to reach 100%, due to the presence of biodegradation products in the form of acidic substances that inhibit bacteria from defending themselves, so the biodegradation process stops. This phenomenon is termed the biodegradation limiting agent [17,28].

When comparing the biodegradation power of the two types of sponge symbionts bacteria, it can be said that PS bacteria have better biodegradation ability than BP bacteria, this can be seen from the number of new peaks formed by 6 pieces and the number of components identified as many as 8-9 types on the chromatogram GC-MS (Figure 4), while the GC-MS chromatogram on the use of BP type bacteria, identified 3 new peaks and 3-4 kinds of components (Figure 2).

*3.2 Biodegradation Products Bacteria symbiont sponge against Naphthalene.*

Analysis of bacterial biodegradation products for PAHs can know with certainty through the observation of the functional group components of organic compounds using the Spectrophotometer - IR instrument, as shown in Figure 5.

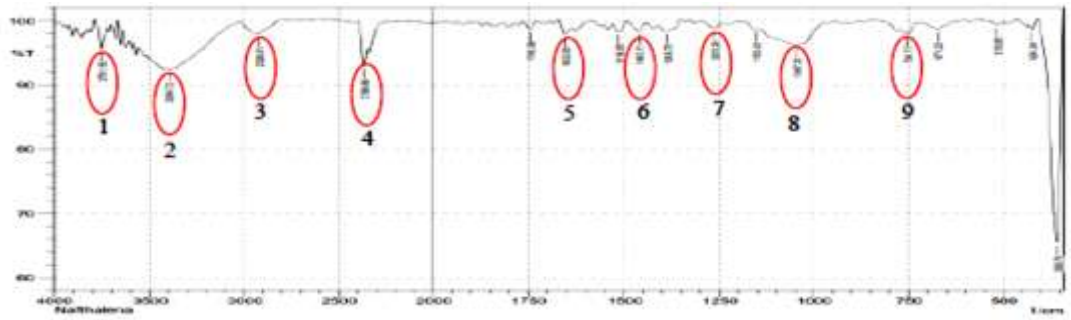


**Figure 5.** Chromatogram of the results of spectrophotometry-IR measurements against naphthalene biodegradation media using *Bacillus pumilus* strain GLB197 (BP) sponge isolate *Niphates Sp* in extreme waste after 30 days contact.

Transmittance data and wave numbers of organic components resulting from naphthalene biodegradation using BP bacterial biodegrading (Figure 5), identified 17 peaks indicate that frequency (cm-1) or wave number (ὺ) successively number 1 is a –OH alcohol and methylene group with vibrations strain, number 2 is estimated to be aromatic CH, is an asymmetric strain, 3 is CH alkanes containing C-H vibrational strain, 4 is methyl from carboxylic acid, 5 alkene (C=C) and aldehyde component, number 6 is a functional group of ketones and ether, 7 group (C=O) of esters and also shows the presence of esters, number 8 is the alcohol component in the form of wobble vibration and 9 is a component of -CH2- CH vibration wobble

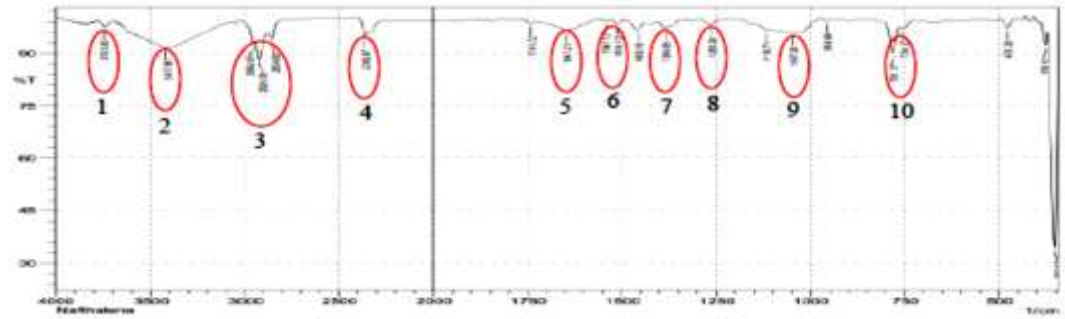


**Figure 6.** Chromatogram of the –IR spectrophotometry measurement results for naphthalene bio-degradation media using Pseudomonas stutzeri strain SLG510A3-8 (PS) sponge isolate *Hyrtios erectus* in extreme waste after 30 days contact

Spectrophotometric-IR chromatogram (Figure 6) peak number 21, showing the results of identification of relative and identical functional groups with chromatogram -IR (Figure 5), at successive frequencies starting sequence number 1 is the heavy chain alkane, number 3 the methylene group or alkanes with vibrations asymmetrical strain and also the presence of methylene vibration symmetric strain, number 4 is estimated to be the -OH carboxylic acid group, number 5 is group C = O ketone and ester, number 6 is group C = C aromatic vibration asymmetric strain and also symmetric strain, number 7 is type methylene group bending vibration CH, number 8 group C-CH3 vibration shake and there are also groups of CO ether and ester, and number 9 is the aldehyde group while number 10 is the tertiary butyl group CC vibration. This -IR data with the alleged cluster as shown in Figures 5 and 6, shows that there are organic compounds of alcohol, aldehyde, ether, ketone, ester and carboxylic acid groups in the interaction media that are expected to be biodegradable products of BP and PS type symbiont sponge bacteria [15,17].

Analysis of data and information Figures 2 and 4, it appears that a new peak which is a biodegradation product compound in the form of a simple organic compound of alcohol, esters,

aldehydes, ketones, turns out to be identical to the results of the IR-analysis data (Figures 5 and 6) at the appropriate frequency also hints new components of biodegradation products are alcohol, aldehyde, ketone and ester compounds.

*3.3 Bio-adsorption power of sponge symbiont bacteria on heavy metal test.*

Bacterial bio-adsorption power of heavy metals is determined based on the calculation of the capacity and efficiency of absorbance measurement data using the AAS instrument. The data shown in Figure 7 shows that the bio-adsorption power of BP bacteria is more dominant against Cd (II) contaminants compared to Cr (VI) contaminants, this can be seen in the capacity and efficiency of BP bacteria bio-adsorption of Cd (II) ions reaching 56.30 % (Figure 7.b), higher than that of Cr (VI) ions reaching only 52.74% (Figure 7.a). Theoretically this result can be reviewed in 2 aspects, namely the aspect of the toxic properties of Cr (VI) which is higher than Cd (II) and the second aspect, namely the position of the two heavy metals of these contaminants in the periodic arrangement of elements causing ionic radii, electron affinity and ion electronegativity Cd (II) tends to be greater than Cr (VI). Based on these two aspects, it is very possible if the bio-adsorption power of BP bacteria against Cd (II) is higher than that of Cr (VI) ions [28]. The capacity and efficiency of bio-adsorption of BP bacteria on the Cr (VI) and Cd (II) heavy metals after contact for 15 days, shown in Figure 7.

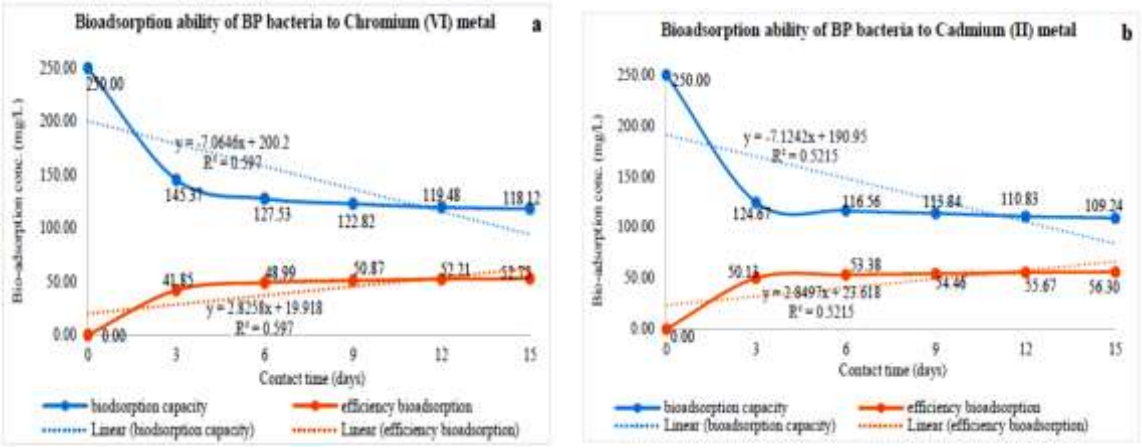


**Figure** 7. Relationship between capacity and efficiency of bio-adsorption of BP bacteria on (a) heavy metal Cr (VI) and (b) heavy metal Cd (II)

The bio-adsorption ability of PS bacteria against Cd (II) heavy metal contaminants was higher than that of Cr (VI). This situation can be seen in the capacity and efficiency of bio-adsorption achieved by PS bacteria against Cd (II) ion contaminants reaching 61.23% (Figure 8.b) higher than the capacity and bio-adsorption of these bacteria against Cr (VI), reaching 57, 80 % (Figure 8.a).

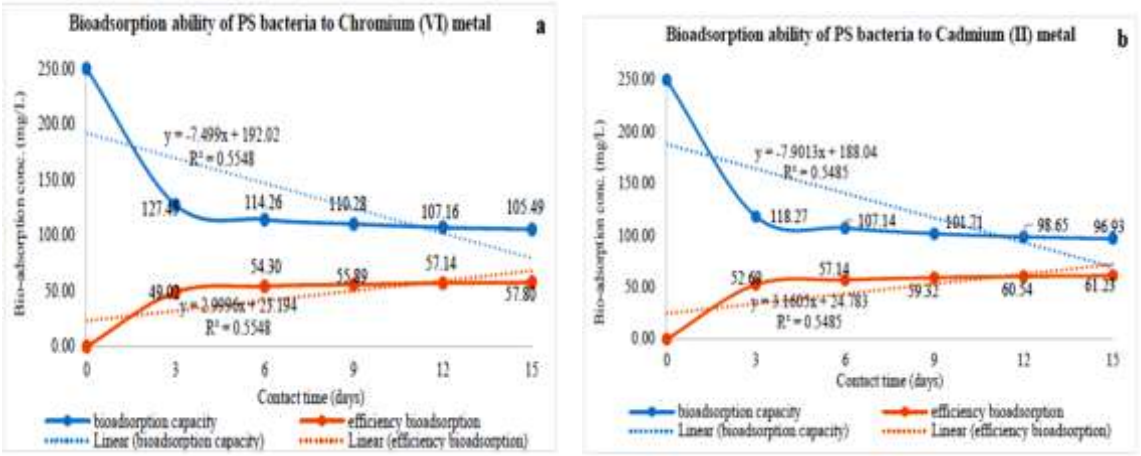


**Figure 8.** The relationship between the capacity and efficiency of bio-adsorption of PS bacteria on (a) heavy metal Cr (VI) and (b) heavy metal Cd (II)

Comparing the bio-adsorption performance between the two types of bacteria to the heavy metal types Cr (VI) and Cd (II), it appears that the bio-adsorbent of PS (*Pseudomonas stutzeri* strain SLG510A3-8) strains is stronger in adsorbing both Cr (VI) ions and Cd ions (II) compared BP bacteria (*Bacillus pumilus* strain GLB197) against the same metal. Based on Figure 9, it shows that PS bacteria have a stronger bio-adsorption power than BP bacteria, both against Cr (VI) ion contaminants (Figure 9.a), and against Cd (II) ion contaminants, (Figure 9.b). The bio-adsorption capacity and efficiency achieved by BP and PS bacteria against Cr (VI) and Cd (II) ion contaminants in this experiment are much smaller than the achievements in previous studies using PS and BP bacteria against single metal heavy chromium and cadmium contaminants with their respective efficiencies. respectively reaching 78.17% and 81.42% [25,29]. This result can be seen in Figure 9.

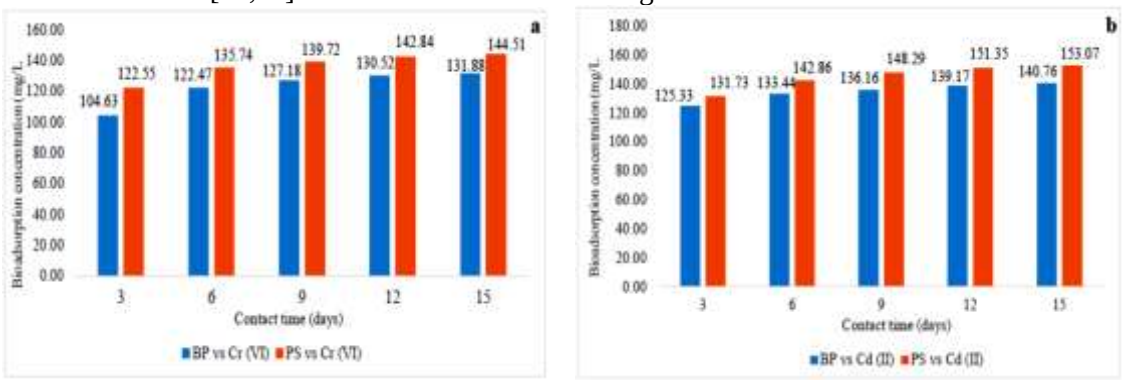


**Figure 9.** The relationship of bio-adsorption strength of BP bacteria and PS symbiont sponge against (a) heavy metal Cr (VI), and (b) heavy metal Cd (II)

This shows that there is an influence of the presence of naphthalene in extreme waste inhibiting factors of the bio-adsorption activity of BP and PS bacteria on heavy metal contaminants. The effect of naphthalene contaminants that inhibits the bio-adsorption ability of BP and PS bacteria on the test heavy metals can also be seen in the correlation value (R2) of the regression equation of the BP and PS bacteria on the test metal. Bio-adsorption of BP bacteria against Cr (VI) ions with correlation value R2 = 0.5970 and for Cd (II) ions R2 = 0.5125, whereas bio-adsorption of PS bacteria against Cr (VI) ions with correlation value R2 = 0.5548 and with respect to Cd (II) ions, R2 = 0.5485. The bio-adsorption correlation values of these two types of bacteria to the heavy metals chromium and cadmium indicate that there are other factors that influence the bio-adsorption activity of the BP and

PS bacteria on the heavy metals Cr (VI) and Cd (II) ions. The inhibiting factor thought to be due to the presence of naphthalene in extreme waste.

Another effect is the competition between the two types of BP and PS bacteria resulting in biopsy attraction between the two against the heavy metal test to form chelate [30,31]. he superiority of the bio-adsorption of BP and PS bacteria on the test heavy metals compared to biodegradation of naphthalene is due to the reduction of naphthalene by bacteria taking a biodegradation mechanism that requires a reduction in the structure of the naphthalene molecule requires a longer time compared to the reduction of heavy metal toxicity by using the bio-adsorption method through the mechanism of forming bacteria chelating metal ion [32,33]. This phenomenon is a finding as well as a new and interesting case to be studied more deeply, about what effect the presence of a type of PAHs in waste has to do with the performance of bacterial bio-adsorption of heavy metals and vice versa how does the influence of the presence of heavy metals on the ability of certain bacterial biodegradation of PAHs.

## 4. Conclusion

Bacteria *Bacillus pumilus* strain GLB197 (BP), *Niphates Sp* sponge isolate and *Pseudomonas stutzeri* strain SLG510A3-8 (PS), sponge isolates *Hyrtios erectus,* have biodegradation and bio-adsorption activity against extreme waste contaminated with a mixture of naphthalene and Cr (VI) and Cd (II) heavy metal components. The level of biodegradation of BP bacteria to naphthalene was only 7.16% and PS bacteria was 11.24%. The bio-adsorption efficiency of BP bacteria on Cr (VI) ions reached 56.30% and on Cd (II) 61.23%, while the bio-adsorption of PS bacteria on Cr (VI) ions was framed 52.74% and on Cd (II) ions in numbers 57.80%. Naphthalene biodegradation products by BP and PS bacteria are in the form of organic compounds of alcohol, aldehyde, and carboxylic acids. The biodegradation ability of PS bacteria to naphthalene in extreme waste tends to be better than BP bacteria. The bio-adsorption power of PS bacteria on Cr (VI) and Cd (II) ions in modified extreme wastes was more dominant than BP bacteria on heavy metals test. There is an effect of naphthalene on the bio-adsorption performance of BP and PS bacteria and conversely there is an effect of inhibition of heavy metal biodegradation activity on the ability of bacteria to adsorb test heavy metals.

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