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The application of biosurfactant-producing bacteria immobilized in PVA/SA/bentonite bio-composite for hydrocarbon-contaminated soil bioremediation

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Oil spills that contaminate the environment can harm the surrounding ecosystem. The oil contains petroleum hydrocarbon which is toxic to the environment hence it needs to be removed. The use of bacteria as remediation media was modified by immobilizing into a matrix hence the bacteria can survive in harsh conditions. In this research, the ability of biosurfactant-producing bacteria (*Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Ralstonia pickettii*) immobilized in the PVA/SA/bentonite matrix was tested in remediation on oil-contaminated soil. The immobilized beads filled with bacteria were added to the original soil sample, as well as washed soil. The beads were characterized by using FTIR and SEM. Based on FTIR analysis, the PVA/SA/bentonite@bacteria beads had similar functional groups compared to each other. SEM analysis showed that the beads had non-smooth structure, while the bacteria were spread outside and agglomerated. Furthermore, GC-MS analysis results showed that immobilized *B. subtilis* and *R. pickettii* completely degraded tetratriacontane and heneicosane, respectively. Meanwhile, after soil washing pre-treatment, immobilized bacteria could completely degrade octadecane (*P. aeruginosa* and *R. pickettii*) and tetratriacontane (*P. aeruginosa* and *B. subtilis*). Based on those results, immobilized bacteria could degrade oil compounds. The degradation result was influenced by the enzymes produced, the ability of the bacteria, the suitability of the test media, and the matrix used. Therefore, this study can be a reference for further soil remediation using eco-friendly methods.

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Introduction

Crude oil is a non-renewable and irreplaceable source of energy,[1] and oil is processed as a fuel and other products.[2] Total Petroleum Hydrocarbon (TPH) is a term used to represent crude oil which consists of a mixture of thousands of compounds.[3] Most of the scientific literature on crude oil biodegradation refers to the TPH fraction, which includes volatile and extractable crude oil hydrocarbons, such as organic gasoline ($>C_6$–C_{10}), organic diesel ($>C_{11}$–C_{28}), and the organic oil range (C_{29}–C_{35}). All of these compounds are described as toxic to the environment.[4] Increasing demand for crude oil products in various industries and daily life can lead to increased oil costs, increased oil exploitation, and increased oil pollution.[5] Apart from having a positive social and economic impact on society, the use of oil can also have a negative impact on the environment, especially in the case of oil spills which can pollute the environment. The waste produced by oil refineries can be in the form of liquid and solid waste. Soil contaminated with high concentrations of crude oil hydrocarbons can be a source of groundwater contamination. Most of this waste is mutagenic for humans and the surrounding environment.[6] One method of eliminating this waste is using bioremediation. Bioremediation is a process mainly by using microorganisms and plant to remove pollutant in the soil and other environment.[7] This method is effective and low-cost as well as can be used as a solution to removing contaminants in the environment.[8]

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The use of bacteria culture as a bioremediation agent is more widely used because of its fast growth and ability to adapt to toxic conditions.[9] Furthermore, bacteria are the most active agents in crude oil degradation because they act as the main decomposers of oil spills in the environment.[10] Some bacteria were reported to be able to degrade hydrocarbons because they are capable of producing biosurfactants such as *Pseudomonas aeruginosa*,[11] *Bacillus subtilis*,[12] and *Ralstonia pickettii*.[13] The synergistic effect of surfactants can occur through two mechanisms, namely changes in the bioavailability of the substrate for microorganisms and changes in the hydrophobicity of the cell surface which allows the substrate to associate more easily with bacteria cells.[14]

In previous research, *B. subtilis* could degrade crude oil in artificial seawater medium, in which 91% of a compound in crude oil was degraded.[15] On the other hand, bioremediation using *B. subtilis*-free cells can degrade crude oil by 65% under optimal conditions.[1] Bharali *et al.*[11] reported oil contaminated soil by *P. aeruginosa* recovered 73.5–63.4% of residual oil from the sludge through its biosurfactant ability. In addition, the bacterium *R. pickettii* is also capable of degrading crude oil in the high-salinity water, which degraded about 99%.[13] The biosurfactant-producing bacteria have high ability to degrade crude oil, however, it requires a long duration for the incubation process.[14]

Recent research showed that immobilized bacteria in the matrix could survive longer under extreme conditions.[16] One technique widely used for immobilizing bacteria is entrapment where bacteria, as living cells, are encased in a polymeric matrix with a pore capacity that allows diffusion of the substrate to the bacteria. Materials that have been used successfully for cell entrapment include agar, agarose, carrageenan, collagen, alginate, chitosan, polyacrylamide, polyurethane, and cellulose.[17] Nhi-Cong *et al.*[18] used immobilization technique on wastewater polluted by oil. The result showed, within immobilized bacteria using coconut fiber matrix, it could degrade near 100% after 14 days. On the other hand, sodium alginate (SA) is also being a generally biopolymer for building an organic immobilization matrix that is non-toxic to microbes.[19] Furthermore, combining SA within polyvinyl alcohol (PVA) could increase the mechanical strength of the beads matrix.[20]

Moreover, the addition of bentonite clay to the matrix also aims to increase the adsorption capacity of the beads.[21] A previous study reported bentonite that designed into alginate matrix could enhance adsorption capacity on removal wastewater pollutant.[22] Furthermore, Baigorria *et al.*[23] used bentonite composite PVA/SA on arsenic removal.[2] Hence, in this study, PVA/SA/bentonite@bacteria bio-composite beads were used to degrade hydrocarbons. This study expected that this system can degrade hydrocarbons more efficiently. Based on this hypothesis, it is necessary to conduct research on the synthesis of PVA/SA/bentonite@bacteria bio-composite and test its ability to degrade hydrocarbons.

Experimental

Materials

Bacteria *P. aeruginosa* NBRC 3080, *B. subtilis* NBRC 3009, and *R. pickettii* NBRC 102503 were purchased from Biological Resource Center (NBRC), Japan. Furthermore, demineralized water and 70% alcohol were obtained from SAP Chemicals, Indonesia. Nutrient Agar (NA), Luria Bertani Broth (LB), and Tween 80 were obtained from Merck, Germany. Moreover, contaminated soil sample was obtained from oil refinery wells in Bojonegoro, Indonesia.

Soil pre-treatment

Washing soil was conducted to remove impurities and decrease TPH in original soil. Approximately 100 g L^{-1} of contaminated soil was mixed within 0.2% Tween-80 for two times repeated cycles. The mixture was centrifuged for 20 min under 3000 rpm rotary speed, then took off the supernatant.[24] The sediment of soil pre-treatment was used for the next bioremediation procedure.

Bacterial culture

Synthetic stock culture of biosurfactant-producing bacteria (BPB), namely *P. aeruginosa* (2.11×10^8 CFU per mL), *B. subtilis* (2.47×10^8 CFU per mL), and *R. pickettii* (2.46×10^8 CFU per mL) were inoculated on NA media and incubated at 37 °C for 24 hours.[25] Subsequently, the regenerated bacteria colony was inoculated into a 500 mL Erlenmeyer with 200 mL of sterile LB media.[25] The culture was incubated on an incubator shaker at 180 rpm for 24 hours for *P. aeruginosa* and *B. subtilis*[25] as well as 44 hours for *R. pickettii*.[26] Then, the bacteria were carried out for 50 mL from each species and centrifuged to obtain their biomasses. After that, they were added by 5 mL demineralized water individually.

Bacteria immobilization process

Immobilization gel was prepared by mixing 2 g PVA, 1 g SA, and 0.5 g bentonite into 45 mL demineralized water.[25] The mixture was heated for an hour at 150 °C and stirred using magnetic stirrer bar.[27] Then, it sterilized by autoclaving (121 °C, 15 min) to kill out other contaminants. After the hydrogel was cooling down, then 5 mL bacterium that prepared before was added. This study was not examined for consortium bacteria. Hence, one hydrogel solution was mixed within one species of bacteria.

The variable used in this process was the presence of live bacteria treatment. Beads were formed by dripping the hydrogel solution into 4% (w/v) $CaCl_2$ solution (cross-linking agent) using a sterile disposable syringe.[28] The formed beads were immersed in 4% (w/v) $CaCl_2$ solution for 24 hours (cross-linking time). Subsequently, the beads were washed with sterile demineralized water and ready to be used.

Hydrocarbon degradation by immobilized BPB on PVA/SA/bentonite matrix

Hydrocarbon degradation by PVA/SA/bentonite matrix immobilized bacteria was carried out into 100 mL Erlenmeyer containing 5 g of soil and 10 g immobilized bacteria beads. The solution was incubated for 14 days at 37 °C under static conditions.[14,15]

Calculation of total petroleum hydrocarbon (TPH) and product metabolite identification

The culture was then separated from the soil media by dissolving it in 10 mL organic solvent *n*-hexane using a centrifuge at 2000 rpm for 10 minutes.[29] The resulting oil was dried using an oven at 70 °C for 10 min to determine the mass of the bioremediated oil and obtain the TPH value by gravimetric method.[30] Degradation results from oil bioremediation process were identified by analyzing oil samples. Analysis was carried out using a hydrocarbon profile test with a Gas Chromatography-Mass Spectrometry (GC-MS) instrument. The oil samples obtained were diluted 20× using *n*-hexane before being analyzed. GCMS was performed using an Agilent Technologies 7890 GC System linked to an Agilent Technologies 5975C VL MSD Detector with a 30 m × 50 μm × 0.25 μm Agilent 19091S-433 column. The oven temperature was programmed to start from 80 °C and held for 2 min, and then the temperature was increased to 280 °C at 5 °C min^{-1} and held for 15 min greater.[13]

Statistical analysis

The results were calculated by obtaining mean result of triplicate sample per each various bacterium. Analysis of Variance (ANOVA) was used to determine the significant difference between TPB value before and after washing soil pre-treatment bioremediation result, by each BPB. Differences between mean values at a confidence level of 5% ($P < 0.05$) were considered to be statistically significant.[31]

Results and discussion

Crude oil bioremediation and degradation product identification

Immobilized bacteria in the PVA/SA/bentonite matrix were tested for their ability to degrade crude oil contaminants in soil samples, during a 14-day incubation period. GCMS chromatograms of the degradation test results of non-pre-treatment soil are shown in Fig. 1. The chromatogram shows an increase in intensity at various points for each immobilized bacterium. The increase/decrease in intensity affects the ability of the bacteria to degrade crude oil compounds. The greater increases in intensity, the greater of percentage recovery produced or the lower of percentage degradation produced by the bacteria. Conversely, a decrease in intensity indicates a higher degradation ability of the immobilized bacteria. Fig. 2 shows the % recovery of immobilized bacteria in degrading compounds in original soil samples, where *P. aeruginosa* had the highest % recovery, exceeding 650%. This was due to the accumulation of compounds where the bacteria had not been able to degrade them. The accumulation of these compounds could mean the production of simple compounds resulting from the degradation of higher molecular weight compounds. This was because microorganisms are able to involve aliphatic or aromatic fractions of oil by degrading complex compounds into simpler ones.[32]

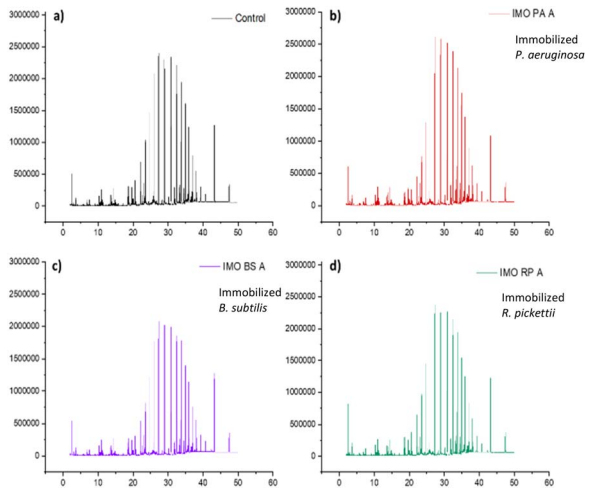

Fig. 1 Chromatograms of crude oil recovery (a) control, and immobilization by bacteria (b) *P. aeruginosa*, (c) *B. subtilis*, and (d) *R. pickettii* after treatment 14 days.

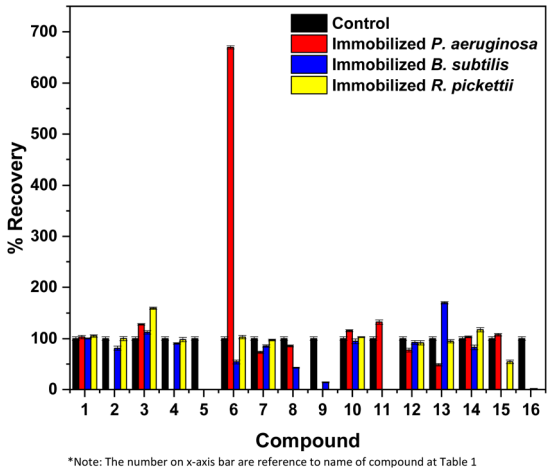

Fig. 2 Crude oil recovery results by immobilized bacteria of non-pre-treatment soil bioremediation. Data showed the mean of triplicate analysis ± standard deviation (SD).

Generally, biosurfactant produced by bacteria enhances hydrophobicity of cell surface, causing hydrophobic substrate can interact within bacteria cell. After that, the degradation process will be taken further action. This degradation came out because of the availability of catechol 2,3-dioxygenase or ortho-cleavage dioxygenase enzymes. These enzymes have a role on making oxygen incorporation within hydrocarbon substrate and break hydrocarbon chain.[14] While on degradation of aliphatic chain, terminal monooxygenase enzyme had a role on converting substrate become fatty acid and acetyl CoA.[33] Among of these bacteria *P. aeruginosa*, *B. subtilis*, and *R. pickettii* had been reported could produce biosurfactant and secreted dioxygenase enzymes.[34–36] *Pseudomonas* sp. had been reported as oil degraded bacteria. Study by Rodríguez-Uribe *et al.* (2021) reported that *Pseudomonas* sp. was a bacterium that had been isolated from oil contaminated soil.[37] However, Bharali *et al.*[11]

Table 1 Recovery results for metabolite compound of non-pre-treatment soil bioremediation

No.	Name of compound	*P. aeruginosa*		*B. subtilis*		*R. pickettii*	
		Recovery (%)	Degradation (%)	Recovery (%)	Degradation (%)	Recovery (%)	Degradation (%)
1	1,4-Epoxynaphthalene-1(2*H*)-methanol, 4,5,7-Tris(1,1-dimethylethyl)-3,4-dihydro-	103.217	0	100.257	0	104.968	0
2	1-Heptacosanol	0	100	81.125	18.875	100.008	0
3	Methylbenzene	127.943	0	112.245	0	159.571	0
4	Cyclohexane, nonadecyl-	0	100	90.434	9.566	97.947	2.053
5	Ditetradecyl ether	0	100	0	100	0	100
6	Dotriacontane	669.518	0	54.231	45.769	102.951	0
7	Eicosane	73.061	26.939	85.327	14.673	97.217	2.783
8	Heneicosane	85.604	14.396	42.942	57.058	0	100
9	Hexadecane, 2,6,10,14-tetramethyl-	0	100	14.137	85.863	0	100
10	Hexatriacontane	115.309	0	94.723	5.277	102.925	0
11	Octacosanol	132.283	0	0	100	0	100
12	Silane, dimethyl(docosyloxy)butoxy-	77.438	22.562	92.315	7.685	91.630	8.370
13	Tetracosane	48.644	51.356	170.223	0	94.640	5.360
14	Tetrapentacontane	103.246	0	82.686	17.314	117.278	0
15	Tetratriacontane	107.619	0	0	100	54.433	45.567
16	Tetratriacontyl heptafluorobutyrate	0	100	1.339	98.661	0	100

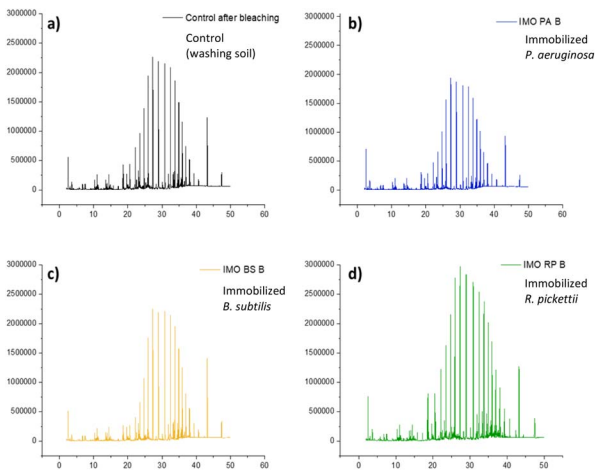

Fig. 3 Chromatogram of crude oil recovery (a) control after washing pre-treatment, and immobilization by bacteria (b) *P. aeruginosa*, (c) *B. subtilis*, and (d) *R. pickettii* treatment 14 days.

also used four strains of *Pseudomonas aeruginosa* in oil degradation by role of rhamnolipid biosurfactant producing. Furthermore, *B. subtilis* also had been reported isolate from oil product.[38] Zhao *et al.*[39] showed *B. subtilis* produced C13-, C14- and C15-surfactin congeners and Tao *et al.*[40] examined *B. subtilis* degraded crude oil within degradation ratio approximately 85%. Meanwhile study about *R. pickettii* was still minority. However this bacteria reported could examine crude oil under high salinity medium.[13]

Table 1 shows the %recovery and %degradation produced by each immobilized bacteria in the PVA/SA/bentonite matrix on native soil test media. Immobilized *P. aeruginosa* produced the highest %recovery of dotriacontane compound. These bacteria could completely degrade 1-heptacosanol, cyclohexane, nonadecyl-, ditetradecyl ether, hexadecane, 2,6,10,14-tetramethyl-, and tetratriacontyl heptafluorobutyrate compounds up to 100%. Meanwhile, immobilized *B. subtilis*, compounds that completely degraded were ditetradecyl ether and tetratriacontane. Furthermore, immobilized *R. pickettii*, compounds that completely degraded include tetratriacontyl heptafluorobutyrate, octacosanol, ditetradecyl ether, hexadecane, 2,6,10,14-tetramethyl-, and heneicosane. The difference in compounds degraded by bacteria was influenced by the enzymes produced by each bacterium.[15]

Based on oil compositions, it consists of aliphatic and polyaromatic hydrogen (PAH). The degradation of oil was through enzymatic system within ring cleavage by dioxygenase[14] and aliphatic chain degradation by terminal monooxygenase through production fatty acid and acetyl CoA.[33] In Table 1, the oil compounds were identified by dotriacontane ($C_{32}H_{66}$), eicosane ($C_{20}H_{42}$), heneicosane ($C_{21}H_{44}$), tetracosane ($C_{24}H_{50}$),

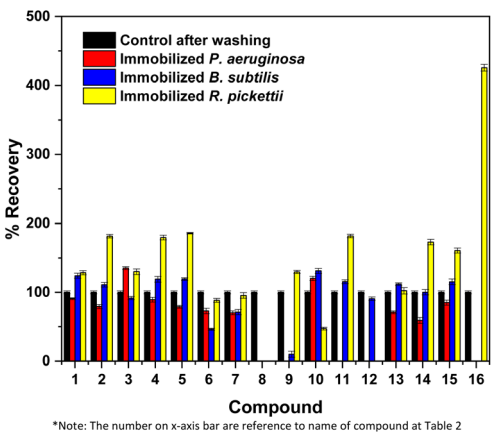

Fig. 4 Crude oil recovery of washing soil by immobilized bacteria. Data showed the mean of triplicate analysis ± SD.

Table 2 Recovery results for metabolite compounds of washing soil treatment by immobilized bacteria

		P. aeruginosa		*B. subtilis*		*R. pickettii*	
No.	Name of compound	Recovery (%)	Degradation (%)	Recovery (%)	Degradation (%)	Recovery (%)	Degradation (%)
1	1,4-Epoxynaphthalene-1(2*H*)-methanol, 4,5,7-Tris(1,1-dimethylethyl)-3,4-dihydro-	90.860	9.140	124.181	0	128.479	0
2	1-Heptacosanol	79.403	20.597	111.055	0	181.757	0
3	Benzene, methyl-	135.260	0	91.766	8.234	130.041	0
4	Cyclohexane, nonadecyl-	89.372	10.628	119.015	0	179.622	0
5	Cyclohexane, octadecyl-	79.071	20.929	119.356	0	185.920	0
6	Dotriacontane	73.029	26.971	46.362	53.638	88.439	11.561
7	Eicosane	70.196	29.804	71.167	28.833	95.504	4.496
8	Hexacontane	0	100	0	100	0	100
9	Hexadecane, 2,6,10,14-tetramethyl-	0	100	10.062	89.938	129.679	0
10	Hexatriacontane	120.272	0	131.175	0	47.348	52.652
11	Octacosanol	0	100	115.487	0	181.951	0
12	Octadecane	0	100	90.825	9.175	0	100
13	Silane, dimethyl (docosyloxy)butoxy-	71.088	28.912	111.998	0	102.314	0
14	Tetracosane	59.184	40.816	100.119	0	172.930	0
15	Tetrapentacontane	85.086	14.914	115.267	0	160.777	0
16	Tetratriacontane	0	100	0	100	425.556	0

and tetratriacontane ($C_{34}H_{70}$).[11] Generally, the result shows that the majority of those compounds were degrade. However immobilized *P. aeruginosa* did not degrade dotriacontane and tetratriacontane neither with *R. pickettii*, while *B. subtilis* degraded tetratriacontane completely, in contrast within tetracosane. Immobilized *R. pickettii* had completely degraded heneicosane. Moreover, the highest %recovery, which was obtained by immobilized *P. aeruginosa* had zero %degradation on dotriacontane. Dotriacontane is a natural product and can find in soil composition synthesized by plant or actinobacterium.[41] However, this compound also could be a degraded compound from another complex compound.

Soil contaminated oil bioremediation after washing pre-treatment

Washing soil treatment was declared as a pre-treatment method for reducing TPH value. So, it was applicable on bioremediation by using microorganism.[11] The highest TPH value could inhibited microorganism ability on producing oxidizing-oil enzymes.[14] In this study, the degradation results after washing process soil pre-treatment are shown in Fig. 3 and 4. Based on the chromatogram in Fig. 3, there was an increase in the intensity of the compounds produced by immobilized *R. picketti*, which exceeded the soil sample after washing pre-treatment. Meanwhile, in immobilized *P. aeruginosa* and *B. subtilis*, there was a decrease in intensity in the chromatogram. Fig. 4 shows the graphic of %recovery analysis compounds based on degradation results. Mostly, %recovery of immobilized *R. pickettii* were higher than control washing soil. However, some of result were exhibited zero %recovery.

Meanwhile, Table 2 shows the %recovery and %degradation produced by immobilized bacteria in degrading oil compounds in soil samples after washing pre-treatment. Based on that table, the oil compounds were dotriacontane ($C_{32}H_{66}$), eicosane ($C_{20}H_{42}$), octadecane ($C_{18}H_{38}$), tetracosane ($C_{24}H_{50}$), and tetratriacontane ($C_{34}H_{70}$).[11] *R. pickettii* shows high value up to 425% recovery. This was an accumulation of other compounds degraded to form tetratriacontane and it was a natural product synthesized by plant.[42] Based on Table 2, *P. aeruginosa* can completely degrade hexacontane, hexadecane, 2,6,10,14-tetrametyl, octacosanol, octadecane, and tetratriacontane. Meanwhile, *B. subtilis* can only completely degrade hexacontane and tetratriacontane.

The ability of each bacterium during the degradation process was influenced by its ability to face resistant conditions in the oil.[38] This condition caused bacteria was able to die/in-active, then involved in decreasing performance in degradation. Also, the performance of bacteria was influenced by the degradative enzymes produced by each bacterium.[43] Besides being

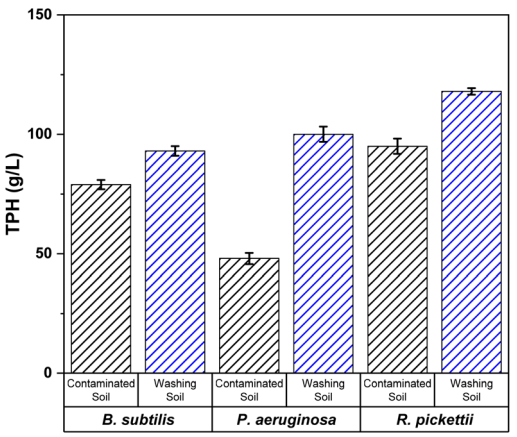

Fig. 5 Involvement of soil washing on growth of BPB at PVA/SA/bentonite. This result represented mean ± SD of triplicate experiments. Statistically significant ($p < 0.05$) were observed among pre-treatment and each BPB result.

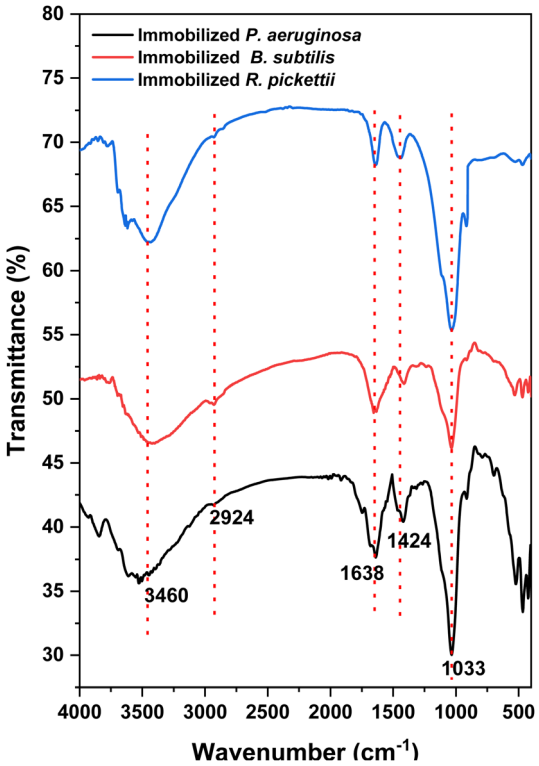

Fig. 6 FTIR analysis of *P. aeruginosa*, *B. subtilis*, and *R. pickettii* immobilized into PVA/SA/bentonite matrix.

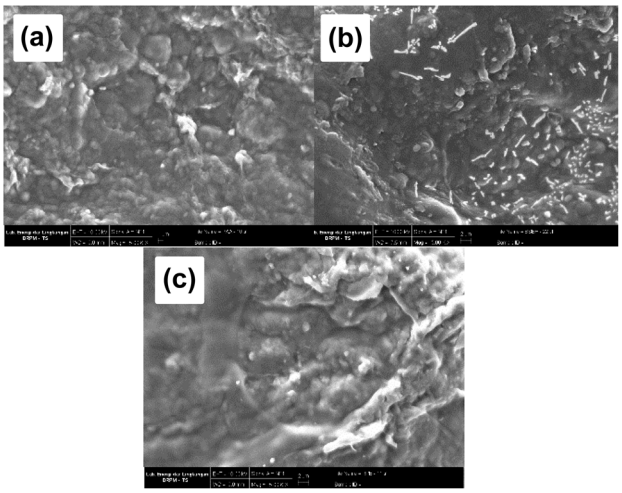

Fig. 7 The SEM results of the PVA/SA/bentonite beads at 10.000× magnification (a) *P. aeruginosa*, (b) *B. subtilis*, and (c) *R. pickettii* immobilized bacteria.

influenced by enzymes, the limited ability of bacteria to penetrate immobilized beads also plays an important role in the low %degradation. This is in line with a previous study, that the immobilization method was more suitable for liquid media test samples, unlike this research that uses dry soil as test media.[44] Furthermore, the mismatch of the immobilization matrix used also plays a role in this factor, where the matrix could inhibit the entry of molecules into the beads, hence bacteria were unable to completely degrade. However, based on Tables 1 and 2, all three bacteria had been proven to be capable of degrading oil compounds, but further research is needed to determine how effective these bacteria in another degradation.

Effect of washing pre-treatment on bacterial growth

By using gravimetric method, TPH values were obtained from the treatment results, which represent bacterial growth.[11] From the results which are shown in the Fig. 5, it was found that there was an increasement of all bacterial species in the soil after washing process. Therefore, the soil washing process was proven to be able to eliminate impurities or high concentrations of hydrocarbon compounds, so that BPB was easier to grow and remediate oil pollutants that were contaminated in soil.

Beads synthesis and characterization

Fourier transform infrared spectroscopy (FTIR). Fourier Transform Infra-Red (FTIR) analysis on the characterization of PVA/SA/bentonite@bacteria beads were used to identify the functional groups. The FTIR results are shown in Fig. 6. Wavenumber at broad peaks around range 3500–3300 cm^{-1} refer to OH stretching overlapping within NH group, while peaks at around 2924 cm^{-1} refer to CH aliphatic stretching vibration, which identified as functional groups of bacteria, SA, and PVA components.[23] Furthermore, wavenumber peaks spectra range around of 1638 cm^{-1} show C=C and C=O of SA and bacteria.[45,46] Then, Si–O functional group of bentonites were exhibit at around 1033 cm^{-1}.[47]

Different bacteria species additions were not shown a different significant peak. It was involved by the characteristic of bacteria which generally consist of organic biomolecule (lipid, protein, polysaccharides, *etc.*).[46] A study reported that bacteria was arranged by OH, CH, NH, C=O and C–O functional groups.[46] Hence, the FTIR spectra of synthesized beads within different bacteria addition had similarity of each peak.

Morphological analysis. Besides being characterized using FTIR, the beads were also analyzed for their topological and morphological shape using Scanning Electron Microscopy (SEM). Fig. 7a–c exhibit SEM images of PVA/SA/bentonite surface visuals each bacterium. The bacteria were stick and agglomerate within their matrix synthesized. It also showed that the bacteria were spread evenly at their matrix.

Conclusion

BPB (*P. aeruginosa*, *B. subtilis*, and *R. pickettii*) had been analyzed for degrading TPH contaminated soil. They were successfully immobilized into PVA/SA/bentonite, which could enhance their ability to face stress level contaminant. GCMS analysis showed that immobilized bacteria completely degraded tetratriacontane by immobilized *B. subtilis* and heneicosane by immobilized *R. pickettii*. Furthermore, after washing soil pre-treatment, immobilized bacteria could completely degrade octadecane (immobilized *P. aeruginosa* and immobilized *R. pickettii*) and tetratriacontane (immobilized *P.*

aeruginosa and immobilized *B. subtilis*). Therefore, this study proved that all these immobilized BPB could degrade hydrocarbon pollutant by their eco-friendly ways.

Author contributions

Adi Setyo Purnomo: conceptualization, methodology, investigation, validation, resources, writing – original draft, writing – review & editing, funding acquisition. Surya Rosa Putra: methodology, validation, supervision. Herdayanto Sulistyo Putro: supervision, validation. Afan Hamzah: supervision, validation. Nova Ainur Rohma: methodology, investigation, writing – original draft. Alya Awinatul Rohmah: revision and editing. Hamdan Dwi Rizqi: validation, formal analysis. Asranudin: visualization, writing – review & editing. Bieby Tangahu: conceptualization, methodology, resources. IDAA Warmadewanthi: conceptualization, methodology, resources. Kuniyoshi Shimizu: validation, supervision, writing – review & editing.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

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