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Biodegradation of phenol, anthracene and acenaphthene singly and consortium culture of indigenous microorganism isolates from underground coal gasification area

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Abstract. *The gasification of Coal has a potential for long term energy supply in the future by conversion of non-mineable coal seams to syngas for various industrial applications. However, recalcitrant aromatic compounds such as phenol, anthracene, and acenaphthene from UGC are potential to contaminate groundwater, soil and the broader environment. Therefore, the polluted environment required bioremediation. The purpose of this study was to determine the capability of Bacillus velezensis and Stenotrophomonas maltophilia isolated from the area of Underground Coal Gasification (UCG) on biodegradation of phenol, anthracene, and acenaphthene in the single and mixed cultures. The degradation of pollutants in the concentration range of 250-500 ppm evaluated by measuring of the ability of bacteria to grow in single component supplemented mineral salt media, screening tests for hydrocarbon-degrading bacteria and biodegradation ability by gravimetric analysis method. The results showed biodegradability of phenol, anthracene, and acenaphthene at concentration 500 ppm after 14 days by S. maltophilia were 60,2%, 80,4%, and 82,5%. B. velezensis showed 79% of biodegradability of phenol, anthracene 79,8%, and acenaphthene 81,1%. The consortium of both isolates has the highest percentage of phenol, anthracene, and acenaphthene biodegradation 83,4 %, 83, 53 % and 83,47 % respectively.*

Keywords : *Biodegradation, mixed culture, S. maltophilia, B. velezensis, phenol, anthracene, acenaphthene*



1. Introduction

Coal is a dominant energy source in electricity generation. The world's total energy output is approximately 27%, and more than 39% of all electricity is produced by coal-fired power plants [1]. In the coal mining industry, there are combustion and pyrolysis processes that produce coal tar containing phenol compounds and Polycyclic aromatic hydrocarbons (PAHs). Phenol and PAHs are pollutants of organic compounds that are widespread. Phenol has a composition of 20-30% in coal tar and coal liquefied oil. As for anthracene and acenaphthene which include PAHs with three aromatic rings, it is known to have a composition of 21.6% -52.5% of the total balance of coal [2]. Pollutants from coal industry process cause environmental damage and changes in land use and morphology.

Pollutants have the potential to accumulate in biota that lives in waters. PAHs contained in coal can also cause cancer if exposed to humans in a certain amount, have mutagenic effects, are persistent, carcinogenic and teratogenic in various organisms. PAHs can accumulate in low-level animals at high levels because of their nature which is difficult to digest by the body. Anthracene is a three-ring PAH with relatively serious toxicity. Once anthracene enters the body, it appears to target the skin, stomach, intestines and the lymphatic system, and it is a probable inducer of tumors [3].

The use of microorganisms derived from coal has been shown to reduce levels of phenols and PAHs such as anthracene and acenaphthene so that they can be used as an alternative to prevent or reduce coal waste in the environment. The results of [4] showed that *Acinetobacter calcoaceticus* was able to degrade phenol up to 91.6% within 48 hours. Research by Mirnawati et al.[5] revealed that the degradation power of *Bacillus cereus* against anthracene compounds isolated from aquatic waste could reach 98.11% within 12 days of incubation. This research is essential to do because of the lack of biodegradation research that utilizes indigenous bacteria isolates from the coal itself.

Indigenous bacteria are known to have good environmental adaptability so that the decomposition process of pollutant compounds can run optimally. In the previous preliminary test, we obtained coal-indigenous bacteria isolates one seam. The UCG14 drill point has the potential to degrade anthracene and acenaphthene compounds. Therefore, this study was conducted to deepen the ability to test the ability of indigenous bacteria isolates from coal to degrade phenol, anthracene, and acenaphthene. This study aims to evaluate the potential of indigenous coal bacteria in degrading phenol, anthracene, and acenaphthene in singly and consortium. The results of the study are expected to become scientific information especially about biodegradation and support environmentally friendly waste treatment systems in the mining or coal processing industry.

2. Material And Methods

The method used in this study is an experimental method with Completely Randomized Design (CRD) with two factors. Parameters measured using the experimental method included measurement of bacterial growth using a spectrophotometer at a wavelength of 600 nm and the Total Plate Count (TPC) method. Evaluation of the potential for biodegradation of hydrocarbons was carried out using a secondary screening test of hydrocarbon-degrading bacteria by agar diffusion method in well, and the examination of bacterial biodegradation ability by gravimetric analysis method [6]. Each treatment was carried out with three replications. The bacterial isolates used in this study were *Stenotrophomonas maltophilia* and *Bacillus velezensis*, which were isolates of original coal one seam D drill point UCG14 came from a coal mine in Sekayu, Musi Banyuasin Regency, South Sumatra. The research data obtained were analyzed statistically by Variance Analysis (ANOVA) F test with a 95% confidence level and if there were significant differences followed by Duncan's Multiple Range Test.

3. Results And Discussion

3.1 Tolerance of *S. maltophilia* and *B. velezensis* against Phenol, Anthracene, and Acenaphthene

The growth ability of *S. maltophilia* and *B. velezensis* was carried out using the Total Plate Count (TPC) method. At this stage, the number of *S. maltophilia* and *B. velezensis* colonies calculated on mineral salt medium (MSM) which each contained phenol, anthracene, and acenaphthene in a

concentration of 250 mg L⁻¹ and 500 mg L⁻¹. Colonies of bacteria grown on the medium indicated that both types of bacteria were able to degrade phenol, anthracene, and acenaphthene at concentrations of 250 ppm and 500 ppm. The results of the TPC were statistically analyzed using Variance Analysis (ANAVA) with a confidence level of 95% and continued with Duncan's Multiple Range Test.

Table 1. Duncan's Multiple Range Test on *S. maltophilia* and *B. velezensis* populations on phenols, anthracene, and acenaphthene at concentrations of 250 mg L⁻¹ and 500 mg L⁻¹

Species (x 10 ⁶) CFU	Concentration mg L ⁻¹	Hydrocarbon		
		Phenol	<i>Anthracene</i>	<i>Acenaphthene</i>
<i>S. maltophilia</i> (x 10 ⁶)	250	4,28 c C	4,88 b C	5,99 a C
	500	6,02 c B	7,48 a A	6,45 b B
<i>B. velezensis</i> (x 10 ⁶)	250	5,79 b B	5,75 b B	7,23 a A
	500	8,27 a A	7,36 b A	7,37 b A

Annotation : The same letter (horizontal/horizontal direction) and the same capital letter (down / vertical direction) indicates that the value is not significantly different.

Based on Duncan's Multiple Range Test the highest number of *S. maltophilia* bacterial cells was found in medium containing acenaphthene at a concentration of 250 mg L⁻¹, with a number of 5,99 x 10⁶ CFU / ml. At a concentration of 500 mg L⁻¹, the highest average number of *S. maltophilia* bacterial cells was found in medium containing anthracene with a population of 7,48 x 10⁶ CFU / ml. The highest average number of *B. velezensis* bacterial cells was observed in medium containing acenaphthene at a concentration of 250 mg L⁻¹, with a population of 7,23 x 10⁶ CFU / ml. At a concentration of 500 mg L⁻¹, the highest average number of *B. velezensis* bacterial cells was found in medium containing phenol with a population of 8,27 x 10⁶ CFU / ml. Overall, comparisons between the two species show that *B. velezensis* produces an average bacterial cell number higher than *S. maltophilia*. Factors that can influence are the characteristics of the *Bacillus* genus that has endospores.

One of the characteristics of endospores is resistance to high hydrocarbon levels [7]. Besides, as Gram-positive bacteria, *B. velezensis* has thicker peptidoglycan walls. The peptidoglycan wall is reported to be able to absorb contaminant compounds (phenols, anthracene, and acenaphthene) [8]. Based on the high of bacterial populations on medium containing phenol, anthracene, and acenaphthene, it can be indicated that *S. maltophilia* and *B. velezensis* were able to degrade these three types of hydrocarbons, and were tolerant to concentrations of 500 mg L⁻¹. Both types of bacteria can use hydrocarbon compounds as carbon sources and energy for their growth. Tolerance of bacteria toward high levels of phenol may be due to genetic changes in specific mutation in the plasmid carrying genes for phenol degradation [9] or horizontal gene transfer. Tolerance of bacteria to a particular concentration of phenol is not related to the degradation of such amount. the tolerance of *Xanthobacter flavus* to 1100 mg L⁻¹ of phenol, but this strain could not be given in the amount and showed the ability to degrade only 650 mg L⁻¹ phenol [9].

3.2 Growth of *S. maltophilia* and *B. velezensis* Medium Salt Mineral (MSM) containing phenol, anthracene, and acenaphthene at a concentration of 500 mg L⁻¹.

The growth of *S. maltophilia* and *B. velezensis* bacteria was carried out on MSM, each of which was added with phenol, anthracene, and acenaphthene with a concentration of 500 mg L⁻¹, for 5 days (120 hours), at a temperature of 30°C in a rotary shaker with a speed of 120 rpm to increase aeration so that

oxygen can circulate. Oxygen plays a role in the activation of mono- and dioxygenase enzymes so that the initiation of phenolic, anthracene, and acenaphthene oxidation aromatic hydrocarbon rings can occur [10]. Measurement of cell counts is carried out every 12 to 108 hours. The initial inoculant concentration is 10% of the total MSM volume. Growth curves illustrate bacterial growth profiles, and time is most effective in degrading hydrocarbons in MSM medium. The growth curve consists of four phases, namely phase lag, exponential phase, stationary phase, and phase of death. In the lag phase or adaptation phase, microorganisms need time to adapt to growing medium nutrients.

Bacterial cells need time to produce oxygenase enzymes is necessary for PAH in the medium. Bacteria initiate PAH degradation by the action of intracellular dioxygenases. The PAHs must be taken by the cells before degradation can take place. Bacteria of the most often oxidize PAHs to cis-dihydrodiols by the incorporation of both atoms of an oxygen molecule. The cis-dihydrodiols are further oxidized, first to the aromatic dihydroxy compounds (catechols) and then channeled through the ortho-meta cleavage pathways [3].

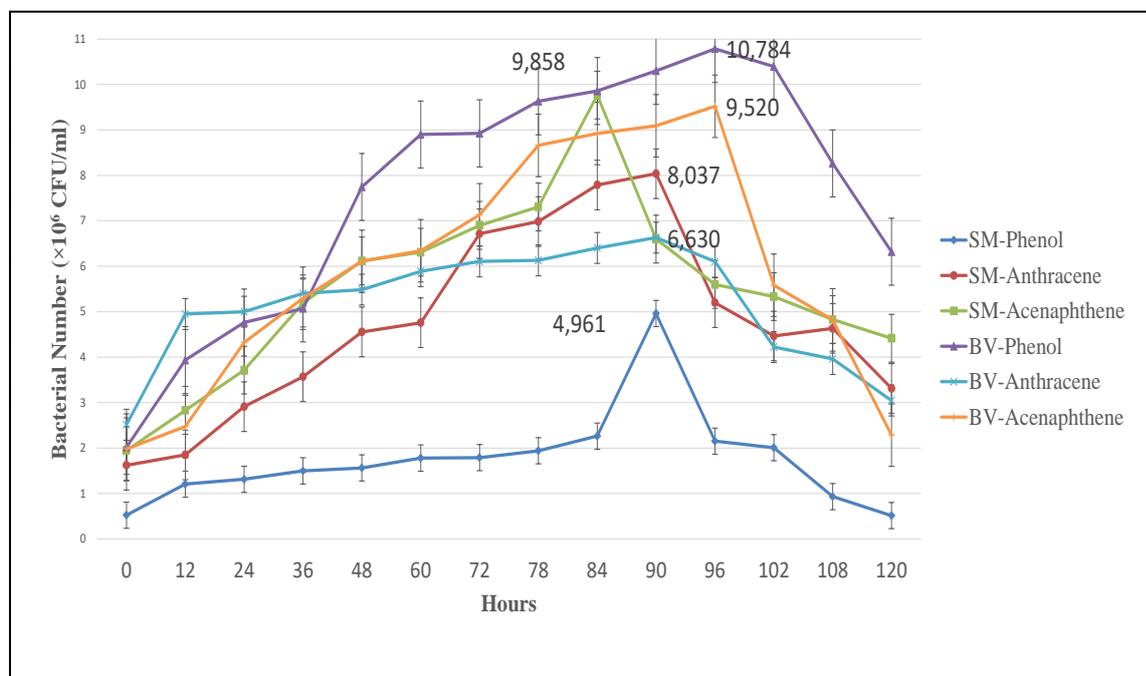
From Figure 1. In phenols, *S. maltophilia* requires adaptation for up to 48 hours, and reaches an exponential peak at 90 hours with a bacterial density of $4,96 \times 10^6$ CFU, in the adaptation phase anthracene reaches 60 hours, the exponential phase at 90 hours with bacterial density $8,37 \times 10^6$ CFU. While the acenaphthene adaptation phase is only 24 hours, the exponential phase at 84 hours with a bacterial density of $9,85 \times 10^6$ CFU. The ability to grow *S. maltophilia* on phenol indicates that *S. maltophilia* can degrade phenol and use it as nutrients, but the growth of *S. maltophilia* in anthracene and acenaphthene is higher than in phenol. According Han et al., in Ahmad, et al. [9], degradation of phenol by *S. maltophilia* can reach 805 mg L⁻¹ within 48 hours. Furthermore, *Stenotrophomonas* sp. NCCP-310 has maximum growth after 49 hours and can degrade phenol for 65 hours. In phenol, the adaptation phase of *B. velezensis* was found at 12 hours, the exponential phase at 96 hours with a bacterial density of $10,78 \times 10^6$ CFU. In the anthracene, the adaptation phase occurred in 24 hours, the exponential phase in 90 hours but the cell population only reached $6,63 \times 10^6$ CFU, whereas in acenaphthene the adaptation phase occurred in 12 hours, the exponential phase at the 96 hours with the population reaching $9,52 \times 10^6$ CFU [9].

Based on bacterial growth, *B. velezensis* can decompose phenol very fast and is more effective than anthracene and acenaphthene. According to Palleroni [11], the growth of *Stenotrophomonas* heterotrophically can high by adding organic compounds as carbon and energy sources. Therefore, it can be interpreted that *S. maltophilia* and *B. velezensis* can use organic compounds contained in hydrocarbons (phenols, anthracene, and acenaphthene) as carbon and energy sources. Hydrocarbons are rich sources of carbon and energy needed for microbial growth, including bacteria [10]. Bacteria obtain energy from the catabolism process which starts from the oxidation process of aromatic hydrocarbon rings. Some *Bacillus* can utilize phenol genera as the sole source of carbon and energy include *Bacillus subtilis*, *Bacillus brevis* [4]. Under aerobic conditions, degradation of phenol was shown to be initiated by oxygenation into catechols as intermediates followed by a ring cleavage at either the ortho or meta position, depending on the type of strain. Aerobically, the first phenol converted to catechol. Subsequently, the catechol degraded via ortho or meta fission to intermediates of central metabolism. The initial ring fission is catalyzed by ortho cleaving enzyme, catechol 1, 2 dioxygenases or by a meta cleaving enzyme catechol 2,3 dioxygenase, where the product of ring fission is cis-muconic acid for the former and 2-hydro cis muconic semialdehyde for the latter [12].

Growth of *B. velezensis* cells is relatively higher compared to *S. maltophilia*. According to Das et al. [7]. *Bacillus* sp. known to have better hydrocarbon degradation power compared to other isolates from areas contaminated with pollutants. The high ability of *Bacillus velezensis* to degrade hydrocarbons is due to the characteristics of *Bacillus* sp. who are tolerant of high levels of hydrocarbons in the soil because the endospores are resistant so that members of the genera can survive and grow in the environment.

It is known that the first step in the bacterial aerobic degradation of PAH requires a dihydroxylation reaction and subsequent formation of *c* / *s*-dihydrodiol which is carried out by membrane-bound enzyme systems. Further oxidation leads to the creation of catechol which becomes

the substrate for other dioxygenases. Bacteria dioxygenases have great potential biotechnology since they participate in biodegradation of many natural and xenobiotic compounds [9].



Annotation : SM: *S. maltophilia*; BV: *B. velezensis*

Figure 1. Graph. of growth curves of *S. maltophilia* and *B. velezensis* on phenols, anthracene, and acenaphthene

The growth of both types of bacteria showed that *S. maltophilia* on the medium containing acenaphthene had the highest number of cells compared to the medium containing phenol and anthracene. *B. velezensis* on a medium containing phenol showed the highest number of cells compared to medium containing anthracene and acenaphthene. When observed as a whole, the two types of bacteria show the highest cell count in a medium containing acenaphthene. Therefore, based on the growth curve, it can be seen that acenaphthene is the most easily decomposed compound by *S. maltophilia* and *B. velezensis*, compared to phenol and anthracene compounds. While the compounds that are most difficult to decompose by *S. maltophilia* and *B. velezensis* are anthracene. Research by Xu et al. [13], revealed the influence of various factors on the degradation of hydrocarbons by various strains of bacteria. These factors include pH, the concentration of pollutants (hydrocarbons), sources of nitrogen and carbon, and the addition of metal ions. As a result, bacteria generally more efficiently degrade hydrocarbons at pH nine than pH 7. The efficiency of hydrocarbon degradation decreases with increasing hydrocarbon concentrations and is limited to the growth of bacterial strains. However, both of strains show high degradation at all concentrations so that it exhibits high resistance to toxicity.

3.3 Secondary Screening of *S. maltophilia* and *B. velezensis* at Phenol, Anthracene, and Acenaphthene Concentrations of 250 and 500 ppm.

Secondary screening is done by agar diffusion method in wells. At this stage, *S. maltophilia* and *B. velezensis* tested for their ability to use hydrocarbons from phenols, anthracene, and acenaphthene at concentrations of 250 ppm and 500 ppm, contained in MSM. The ability to use hydrocarbons of these two bacterial species evaluated by the formation of clear zones around the well. The diameter of the

clear zone was then analyzed using Variance Analysis (ANOVA) with a confidence level of 95% and continued with Duncan's Multiple Range Test (table 2.)

Table 2. Duncan's Multiple Range Test on a secondary screening of *S. maltophilia* and *B. velezensis* on phenols, anthracene, and acenaphthene with concentrations of 250 and 500 mg L⁻¹.

Clear zone on well of Species (mm)	Concentration mg L ⁻¹	Hydrocarbon		
		Phenol	<i>Anthracene</i>	<i>Acenaphthene</i>
<i>S. maltophilia</i> (mm)	250	17,13 b A	16,46 b B	19,13 a B
	500	12,40 a B	13,87 a C	12,73 a C
<i>B. velezensis</i> (mm)	250	16,67 b A	19,93 ab A	22 a A
	500	12,43 a B	11,50 a D	11,80 a C

Annotation : The same letter (horizontal/horizontal direction) and the same capital letter (down / vertical direction) shows the value is not significantly different according to Duncan's Multiple Range Test

Based on the results of Duncan's Multiple Range Test, *S. maltophilia* produced a larger clear zone diameter at a concentration of 250 ppm compared to 500 ppm. Also, at the strength of 250 ppm, the largest width of a clear zone was produced in acenaphthene, which was 19.13 mm. While at a concentration of 500 ppm, the largest clear zone diameter found in anthracene which is 13.867 mm. As with *S. maltophilia*, *B. velezensis* produces a clear zone with a larger diameter at a concentration of 250 ppm compared to 500 mg L⁻¹. At a concentration of 250 ppm, the largest clear zone diameter produced on a medium containing acenaphthene, which was 22 mm. *B. Velezensis* can also degrade phenol concentration of 500 mg L⁻¹, and provide a clear zone diameter of 12.433 mm. *B. velezensis* produced an average clear zone diameter greater than *S. maltophilia* in three types of 250 mg L⁻¹ concentration of hydrocarbons, while *S. maltophilia* in all 500 mg L⁻¹ concentration compounds produced an average diameter of a clear zone that was greater than *B. velezensis*. At the same concentration. Differences in ability in types of compounds and concentrations show that hydrocarbon degradation factors can cause the degradation of hydrocarbons by bacteria by indigenous microorganisms, which have a specific metabolic capacity [14].

3.4 Biodegradation ability of *S. maltophilia*, *B. velezensis*, and Consortium.

The biodegradation ability of phenol, anthracene, and acenaphthene at concentrations of 500 mg L⁻¹ can be seen in table 3.

Table 3. Duncan's Multiple Range Test on the Percentage of Biodegradation of Phenol, Anthracene and Acenaphthene by *Maltophilia B. velezensis* and consortium

Treatment	Hydrocarbons	Time of Fermentation							
		0	2	4	6	8	10	12	14
Control (%)	Phenol	0,00a C	0,20a G	0,00a F	0,06a H	0,00a G	0,00a H	0,00a F	0,00a E
	<i>Anthracene</i>	0,00 a C	0,00a G	0,00a F	0,20a H	0,33a G	0,00a H	0,00a F	0,00a E
	<i>Acenaphthene</i>	0,00b C	0,06ab G	0,26a F	0,00a H	0,00b G	0,00b H	0,00b F	0,06ab E
<i>S. maltophilia</i> (%)	Phenol	0,20 h BC	1,67g F	5,73 f E	16,73 e G	29,73 d F	42,60 c G	52,87 b E	60,27 a D
	<i>Anthracene</i>	1,20g AB	8,53f D	20,9e D	51,00 a D	63,60 c A	72,47 b A	74,73 b B	80,40 a ABC
	<i>Acenaphthene</i>	0,93h ABC	6,80g E	19,4 f D	24,33 e F	42,47d E	59,07 c E	72,60 b C	82,47 a AB
<i>B. velezensis</i> (%)	Phenol	1,53 h A	9,00g D	20,27 f D	32,00 e E	44,47d E	54,53 c F	62,80 b D	79,00 a C
	<i>Anthracene</i>	1,80h A	18,87 g A	33,73 a F	48,07 e B	59,40d B	65,87 c C	72,60 b C	79,80 a BC
	<i>Acenaphthene</i>	1,60h A	11,00 g C	23,20 f C	34,67 e D	50,40 d D	61,13 c D	77,07 b A	81,13a ABC
Consortium (%)	Phenol	1,86h A	16,2g B	28,20 f B	38,73 e C	56,20 d BC	69,40 c B	76,87 b A	83,40 a A
	<i>Anthracene</i>	1,07 h ABC	15,2 g B	34,93 f A	40,00 e C	54,07 d CD	65,66 c C	78,33 b A	83,53 a A
	<i>Acenaphthene</i>	0,87h ABC	9,67 g CD	19,93 f D	30,40 e E	52,80 d CD	61,33 c D	76,93 b A	83,47 a A

Annotation : The same letter (horizontal/horizontal direction) and the same capital letter (down / vertical direction) shows the value is not significantly different according to Duncan's Multiple Range Test

Based on the results of Duncan's Multiple Range Test, prove that *S. maltophilia* and *B. velezensis* can use carbon contained in hydrocarbons as the primary energy and carbon source. The ability of bacteria to degrade hydrocarbons is related to metabolic processes involving catabolic enzymes. The presence of catabolic enzymes supported by bacterial adaptive strategies produced by the pattern of bacterial gene regulation systems. This ability stimulates various catabolic pathways for enzyme production so that biodegradation of hydrocarbons can occur.

S. maltophilia can degrade more than 50% phenol, anthracene or acenaphthene on 12th, 6th and 10th days, while *B. velezensis* can degrade > 50% phenol, anthracene, and acenaphthene on the 10th and 8th days. The consortium showed a degradation percentage of > 50% phenol, anthracene, and acenaphthene on the 8th day. Increasing the proportion of biodegradation is associated with rapid bacterial cell proliferation. Bacterial cell proliferation supported by an adequate supply of carbon sources from hydrocarbon compounds and conditions (pH and temperature) that support [8].

The percentage of biodegradation in each treatment presented in Figure 3. At the fourteen days, *S. maltophilia* and *B. velezensis* were able to degrade anthracene and acenaphthene. However, *B. velezensis* can degrade phenol better than *S. maltophilia*. Degradation capability can be caused by various factors, including reduced bioavailability of hydrocarbons or accumulation of toxic metabolites during the biodegradation process [8]. Besides, intrinsic factors such as specific characteristics or characteristics of related bacteria also play a role. The ability of bacteria to degrade organic compounds is relevant to their genetic role. Enzymes mediate the chemical reactions involved in metabolism. The various types of enzymes possessed by bacteria are the output of specific genetic information in cells. Thus, the ability of each bacterium to degrade hydrocarbons can also be different.

Based on the results obtained, the consortium showed a higher percentage than the single culture of *S. maltophilia* and *B. velezensis*. The consortium can degrade up to 83.4% phenol, 83.53% anthracene, and 83.46% acenaphthene. This capability supported by various research results that report similar results. Inoculation of bacterial consortia, with different catabolic degradation pathways, is said to be more efficient than single-type inoculation with a complete catabolic pathway [15]. Research by Wu et al. [16]. reported the results of the degradation rate by a consortium consisting of *Stenotrophomonas* sp. and *Bacillus* sp. Degrade PAHs up to 85% in 10 days incubation time.

Ahmad et al. [9]. (2016), has reported phenol degradation by *S. Maltophilia* which shows that the results can tolerate 1000 mg L⁻¹ phenol and can degrade 750 mg L⁻¹ phenol within 65 hours. In another study, *S. maltophilia* known can degrade phenol up to 100% at an optimum concentration of 200 ppm for four days [17]. The results of the Arulazhagan et al. [18]. showed that *S. maltophilia* was able to degrade 500 mg L⁻¹ low molecular weight PAHs (LMW PAHs), including anthracene, with a ten-day observation time. It reported that the degradation of *S. maltophilia* through a process involving hydrophobic cell interactions and biosurfactant production. *S. maltophilia* can degrade anthracene at a concentration of 400 mg L⁻¹ for 28 days to a percentage of 85% -90% [19].

Research involving *B. velezensis* as a bioremediation agent is still minimal. However, *B. velezensis* is known to produce biosurfactants in the form of surfactin which can reduce surface tension, increase water solubility, and stabilize pH and temperature [20]. Biosurfactant has a vital role in the biodegradation process of hydrocarbons. So, through this study, it was known that *B. velezensis* was proven to be able to degrade hydrocarbons, specifically phenol, anthracene, and acenaphthene.

Some species of the *Bacillus* genus are known to be able to degrade phenol, and *Bacillus cereus* can degrade phenol to a concentration of 1000 ppm in free strains and reaches 2000 ppm in strain immobilized by alginate [21].

Phenol is toxic to humans and aquatic organisms, often found in industrial wastes such as textiles, paints, petrochemicals, and paper [22]. Although phenol is toxic to several bacteria such as *Bacillus brevis*, *Candida tropicalis*, *Bacillus amyloliquefaciens*, *Pseudomonas putida* and *Bacillus stearothermophilus* it is reported that it can degrade phenol. Phenol can be aerobically degraded to catechol using a single microbe or a combination of microorganisms [22].

The process of biodegradation by bacteria divided into a process of bacterial attachment to hydrocarbon compounds and degradation processes through metabolic pathways. The nature of hydrophobic PAHs causes low bioavailability of PAHs to bacteria. Thus, bacteria induce active surface molecules called biosurfactants. Biosurfactants can work in various ways: 1) increasing the solubility and bioavailability of PAHs by reducing surface tension or interfaces, 2) regulating the attachment and detachment processes on and off the surface [23]. Catabolic reactions of bacteria involve enzymes contained in the cytoplasm. Although each type of bacterium can have a different metabolic pathway, there are the same 'key' enzymes.

Enzymes involved in the degradation of hydrocarbons are generally oxygenase, dehydrogenase, phosphatase, and lignolytic[15]. The first stage of aerobic metabolism in phenol is hydroxylation of phenol to catechol by NADPH-dependent phenol hydroxylase and flavoprotein enzymes. At this stage, one oxygen atom from the molecule enters the aromatic ring to form catechol. The phenol hydroxylase enzyme is very dependent on the presence of NADPH. The second stage catalyzes the enzyme catechol 1,2-dehydrogenase (ortho division) or catechol 2,3-dioxygenase (meta division). Ortho cleavage products are cis, cis muconic acid or its derivative compounds, while meta-cleavage products are 2-hydroxy muconate semialdehyde. Furthermore, products from the ortho and meta pathways metabolize to enter the tricarboxylic acid cycle (TCA cycle).

Similar to the phenol metabolic pathway, the metabolic pathways in anthracene and acenaphthene also involve the enzyme deoxygenase and dehydrogenase. The metabolic pathway of the two compounds also produces catechol compounds which then enter the TCA cycle. Overall, *S. maltophilia* and *B. velezensis* which are coal isolates of 1 seam D indigenous bacteria UGC 14 have good potential for biodegradation of hydrocarbon compounds, especially phenol, anthracene, and acenaphthene to concentrations of 500 ppm. Both single culture and mixed culture showed a percentage of biodegradation > 50% within two weeks of observation. This research is recommended for optimization to achieve a higher rate of biodegradation in a shorter time so that it can be applied on a larger scale.

4. Conclusions

Stenotrophomonas maltophilia and *Bacillus velezensis* isolates of coal indigenous for 14 days were able to degrade phenol 60.27%, anthracene 80.4% and acenaphthene 82.47%, but *B. velezensis* was able to degrade phenol higher than *S. Maltophilia*. Phenol and PAH biodegradation have better performance than biodegradation using a single culture.

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