RESEARCH PAPER

Identification of petroleum degrading bacteria and the status of oil pool in south of Minas field, Central Sumatra basin Indonesia

Tri M. Susantoro [a,*], A'liyatur Rosyidah [b], Suliantara Suliantara [a], Herru L. Setiawan [a], Bambang Widarsono [c], Jonathan S. Hadimuljono [d]

[a] Research Centre for Geoinformatics, National Research and Innovation Agency (BRIN), Indonesia
[b] Research Centre for Vaccine and Drugs, National Research and Innovation Agency (BRIN), Bogor, West Java, Indonesia
[c] Research Centre for Process Manufacturing Industry Technology, National Research and Innovation Agency (BRIN), Puspiptek-Serpong, Indonesia
[d] Lemigas R and D Centre for Oil and Gas Technology, Ministry of Energy and Mineral Resources, Jakarta, Indonesia

Abstract

This study aimed to isolate and identify the oil-degrading bacteria and analyze the origin of the crude oil pool. The oil samples were collected from the oil pool located at Ukai River, South of Minas field. The bacteria were isolated using the serial dilution method on nutrient agar media and identified based on the 16 s rRNA gene sequence. Meanwhile, the oil pool's origin was analyzed using a digital elevation model (DEMNAS) to interpret lineament and drainage patterns and then supported by subsurface geological data to analyze the migration pathway from the subsurface to the surface. The identification result revealed three species of the strains L1, L2, and L3 bacteria, which were similar to *Klebsiella quasipnumoniae* strain 07A044^T, *Bulkhoderia multivorans* strain ATCC BAA-247^T, and *Moraxella osloensis* CCUG350^T, respectively. The analysis of lineaments drainage patterns and supported by subsurface data shows no evidence for the migration pathway of oil seepage. Meanwhile the gas chromatography, and gas chromatography mass spectrometry results show that there are similarities between the oil pool and crude oil from the Minas field, but it has experienced degradation. Based on the results, it can be concluded that the three types of bacteria found in oil pools can be used for bioremediation because they are able to degrade the oil pool and make it heavy, while lineament analysis cannot confirm the origin of the oil pool; however, the results of gas chromatography and gas chromatography mass spectrometry show that the oil pool is proven from the Minas field.

Keywords: Drainage pattern, Heavy oil, Identification, Lineament, Oil-degrading bacteria

1. Introduction

The Central Sumatra Basin (CSB), Indonesia, is a mature sedimentary basin which was first discovered the oil field in 1939, and then the giant Duri and Minas fields were discovered, and both started producing in 1944.[1,2] Furthermore, oil and gas exploration continued to find new oil and gas fields, such as Petapahan, which spudded entirely in 1971,[3] and Beruk field in 1976.[4,5] The CSB is estimated to have generated oil and gas with greater potential than the identified hydrocarbon resources.[5] These include the total original heavy oil potential of 40.6 BBO, and there are seven potential areas for heavy oil found.[6,7]

Studying the oil and gas deposits in the CSB is fascinating since they differ from the typical Indonesian sedimentary basins in that most known deposits exhibit seepage at the basin's borders. The existence of this seepage is closely related to the presence of oil and gas on the basin scale and to petroleum generation and entrapment, but has less

Received 23 February 2023; revised 13 May 2024; accepted 29 May 2024.
Available online 10 July 2024

* Corresponding author at: Research Centre for Geoinformatics, National Research and Innovation Agency (BRIN), Bogor, West Java, 16911, Indonesia.
E-mail address: trim010@brin.go.id (T.M. Susantoro).

https://doi.org/10.62593/2090-2468.1033

associated with the sub-basin scale.[8] The existence of seepage usually follows lineament trends (faults and fractures), which are indicated as migration routes for hydrocarbons reaching the surface.[9,10] Lineament is generally related to topographic features, such as straight valleys or river flow; shifting rock formation layers; regular river bends inducible as straight lines; the relationship between the main river and its tributaries, which are perpendicular to each other; the straightness of triangle-facets rows; and extreme lithological offsets or topographical changes.[11,12] Lineament mapping can be carried out using Landsat 8 and DEM.[11] The random forest application shows that lineament is an essential factor for oil and gas exploration.[13] Based on this literature, the origin of oil seeps (oil pools) can be determined by studying the relationship between surface structures from Landsat and DEM data and subsurface structures from literature studies or interpretations of seismic and gravity data.

The literature studies have shown that fewer studies have reported seepage around the CSB. Even the systemized surface geology map officially issued by the Government of the Republic of Indonesia through the Geological Survey Centre - Geological Agency, Ministry of Energy and Mineral Resources does not inform that there is oil and gas seepage in the basin,[14–16] but in the year 2022 was reported an oil pool, which is located south of Minas field with an API gravity of 11°.[7] The existence of this heavy oil pool is very interesting for further study, especially to analyze the types of oil biodegradation bacteria, which are one of the agents for the formation of heavy oil and the occurrence of oil pools, whether it occurred by natural processes called as oil seep or by human activities, which is referred as oil pool.

The primary and most effective natural method for removing petroleum hydrocarbon contaminants from the environment is microbial degradation. Yeast, bacteria, and fungi are the main biodegraders of hydrocarbons in the environment. Many researchers found that complex combinations of hydrocarbons, like crude oil in the soil, require heterogeneous populations with generally broad enzymatic capacity.[17–19] A bioremediation process, defined as the employment of microorganisms to detoxify or eliminate pollutants due to their wide metabolic capabilities, is an emerging method for the removal and degradation of many environmental contaminants.[20] Additionally, the bioremediation method is thought to be relatively non-invasive and economical.[21] Several genera have been reported to have an important role in the degradation of heavy oil, such as *Acinetobacter Marinobacter, Achromobacter, Alkaindiges, Arthrobacter, Alteromonas, Burkholderia, Dietzia, Streptococcus, Streptobacillus, Enterobacter Mycobacterium, Pandoraea, Rhodococcus,* and *Pseudomonas*.[22] For instance, *Streptomyces* spp. isolated from plants growing in oil-contaminated soil had a hydrocarbon utilisation efficiency of up to 98%. These strains were cultivated on petroleum as a single carbon source and decomposed the aromatic, PAHs, and n-alkanes (C6–C30) hydrocarbons at varying levels within 7 days.[23]

The existence of these bacterial in the oil pool area is strongly influenced by environmental conditions such as temperature, pH, salinity, organic matter, soil moisture, and nutrients.[17] Based on the tolerance of oil biodegradation bacteria to temperature, it can calculate the maximum depth of heavy oil formed.[18] This is because heavy oil is produced as a result of water washing and/or bacterial biodegradation processed.[6] Parameters like surface temperature, temperature gradient, thermal conductivity, rock density, and geothermal heat movement affect the subsurface temperature at a specific depth.[19] Utilizing bacteria as a biodegradation agent for bioremediation procedures in areas contaminated with crude oil is another advantage of bacterial identification.[24,25] Because indigenous bacteria need carbon for development and reproduction, they eventually destroy the majority of petroleum hydrocarbons found in the environment.[22] This study of petroleum degrading bacteria identification and the status of oil pool in South of Minas field is crucial since oil pollution still happens in Indonesia due to tanker accidents, docking waste, leaks or breaks in oil pipes, discharge of bilge water and fuel tanks during the ballasting process, and mishaps involving oil drilling. Several accidents affecting coastal areas have been recorded, including the oil spill on Balikpapan beach,[26] the oil spill from the Montara platform,[27] and oil spills that polluted the Karawang coast and its surroundings.[28]

To the best of our knowledge, there is practically no published report related to the oil-degrading bacteria in the CSB oil pool. Therefore this study aims to identify oil-degrading bacteria isolated from the CSB oil pool and analyze the occurrence of the oil pool based on remote sensing data and subsurface data from the Minas field.[1,29] In this study, oil-degrading bacteria were isolated from an oil pool in the Ukai River, Minas field, then purified and molecularly identified. Furthermore, the geochemical study of the pool and the digital elevation model were also examined. The study's results are expected to be used to explore further heavy oil in the CSB and utilize these bacteria as potential candidates for oil pollutant treatment in order to reduce pollution naturally.

2. Materials and methods

2.1. Oil pool location

The oil pool was located at coordinates 101.47751°E and 0.64018°N in Ukai River, on the southern edge of the Minas field, with the surface condition covered by grass and shrubs. Based on the subsurface map, the oil pool is located among two faults in the north−south (N−S) direction in the Minas field (Fig. 1). This field formed by the tensional and wrench fault systems during the Tertiary.[30] The Minas field experienced three tectonic phases, namely the tensional phase in the Eocene - Oligocene, then changed to *trans*-tensional in the Early Miocene - Middle Miocene, and then the compressional phase in the Middle Miocene - Present. This tectonic process forms many oil and gas structural traps, with the geological structure dominated by N−S and northwest-southeast (NW−SE) trend.[31,32] The N−S trending structure is the oldest, parallel to the Malaysian structure, while the NW−SE trending structure is younger and parallel to the Semangko Fault.[33] The two main structures in this basin were actively moving during the tertiary time.[30]

2.2. Oil sample collection

The oil samples for bacterial analysis were carried out on 25−October 30, 2020 from the oil pool around

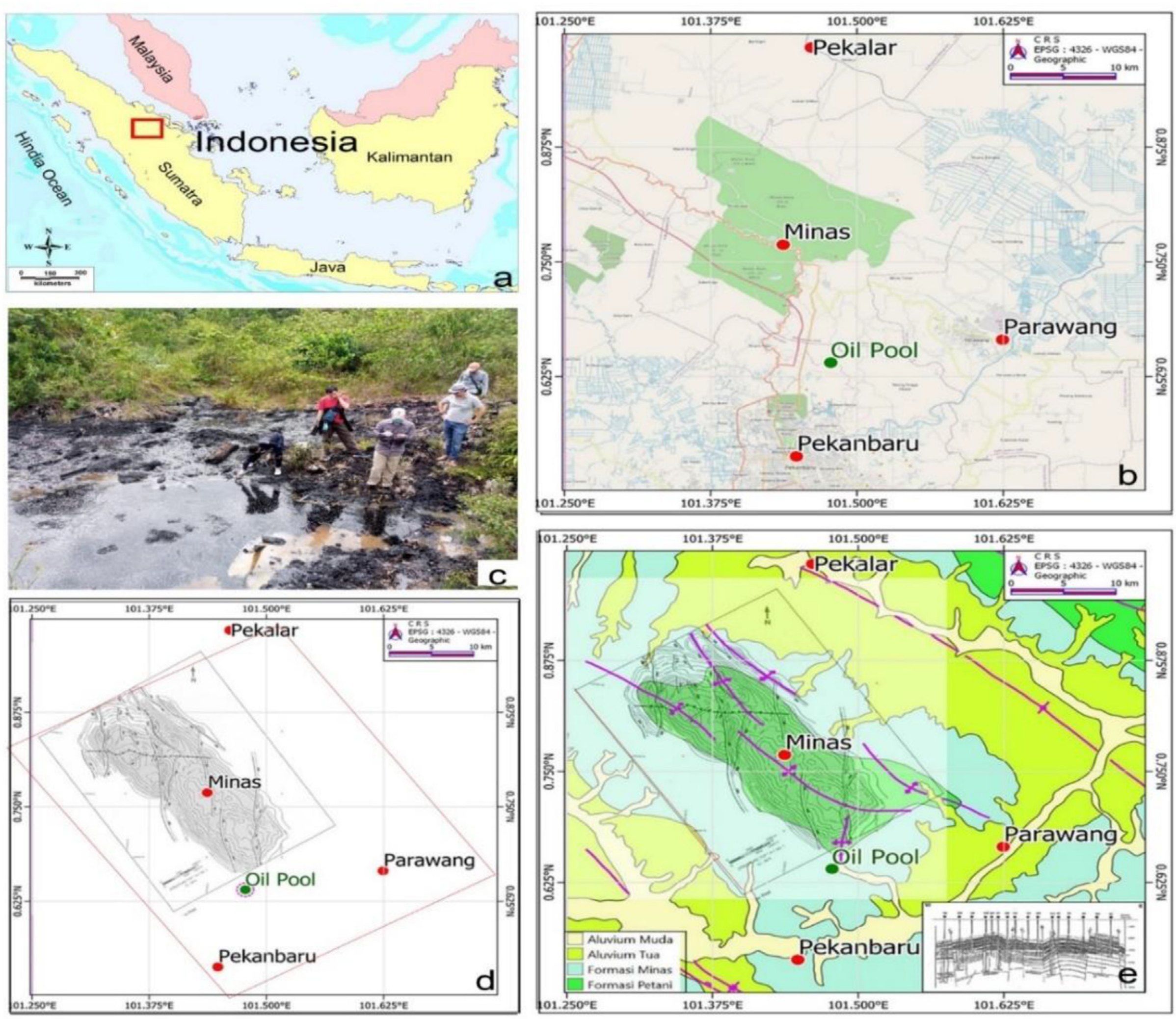

Fig. 1. General information of study areas, a. the location of the study area in Sumatra Island, Indonesia, b. Oil pool location overlaid on Open Street Map, c. Crude of oil pool appearance, d. Overlaid between the oil pool and the Subsurface Map Top of Sihapas Formation, and e. Oil pool overlaid with the Surface Geological Map.

the Ukai River (Fig. 1c), a tributary of the Minas River. About 200 cc of oil samples were taken at coordinates 101.47751°E and 0.64018°N, and stored in two glass vessels for bacterial, gas chromatography (GC), and gas chromatography mass spectrometry (GCMS) analysis. The weather conditions at the time of sampling were sunny and tended to be hot. The field survey also conducted to identify any manifestations of existing geological structures, such as lithological offset, subsidence along the fault, scarp or cliff, drainage pattern change, and vegetation types.

2.3. Isolation and identification of bacteria

Bacterial isolation was carried out at the Process Laboratory, Research and Development Center for Oil and Gas Technology 'LEMIGAS', while bacterial identification was carried out at Research Center for Biology - Indonesian Institute of Sciences (LIPI). Bacteria were isolated from oil samples using the serial dilution method using 0.85% sterile NaCl. Oil-degrading bacteria were isolated by spreading diluted samples on Luria-Bertani (LB) agar containing 1% tryptone, 1% of yeast extract, 0.5% of NaCl, and 1.5% bacteriological agar with pH 7. The plates were incubated at 35 °C until a colony formed. The isolated bacteria were then purified in the same media prior to further investigation. The genomic DNA of each bacterium was extracted based on the GES method with modification.[34] The 16 s rRNA gene sequence was amplified using universal 16 s rRNA gene primers of 27 F (5′AGA GTT TGA TCC TGG CTC AG 3′) and 1492 R (5′GGT TAC CTT GTT ACG ACT T 3′).[35] The PCR products were purified using the PEG precipitation method.[36] The purified PCR products were used for sequencing analysis using an automated DNA sequencer (ABI PRISM 3130 Genetic Analyzer, Applied Biosystems). The raw gene sequence data were trimmed and assembled using the BioEdit program. The assembled sequences were compared with the 16 s rRNA online gene database EzBioCloud (https://www.ezbiocloud.net/). The sequences were aligned with closely related bacterial species obtained from the database using ClustalW. The phylogenetic tree was constructed by Neighbor-Joining Method using MEGA-X software.[37]

The bacteria that have been identified can be used to estimate the depth of heavy oil reservoirs based on their adaptability to subsurface temperatures. These bacteria can live in oil reservoirs and degrade them into heavy oil at a maximum temperature of 60 °C.[38] The maximum depth can be calculated using the formula: (max temperature−surface temperature)/the thermal gradient x 100. Whereas, the average surface temperature in the Central Sumatra Basin is 26.67 °C, and the geothermal gradient is 6 °C/100 m.[18]

2.4. Geochemical analysis of oil pool

Oil pool geochemical analysis using GC and GC-MS was carried out at the Geochemical Laboratory, Exploration Division, Center for Research and Development of Oil and Gas Technology, 'LEMIGAS'. GC analysis was carried out at high temperature by injecting a 0.1 μL seepage oil sample into an Agilent 6890 N GC instrument using a flame ionization detector (FID) with a DB-1 (J and W) 10 m × 0.21 mm i.d. Capillary column. GC-MS analysis of the saturate and aromatic fractions was carried out using an Agilent 6890 N GC by injecting a 0.1 μl seepage oil sample with the addition of a Mass Selective Detector (MSD) and computer applications using Chemstation software. The GC instrument column used by the DB-5MS (J and W) has dimensions of 60 m × 0.25 mm i.d. The automatic injector is used with the Agilent 7673 type with the split/splitless method. EM voltage is 1980 V, electron energy is 70 eV, and the source temperature is 250 °C.[39] The GC and GC-MS results were then compared with Minas Field geochemical data derived from previous studies.

2.5. Digital elevation model processing and interpretation

The sources of digital elevation model (DEM) data were used to assess the origin of seepage from National DEM (DEMNAS) with the numbers 0816−54, 0816−63, 0816−61, and 0816−52, and downloaded from https://tanahair.indonesia,go.id. The geological map of the Pekanbaru quadrangle was downloaded from https://esdm/go.id/geologi (Fig. 1e). DEMNAS data processing includes hill-shade, drainage pattern, and aspect. The parameters for data processing were carried out with azimuth 0°, 315°, 270°, and 225°, and the altitude parameter was used 45°, considering that the research area has a smooth-relief- to hill morphology.

Lineaments were interpreted from a hill shade using four azimuths of light, revealing complete data, drainage pattern, and aspect so that detailed lineament can be identified from various directions than a single azimuth of light.[40] Lineament interpretation was performed manually on hill-shade images with azimuth light 0°, 315°, 270°, and 225°. The interpretation was carried out by considering the understanding of the regional geological conditions of the study area and consistently using a one-hundred-thousandth-scale display. The drainage pattern is produced by hydrologic and climatic in an area, regardless of whether they are permanently occupied rivers or temporary networks.[41] Regional drainage patterns were controlled by the topography, type, and thickness of rock layers or lithology, geological structure, vegetation density, and climate.[42] The aspect was the direction of the slope orientation, calculated according to the clockwise direction in degrees from 0°to 360°, where 0° was facing north, 90° was facing east, 180° was facing south, and 270° was facing west. The results of the lineament interpretation were used to analyze potential oil leaking zones around the study area by examining the relationship between the surface and subsurface lineament that allow for oil migration pathways.

3. Result and discussion

3.1. Bacterial identification based on 16s rRNA gene sequence

Many microorganisms can degrade hydrocarbons and utilize them as a carbon or energy source. These three strains were labeled L1, L2, and L3, respectively. Subsequently, taxonomic identities of the three strains were performed by PCR amplification and comparing the obtained 16 s rRNA gene sequences with the available data in the EzBioCloud database. Based on the molecular analysis, the three isolated bacterial strains were similar to *Klebsiella quasipnumoniae, Burkholderia multivorans,* and *Moraxella osloensis* for L1, L2, and L3, respectively. Fig. 2 show the constructed phylogenetic tree using Neighbor-Joining Method.

The L1, L2, and L3 strains were bacteria isolated from oil samples in the CSB, Indonesia. The 16 s rRNA gene sequence was 1404, 1368, and 1372 bp long and deposited in GenBank under the accession number ON340567, ON340569, and ON340571, for L1, L2, and L3 respectively. The phylogenetic analysis indicated that those three strains belonged to Klebsiella, Bukholderia, and Moraxella genus. Each strain showed 99.86, 99.71, and 99.13% silimarity to *K. quasipneumoniae* subsp. *similipneumoniae* 07A044^T, *B. multivorans* ATCC BAA-247^T, and *M. osloensis* CCUG350^T for L1, L2, and L3, respectively (Fig. 2). Enteric bacteria particularly do not degrade hydrocarbons as an energy source. However, several *Enterobacteriaceae* species, particularly those from the genera *Klebsiella, Enterobacter, Escherichia*, and *Hafnia*, have been reported capable of hydrocarbon degradation. For instance, *Klebsiella pneumoniae* AOR2 was found to have the greatest potential for oil recovery via long-chain hydrocarbon degradation.[43] Furthermore, *K. pneumoniae* AWD5 was isolated from automobile waste-contaminated sites from Silchar, Assam exhibiting degradation of polyaromatic hydrocarbon (PAH) and stimulating plant growth, for rhizosphere-mediated bioremediation. The AWD5 genome also revealed a potential degradation pathway for polyaromatic hydrocarbons, benzoate, phenylacetate, 3-hydroxyphenyl propionate (3-HPP), and 2- and 3-fluorobenzoates.[44] Rodrigues et al. identified four distinct catabolic genes (todC1, ndoB, xylE, and alkB1) in oil-degrading Klebsiella strains isolated from hydrocarbon-contaminated sediments in Santos-São Vicente estuary systems in Brazil. These genes play a role in oil degradation.[45]

Burkholderia multivorans are gram-negative, aerobic, nonfermenting bacillus that is found in soil and water.[46] *Burkholderia* sp. Can use aliphatic and aromatic hydrocarbons as primary carbon and energy sources.[47] Many *Burkholderia* species have plasmids that contribute to their adaptability and produce dioxygenase, enzymes required for the initial oxidation of hydrocarbon chains and aromatic rings.[48–50] This bacterium can also be used as an indicator to map the deepest reservoirs/traps bearing heavy oils based on their adaptability to life at a maximum temperature of 60 °C.[18] In the CSB, calculations show that at this temperature, the depth reaches around 555.5 m, and the biodegradation process of crude oil can still occur in the presence of these bacteria and water contact. Moreover, *M. osloensis* is gram-negative bacteria and is considered a commensal organism on human skin, respiratory tract, and mucosa.[51] This bacteria has previously been reported to have the potential to be used as a bioremediation agent for oil-contaminated environments due to the strain of SBE01's lipolytic and lipase activity.[52] Kusumo et al. also discovered that *Moraxella* sp. bacteria isolated from rivers in Wonocolo, Bojonegoro, can reduce hydrocarbons to 0.67%/hour during the exponential growth phase.[53]

Previous research indicates that the genera Klebsiella, Burkholderia, and Moraxella can breakdown hydrocarbons.[45,54–56] In general, the microbial oil degradation process began with the emulsification of the petroleum pollutant by bacteria-secreted surfactant. The emulsified hydrocarbon was subsequently absorbed by the microorganisms, followed by active or passive transport and endocytosis penetrating the cell membrane. In the end, the petroleum hydrocarbon that enters the cell undergoes an enzymatic reaction with the matching enzyme to degrade the pollutant.[57] Microbial communities

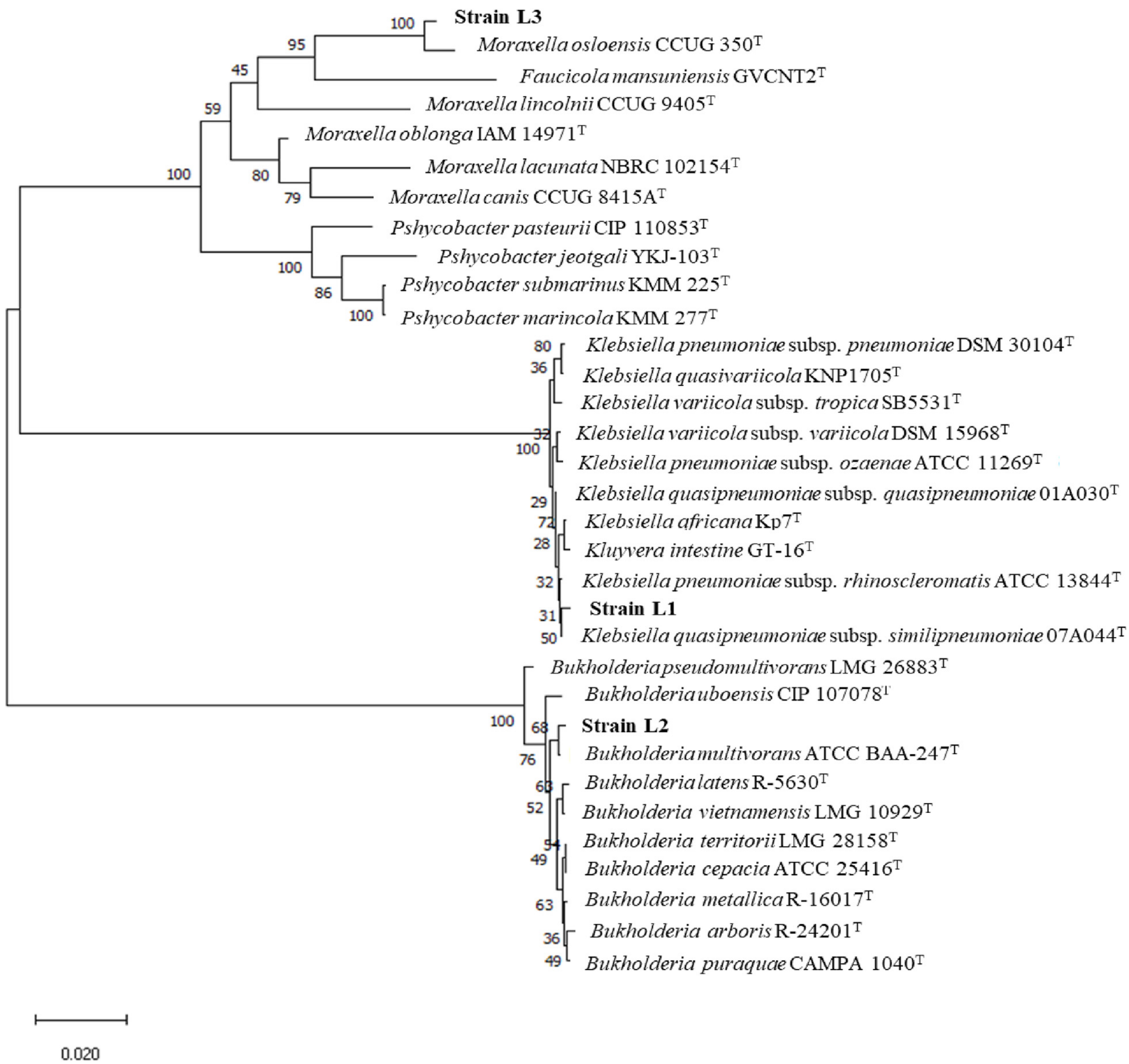

Fig. 2. Phylogenetic tree of strain L1, L2, and L3 and closely related species based on 16 s rRNA gene sequence. The calculations were performed according to the neighbor-joining algorithm (bootstrap number = 1000), and the scale bar represents 0.020 sequence divergence.

with high enzymatic capacity can digest complex hydrocarbons (aliphatics and polyaromatics), and knowing microbial functional diversity and the factors influencing microbial functions is vital for bioremediation investigations.[58] Several processes promote its breakdown, which can be identified by genes related to their hydrocarbon routes, monooxygenase (alkB1) and dioxygenase (nodB, todC1, and xylE).[58]

3.2. Lineament and drainage pattern interpretation

The interpretation results identified 372 lineaments with varying lengths between 400 and 4000 m and then plotted them on a rose diagram (Fig. 3a). Plotting the results show that the general direction of lineaments developing in the Minas field and its surroundings can be grouped into NW–SE, N–S, and NE–SW directions (Fig. 3a). Lineaments in the N–S and NW–SE directions correspond to the main of structures that developed in the CSB, where structures trending N–S are known as old structures and NW–SE as young structures.[31,32] The structure in the N–S and NW–SE directions is rejuvenated by the compression forces and the extension of the wrench fault system during the tertiary.[31] Lineaments with the NE–SW direction are thought to have occurred by tectonics in the

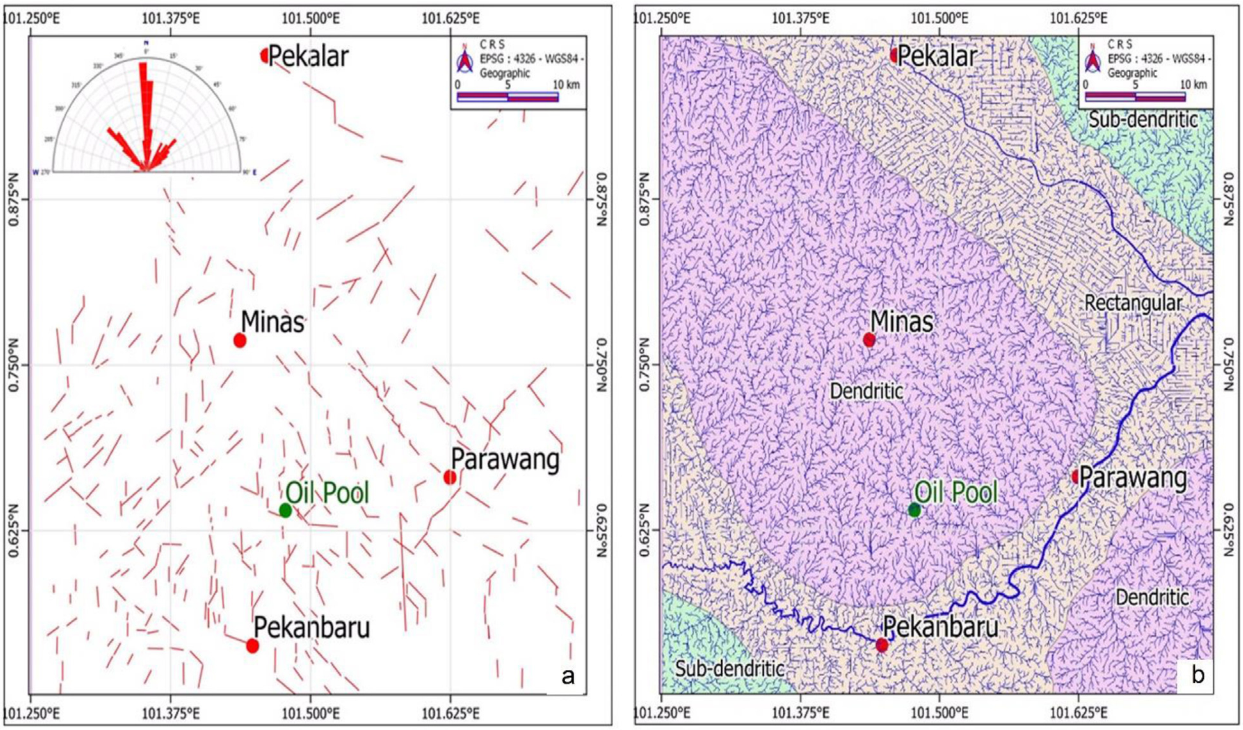

Fig. 3. The interpretation results of DEMNAS Data, a. Lineaments maps of Minas Area and rose diagram, b. Drainage pattern map.

Plio-Pleistocene. The direction of these lineaments intersects the Minas anticline axis perpendicularly, so it is thought to be the result of the tectonic extension forces mentioned above and forms joints on the surface. It is suspected that the surface lineaments are not connected to the oil and gas reservoirs zone.

The interpretation of the drainage pattern that developed in Minas shows a dendritic pattern, a sub-dendritic pattern, and a rectangular pattern. The dendritic drainage pattern stretches in the NW–SE direction, surrounded by a rectangular drainage pattern, and then a sub-dendritic drainage pattern is found on the southwest and northeast sides (Fig. 3b). The dendritic drainage pattern developed in the Minas anticline structure is classified as a broad and gentle sloping anticline, which stretches towards the southeast, cut by the Siak River. This drainage pattern flows in the Petani Formation, the Minas Formation, and some alluvium deposits composed of sandstone, claystone, mudstone, sand, and gravel. The sub-dendritic drainage pattern develops in the northeast and southeast of the study area. This drainage pattern develops over the Minas Formation and Alluvial Deposits, composed of sandstone, claystone, mudstone, sand, clay, and gravel, with a gently sloping morphology. The rectangular drainage pattern develops around the Minas anticline structure, along the Siak River and its tributaries, from Pekanbaru–Perawang–Pekalar. This drainage pattern dominantly flows over alluvial deposits composed of sand, clay, and gravel, with a flat morphology. Human activities with the construction of irrigation networks for agriculture heavily influence the drainage pattern in this unit. This result showed no faults develop in the area.

3.3. The status of oil pool in Minas field

The research area topographically is located at an elevation of 110 to 10 m above sea level, with slopes ranging from 0 to 30%, includes flat to moderately steep slopes.[59] The phenomenon of oil seepage is located on a gentle slope, and at 1 km to north direction, moderately steep slopes are found, with an elevation difference of 20 m. So, there were no sudden changes in elevation, which are believed to be evidence of a fault. The analysis shows the phenomenon of oil pool on the Ukai river bank is located in a dendritic pattern, with general shapes, such as leaves, developing on rocks with relatively the same hardness that are flat and resistant to weathering. The surface geology of the study area is covered by the Petani, Minas, Old Alluvial, and Young Alluvial Formations, which are composed of sedimentary rocks, namely sandstone, claystone, gravel, sand, and shale. Based on this analysis, the study area has a linear relationship between the rock types exposed, the slopes, and the drainage patterns that develop, and not found the structure of geology controlled this area. This result is by the results of the topographical analysis described in the previous paragraph, where no faults develop in the area.

Lineament analysis performed on DEMNAS data by hill-shade processing with light azimuth variations of 0°, 315°, 270°, and 225° and altitude of light 45° produces three main lineament directions, namely NW–SE, N–S, and NE–SW. These results are in accordance with previous research in a

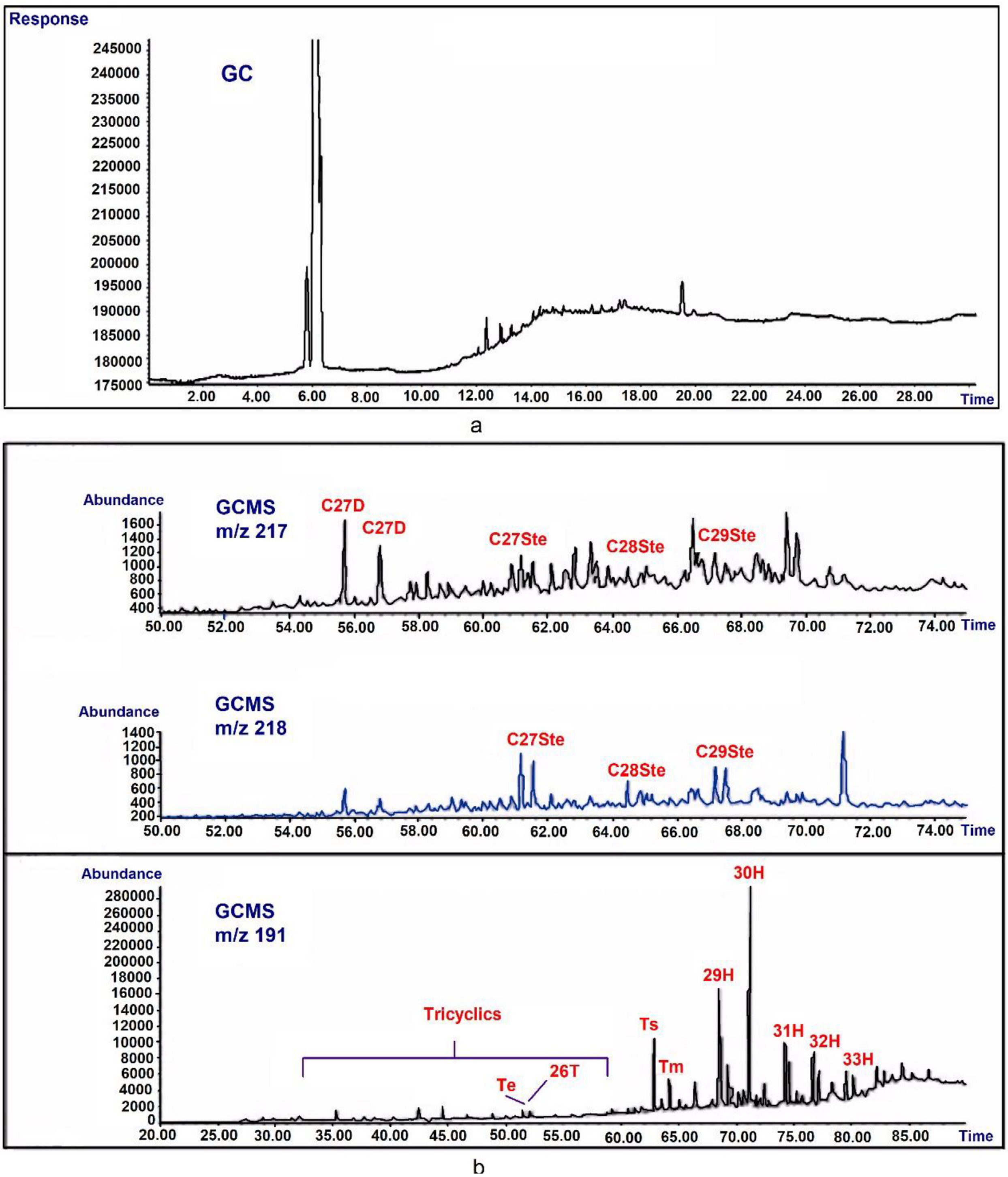

Fig. 4. Chromatogram analysis results, a. GC analysis of Ukai River oil seepage showing loss of light fraction due to biodegradation, b. GCMS analysis results show a wide distribution of n-alkanes.

regional study of the CSB. Locally, lineaments with the NE–SW direction identified intersect perpendicular to the Minas anticline axis.[7,11] The subsurface map of Top Sihapas in the Minas field shows a normal fault structure in the N–S and NW–SE directions with a vertical displacement of about 125 feet.[1,29] The seismic trajectory with the W–E direction shows a fault interpretation that indicates a termination fault in the Petani Formation and does not reach the surface. Previous research also shows that the Minas field structure does not have long and complex faults and is not continuous on the surface.[60] The oil seep phenomenon is located about 3 km from the southern tip of the two normal faults.

Based on the analysis of surface and subsurface data, it can be concluded that there is no evidence to support that the oil pool to the south of the Minas field is a natural seepage. This result showed the existing extensional structures are less disturbed, so that very little seepage occurs, despite high oil reserves. The lack of seepage was due to the limited inversion and erosion that occurred in the basin, and not many lineaments play a role in providing seepage pathways and closed rock pores.[61] Natural seepage often occurs through faults and fractures, and the seal has been eroded, causing the reservoir outcrops.[62] In general, seepage intensity is largely controlled by current tectonics and the timing of petroleum migration.[63,64] Seepage is dominant in tectonic features such as active diapers, faults, and uplifted basin margins at the sub-basin scale. It is explained further that seepage rarely occurs in traps buried under a thick, undisturbed layer of overburden. However, the fault systems in the subsurface of Minas field tend to be sealing or a barrier to hydrocarbon migration.[65]

Geochemical (GC) analysis shows that the oil pool at the study location has experienced degradation characterized by the loss of light-chain alkane fractions (Fig. 4a). The GCMS results show that the oil pool at this location has a wide distribution of n-alkanes, which have been degraded by microbes or washed away by rainwater over a long exposure time (Fig. 4b). The results of the comparison of biomarker parameters with Minas oil, which have been studied by the Research Center for Oil and Gas Technology, LEMIGAS show that the characteristics of the oil pool are similar to those of Minas oil.[66] The results of analysis carried out in previous research show that the API gravity of Minas's oil is 33°API,[67] while the API gravity of oil pools that have been further degraded is 11°API.[68] There is a slight difference with the oil in the field reservoir due to the biodegradation process by bacteria that occurs on the surface resulting in the loss of the light fraction of the alkane chain.

Based on this study, it can be ascertained that the oil pool originates from the Minas field. Klebsiella, Burkholderia, and Moraxella bacteria found in the oil pool are able to live and degrade light hydrocarbons into heavy hydrocarbons. The presence of bacteria is not related to the status of oil pools; the migrating of the oil to the surface is naturally due to geological conditions such as the presence of joints or faults, and it is called an oil seepage. However, the migrating of oil to the surface in the south of the Minas field cannot be explained conclusively based on remote sensing data and is supported by subsurface reservoir data from the Minas field.

3.4. Conclusions

According to the study, three bacteria, *K. quasipnumoniae* strain L1, *B. multivorans* strain L2, and *M. osloensis* strain L3 have been isolated from the CSB, Indonesia. These three bacteria can break down heavy oil, making them suitable bioremediation agents for oil pollution in the soil, notably in Indonesia. These bacteria can also be used to explore the potential for heavy oil in the CSB based on its life characteristics at specific temperatures. If these bacteria can live at a maximum temperature of 60 °C, then they can degrade crude oil into heavy oil up to a depth of 555.5 m. Based on the geochemical data, the crude oil contained in the oil pool is identical to the oil from the Minas oil field. With surface geological data in the form of lithology types, drainage patterns, and lineaments, as well as surface morphology, the status of the presence of an oil pool cannot be ascertained when oil flows from the reservoir through the oil flow paths. In the future, research on the mechanism of oil degradation employing these three isolates should be conducted. Furthermore, full genome sequence analysis is necessary to obtain complete sequence data and identify probable gene clusters that encode the capacity for oil breakdown. So it can be applied to remediation programs in environments affected by oil spills. These results can also be a guide in identifying the presence of layers (traps) containing heavy oil as a result of biodegradation. By combining it with knowledge of local regional geology, it is hoped that heavy oil accumulations can be found in the Central Sumatra basin.

Funding

This work was funded by the Advanced Indonesia Research and Innovation (RIIM) under grant No. B -1810/III.1/FR/11/2022, and R&D Centre for Oil and Gas Technology LEMIGAS-Ministry of Energy and

Mineral Resources, Republic of Indonesia No. 14.K/73/BLM/2020.

Author contributions

TMS contributed to conceptualization, methodology, formal analysis, investigation, writing-original draft, project administration, and funding acquisition. AR contributed to conceptualization, methodology, formal analysis, investigation, data curation, writing-original draft, visualization. SS contributed to software, formal analysis, investigation, resources, writing-original draft and visualization. HLS contributed to validation, investigation, and data curation. BW contributed to writing-review & editing, supervision, and funding acquisition. JSH contributed to software, validation, investigation, resources, data curation, and visualization.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We would like to thank: (1) National Research and Innovation Agency-BRIN and Indonesia Endowment Fund for Education Agency-LPDP through the Advanced Indonesia Research and Innovation (RIIM) funding program for providing financial support for this research; (2) R&D Centre for Oil and Gas Technology LEMIGAS, Energy and Mineral Resources Agency, Ministry of Energy and Mineral Resources, for funding and supporting heavy oil research in the Central Sumatra basin by allowing the use of geochemical laboratory facilities and process laboratories; (3) The head and staff of the Research Center for Geoinformatics who have supported this study; and (4) The E-Layanan Sains, National Research and Innovation Agency (BRIN), for identifying the bacteria found in the field samples. We also thank all parties who have helped in this study.

References

1. Indonesia Petroleum Association (IPA). *Indonesia-oil and gas fields Atlas, Vol. 2: Central Sumatra. 2nd ed. Jakarta, Indonesia: IPA Professional Division Oil & Gas Fields Altas Sub-Committee*. 2006.
2. Koning T, Cameron N, Clure J. Undiscovered potential in the basement : exploring in Sumatra for oil and gas in naturally fractured and weathered basement reservoirs. *Berita Sedimentologi*. 2021;47:67−79.
3. Roezin S. The discovery and development of the Petapahan oil field, Central Sumatra. In: *The 3rd Annual Convention Proceedings*. 1974:111−127.
4. Koning T, Darmono FX. The geology of the Beruk northeast field, Central Sumatra: oil production from pre-tertiary basement rocks. In: *13th Annual Convention Proceedings*. 1984: 385−406.
5. Umri NH, Indrawardana. Basement reservoir opportunity in central Sumatra Basin. In: *Thirty Eighth Annual Connvention & Exhibition*. 2014:IPA14-G−57.
6. Meyer RF, Attanasi ED, Freeman PA. Heavy oil and natural bitumen resources in geological basins of the world. In: *Open File Report 2007 - 1084*. 2007:36.
7. Susantoro TM, Suliantara Setiawan HL, Widarsono B, Wikantika K. Heavy oil potentials in Central Sumatra Basin, Indonesia using remote sensing, gravity, and petrophysics data: from literature review to interpretations and analyses. *Indonesian J Sci Technol*. 2022;7:363−384.
8. Abrams MA. Significance of hydrocarbon seepage relative to petroleum generation and entrapment. *Mar Petrol Geol*. 2005; 22:457−477.
9. Williams HH, Eubank RT. *Hydrocarbon Habitat in the Rift Graben of the Central Sumatra Basin, Indonesia*. vol. 80. Geological Society, London, Special Publications; 1995:331−371.
10. Salati S, van Ruitenbeek FJA, Carranza EJM, van der Meer F, Tangestani MH. Conceptual modeling of onshore hydrocarbon seep occurrence in the Dezful Embayment, SW Iran. *Mar Petrol Geol*. 2013;43:102−120.
11. Suliantara Susantoro TM, Setiawan HL, Firdaus N. A preliminary study on heavy oil location in Central Sumatra using remote sensing and geographic information system. *Scientific Contributions Oil and Gas (SCOG)*. 2021;44: 39−54.
12. Ibanez DM, Pestilho ALS, Miranda FP, Penteado HLB, Dias Filho DC. An integrated remote sensing and biomarker maturity parameter-based approach to trace petroleum migration in a complex fault zone lineament of the Brazilian Equatorial Margin. *Mar Petrol Geol*. 2022;144:105844.
13. Susantoro TM, Wikantika K, Suliantara S, Setiawan HL, Harto AB, Sakti AD. Applying random forest to oil and gas exploration in Central Sumatra basin Indonesia based on surface and subsurface data. *Remote Sens Appl: Society and Environment*. 2023;32:101039.
14. Cameron NR, Ghazali SA, Thompson SJ. *Geological Map of the Siak Sriindrapura & Tanjung Pinang Quadrangles*. Bandung: Sumatera; 1982.
15. Clarke MCG, Kartawa W, Djunuddin A, Suganda E, Bagdja M. *Geological Map of the Pakanbaru Quadrangle*. Bandung: Sumatera; 1982.
16. Suwarna N, Budhitrisna T, Santosa S, Mangga SA. *Geological Map of Rengat Quadrangle*. Bandung: Sumatera; 1994.
17. Rahayu YS, Yuliani, Trimulyono G. Isolation and identification of hydrocarbon degradation bacteria and phosphate solubilizing bacteria in oil contaminated soil in Bojonegoro, East Java, Indonesia. *Indonesian JSci Technol*. 2019;4:134−147.
18. Hadimuljono JS, Firdaus N. Determination of biodegradation zone in central Sumatra Basin. *Scient Contribution Oil Gas*. 2021;44:55−63.
19. Nurhandoko BEB, Kurniadi R, Susilowati S, et al. Integrated subsurface temperature modeling beneath Mt. Lawu and Mt. Muriah in the northeast Java basin, Indonesia. *Open Geosci*. 2019;11:341−351.
20. Medina-Bellver JI, Marin P, Delgado A, et al. Evidence for in situ crude oil biodegradation after the Prestige oil spill. *Environ Microbiol*. 2005;7:773−779.
21. April T, Foght J, Currah R. Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in northern and western Canada. *Can J Microbiol*. 2000;46:38−49.
22. Xu X, Liu W, Tian S, et al. Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. *Front Microbiol*. 2018; 9:2885.

23. Chunyan X, Qaria MA, Qi X, Daochen Z. The role of microorganisms in petroleum degradation: current development and prospects. *Sci Total Environ*. 2023;865:161112.
24. Yani M, Charlena C, Mas'ud ZA, Anas I, Setiadi Y, Syakti AD. Isolation, selection and identification of polyaromatic hydrocarbons (Pahs) degrading bacteria from heavy oil waste (how)-contaminated soil. *HAYATI J Biosci*. 2020;27:142–153.
25. Cahyani CN, Ismayana A, Yani M. Crude oil biodegradation potential using acinetobacter baumannii CYA20 and Bacillus subtilis CYA27 from the Bekasi coast, Indonesia. *HAYATI J Biosci*. 2022;29:701–711.
26. Setiani P, Ramdani F. Oil spill mapping using multisensor Sentinel data in Balikpapan Bay, Indonesia. In: *2018 4th International Symposium on Geoinformatics (ISyG)*. 2018: 1–4.
27. Spies RB, Mukhtasor M, Burns KA. The Montara oil spill: a 2009 well Blowout in the timor sea. *Arch Environ Contam Toxicol*. 2017;73:55–62.
28. Matahelemual GJS, Harto AB, Susantoro TM. Oil spill detection using Sentinel-1 multitemporang data in offshore Karawang. *Scient Contribution Oil Gas*. 2020;43:69–79.
29. Willis B, Fitris F. Sequence stratigraphy of Miocene tide-influenced sandstones in the Minas field, Sumatra, Indonesia. *J Sediment Res*. 2012;82:400–421.
30. Setiawan HL, Suliantara, Widarsono B. Relationship between tectonic evolutions and presence of heavy oil in the central Sumatra Basin. *Scient Contribution Oil Gas*. 2021;44: 21–37.
31. Yarmanto, Noeradi D, Hendar. Telisa deposition model in the central Sumatra Basin. In: *Proceeding of the Indonesia Petroleum Association, 34th Annual Convention*. 2011:IPA10-G–204.
32. Siringoringo LP, Sapiie B, Rudyawan A, Sucipta IGBE. Origin of high heat flow in the back-arc basins of Sumatra: an opportunity for geothermal energy development. *Energy Geosci*. 2024;5:100289.
33. Mertosono S, Nayoan GAS. The tertiary basinal area of Central Sumatra. In: *Indonesian Petroleum Association Proceeding*. 1974:63–76.
34. Pitcher DG, Saunders NA, Owen RJ. Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol*. 1989;8:151–156.
35. White TJ, Bruns T, Lee S, Taylor J. Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. *PCR Protocols*. 1990;31:315–322.
36. Hiraishi A, Kamagata Y, Nakamura K. Polymerase chain reaction amplification and restriction fragment length polymorphism analysis of 16S rRNA genes from methanogens. *J Ferment Bioeng*. 1995;79:523–529.
37. Rosyidah A, Weeranantanapan O, Chudapongse N, Limphirat W, Nantapong N. Streptomyces chiangmaiensis SSUT88A mediated green synthesis of silver nanoparticles: characterization and evaluation of antibacterial action against clinical drug-resistant strains. *RSC Adv*. 2022;12: 4336–4345.
38. Pannekens M, Kroll L, Müller H, Mbow FT, Meckenstock RU. Oil reservoirs, an exceptional habitat for microorganisms. *N Biotech*. 2018;49:1–9.
39. Falaki F. Sample preparation techniques for gas chromatography. In: Kusch P, ed. *Gas Chromatography. London*. IntechOpen; 2018:31.
40. Ahmadi H, Pekkan E. Fault-based geological lineaments extraction using remote sensing and GIS-a review. *Geosci*. 2021;11:183–214.
41. Durighetto N, Vingiani F, Bertassello LE, Camporese M, Botter G. Intraseasonal drainage network dynamics in a headwater catchment of the Italian alps. *Water Resour Res*. 2020;56:e2019WR025563.
42. Sangireddy H, Carothers RA, Stark CP, Passalacqua P. Controls of climate, topography, vegetation, and lithology on drainage density extracted from high resolution topography data. *J Hydrol*. 2016;537:271–282.
43. Afifi A, Motamedi H, Alizadeh B, Leilavi H. Isolation and identification of oil degrading bacteria from oil sludge in Abadan oil refinery. *Environ Exp Biol*. 2015;13:13–18.
44. Rajkumari J, Paikhomba Singha L, Pandey P. Genomic insights of aromatic hydrocarbon degrading Klebsiella pneumoniae AWD5 with plant growth promoting attributes: a paradigm of soil isolate with elements of biodegradation. *3 Biotech*. 2018;8:118.
45. Rodrigues DF, Sakata SK, Comasseto JV, Bícego MC, Pellizari VH. Diversity of hydrocarbon-degrading Klebsiella strains isolated from hydrocarbon-contaminated estuaries. *J Appl Microbiol*. 2009;106:1304–1314.
46. Peralta DP, Chang AY, Ariza-Hutchinson A, Ho CA. Burkholderia multivorans: a rare yet emerging cause of bacterial meningitis. *IDCases*. 2018;11:61–63.
47. Bacosa HP, Inoue C. Heavy oil degrading Burkholderia and Pseudomonas strains: insights on the degradation potential of isolates and microbial consortia. *Palawan Scientist*. 2020;12: 74–89.
48. Cauduro GP, Leal AL, Lopes TF, Marmitt M, Valiati VH. Differential expression and PAH degradation: what Burkholderia vietnamiensis G4 can tell us? *Internet J Microbiol*. 2020;2020:8831331.
49. Morya R, Salvachúa D, Thakur IS. Burkholderia: an untapped but promising bacterial genus for the conversion of aromatic compounds. *Trends Biotechnol*. 2020;38:963–975.
50. Travkin VM, Solyanikova IP. Salicylate or phthalate: the main intermediates in the bacterial degradation of naphthalene. *Processes*. 2021;9:1–19.
51. Koleri J, Petkar HM, Husain AAM, Almaslamani MA, Omrani AS. Moraxella osloensis bacteremia, a case series and review of the literature. *IDCases*. 2022;27:e01450.
52. Soleha S, Retnaningrum E. Screening and molecular identification of lipolytic bacteria from spent bleaching earth. *Biodiversitas*. 2020;21:4155–4161.
53. Kusumo BD, Wilopo W, Retnaningrum E. Preliminary study of liquid hydrocarbon biodegradation by indigineous bacteria isolated from Wonocolo village, Bojonegoro district, east java province. *J Appl Geol*. 2018;2:98.
54. Revathy T, Jayasri MA, Suthindhiran K. Biodegradation of PAHs by Burkholderia sp. VITRSB1 isolated from marine sediments. *Scientifica*. 2015;2015:1–9.
55. Bilen Ozyurek S, Seyis Bilkay I. Biodegradation of petroleum by Klebsiella pneumoniae isolated from drilling fluid. *Int J Environ Sci Technol*. 2018;15:2107–2116.
56. You Z, Xu H, Zhang S, et al. Comparison of petroleum hydrocarbons degradation by Klebsiella pneumoniae and Pseudomonas aeruginosa. *Appl Sci*. 2018;8:1–19.
57. Li X, Li H, Qu C. A review of the mechanism of microbial degradation of petroleum pollution. *IOP Conf Ser Mater Sci Eng*. 2019;484:1.
58. Ehis-Eriakha CB, Chikere CB, Akaranta O. Functional gene diversity of selected indigenous hydrocarbon-degrading bacteria in aged crude oil. *Internet J Microbiol*. 2020;2020.
59. Qiu H, Regmi AD, Cui P, Cao M, Lee J, Zhu X. Size distribution of loess slides in relation to local slope height within different slope morphologies. *Catena*. 2016;145:155–163.
60. Julikah, Rahmat G, Wiranatanegara MB. Subsurface geological evaluation of the central Sumatra Basin in relation to the presence of heavy oil. *Scient Contribution Oil Gas*. 2021;44: 65–81.
61. Boruah A, Verma S, Rasheed A, Siddharth Gairola G, Gogoi A. Macro-seepage based potential new hydrocarbon prospects in Assam-Arakan Basin, India. *Sci Rep*. 2022;12:2273.
62. Leifer I. A synthesis review of emissions and fates for the coal oil point marine hydrocarbon seep field and California marine seepage. *Geofluids*. 2019;2019.
63. Ciotoli G, Procesi M, Etiope G, Fracassi U, Ventura G. Influence of tectonics on global scale distribution of geological methane emissions. *Nat Commun*. 2020;11:1–8.
64. Kroeger KF, Bischoff A, Nicol A. Petroleum systems in a buried stratovolcano: maturation, migration and leakage. *Mar Petrol Geol*. 2022;141.

65. Richmond WC, Dwidjojuwono H, Tastari A, Toha B. Reservoir compartmentalization: an integrated evaluation of supermature Minas oil field, central Sumatra. In: *28th Annual Convention Proceedings Indonesian Petroleum Association*. vol. 2. 2012:137–156.
66. Research and Development Centre for Oil and Gas LEMIGAS. *The Study of Heavy Oil (Oil Sands) Potential and Bitument in Central Sumatra Basin. Jakarta*. 2020.
67. Benard JH, Richardson WC, Sitton GM. Steam oil displacement and vaporization of Minas oil in slimtubes. In: *The SPE/DOE Improved Oil Recovery Symposium, Vol. All Days, P. SPE-39639-MS*. 1998.
68. Widarsono B, Setiawan HL, Susantoro TM, et al. An integrated approach for revisiting basin-scale heavy oil potential of the Central Sumatera Basin. *Scient Contribution Oil Gas*. 2021;44:1–20.