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Research Article

Degradation of diesel fuel by *Pseudomonas aeruginosa* B031 with expression of the *alkB* gene in a column bioreactor

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Abstract

Diesel fuel pollutants contain toxic hydrocarbons dominated by aliphatic and aromatic hydrocarbons. Hydrocarbon pollutants can be degraded in an environmentally friendly manner through bioremediation using hydrocarbonoclastic bacteria, i.e., *Pseudomonas aeruginosa* B031, which harbors the *alkB* gene that encodes an alkane hydroxylase that degrades alkane chains in hydrocarbons. This study compared the ability and efficiency of *P. aeruginosa* B031 to degrade diesel pollutants in a batch system and a continuous system using a column bioreactor, as well as the expression of *alkB*. *P. aeruginosa* B031 could more efficiently degrade diesel fuel in a continuous system in a column bioreactor than in the batch system. The concentrations of phenol, total organic carbon, chemical oxygen demand, and biological oxygen demand in the column bioreactor underwent a greater decrease than those in the batch system, namely 1.5-fold, 1.7-fold, 1.4-fold, and 1.3-fold, respectively. The decrease in these concentrations was followed by changes in functional groups, as shown via Fourier transform infrared (FTIR) spectroscopy. The number of bacteria and the concentration of exopolysaccharide increased in the column bioreactor by 4-fold and 2.3-fold more than the increase in the batch system. The ability of *P. aeruginosa* B031 to degrade diesel fuel in the column bioreactor was also demonstrated by the higher *alkB* expression of *P. aeruginosa* B031 than that in the control.

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Introduction

Several cases of diesel fuel pollution in the environment Diesel fuel pollution in the environment can be caused by exploration, refining, processing, transfer, and storage activities (Hossain et al., 2022; Wang et al., 2022). The diesel fuel pipeline of the Energy Company was reported to have leaked, with approximately 80,000 L of diesel fuel spilled as environmental pollution (Puspoayu et al., 2019). Soil pollution due to diesel fuel can affect water organoleptic properties as well as cause genetic mutation and death of organisms due to toxic compounds found in hydrocarbons (Kumar et al., 2022). The hydrocarbons contained in diesel fuel are dominated by aliphatic and aromatic hydrocarbons. Aliphatic hydrocarbons are composed of linear and cyclic carbon chains (e.g., paraffins and alkynes), while aromatic hydrocarbons are composed of six carbon atoms with a hydrogen atom attached to each carbon atom (e.g., polycyclic aromatic hydrocarbons and phenolic compounds). The toxicity of phenolic

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compounds varies depending on the number of atoms or molecules attached to the benzene chain. For chlorinated phenols, the more chlorine atoms attached to the benzene chain, the higher the toxicity of the compound (Liu and Zhang, 2019). Aromatic hydrocarbons have a higher level of toxicity to organisms than aliphatic hydrocarbons because they can eliminate the cell fluids of organisms (Al-Wasify and Hamed, 2014; El-Borai et al., 2016). Thus, optimal and eco-friendly treatment is required to reduce diesel fuel contamination as well as prevent damage to the environment caused by this. Bioremediation is an alternative treatment for diesel fuel spills because this can degrade diesel fuel pollutants in the environment into simpler molecules that can be used as a source of energy for microorganisms (Ali, 2019; Sari and Retnaningrum, 2019; Benget and Retnaningrum, 2020; Rehman et al., 2021).

Bioremediation can be performed in two ways, i.e., in-situ and ex-situ. In-situ bioremediation is performed directly in polluted areas using indigenous microorganisms so that the remediation occurs naturally, whereas ex-situ bioremediation uses a replica of the contaminated environment. Both techniques are optimal in degrading contaminants, but the ex-situ technique offers more benefits because it is safer, faster, and more effective in treating contaminated soil to prevent toxic effects on flora and fauna in a polluted environment. A commonly used ex-situ technique is the use of a bioreactor, particularly a column bioreactor, because this can control multiple factors including temperature, pH, aeration, and the microorganisms used, thus optimizing the remediation process (Balseiro-Romero et al., 2019; Ugochukwu et al., 2021; Gonçalves and Delabona, 2022; Kumar et al., 2022; Basu and Guha, 2023). The microorganisms used in column bioreactors for the optimal treatment of hydrocarbons are hydrocarbonoclastic bacteria (Kadri et al., 2021). *Pseudomonas aeruginosa* is a hydrocarbonoclastic bacteria that can degrade large amounts of hydrocarbons through the activities of alkane hydroxylase and alcohol dehydrogenase enzymes that degrade the alkane chains in hydrocarbons. Alkane 1-monooxygenase is encoded by the *alkB* gene (Kuppusamy et al., 2016; Meng et al., 2018; Muthukumar et al., 2022). Based on the potential of these bacteria and bioreactors, this study sought to determine the ability and efficiency of *P. aeruginosa* B031 bacteria used in bioreactors to degrade diesel fuel in the environments and to evaluate the expression of *alkB* genes from *P. aeruginosa* B031 bacteria that have a role in degrading diesel fuel.

Materials and Methods

Enrichment and acclimatization of P. aeruginosa B031

The *P. aeruginosa* B031 strain used in this study was isolated from the rhizosphere of *Paraserianthes falcataria* L. Nielsen on land where phytoremediation was conducted (Retnaningrum and Wilopo, 2018). Bacteria were first subcultured on nutrient agar media for propagation, which were then used as stock cultures. Bacteria were acclimatized on mineral salt medium (MSM) supplemented with diesel with varying concentrations (1%, 2%, 4%, 6%, 8%, and 10%) with an incubation time of 14 days in a water bath shaker at 45 rpm and 37 °C; the MSM comprised 1% K_2HPO_4, 1% KH_2PO_4, 1% $(NH_4)_2SO_4$, 0.02% $CaCl_2$, 0.2% $MgSO_4.7H_2O$, and 0.05% $FeCl_3$ (W/V).

Preparation of column bioreactor

The column bioreactor was designed as a tube (using PVC pipe) with a height of 100 cm and a diameter of 11 cm. The test reactor contained solid materials such as medium gravel, small gravel, fine sand, and silt, as shown in Figure 1. The reactor was then filled with 3,617 mL of liquid media, 1% *P. aeruginosa* culture, and 10% hydrocarbons. The hydraulic retention time of the reactor used in this study was set for 4 days (Zahraeifard and Deng, 2011). The flow rate was set at 0.63 mL/min in accordance with the following calculation:

$$Q = {}^{V}/_{t} = {}^{3,617\ mL}/_{5,760\ min} = 0.63\ {}^{mL}/_{min}$$

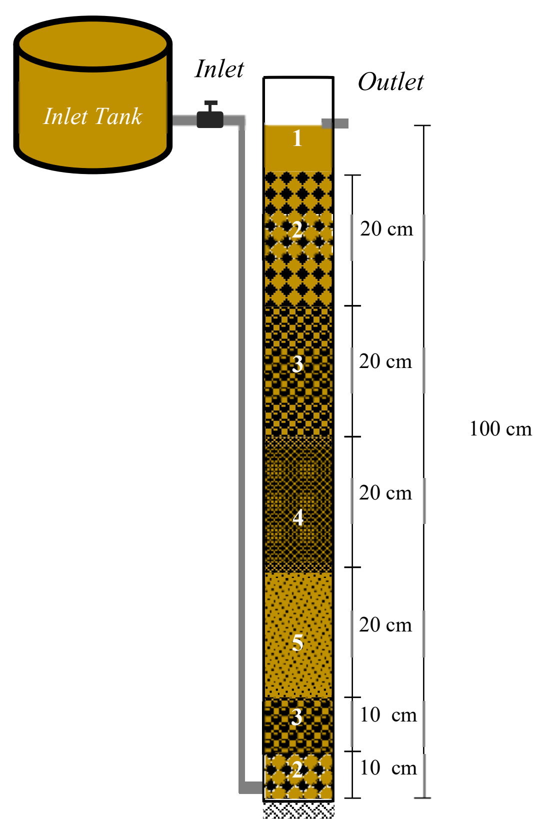

Figure 1. Design of column bioreactor in continuous system: 1. Maximum limit of diesel fuel, 2. Medium gravel, 3. Small gravel, 4. Fine sand, 5. Silt.

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Test of diesel fuel biodegradation

The biodegradation of diesel fuel pollutants by *P. aeruginosa* B031 was tested using two methods. The first method was conducted in a batch system in a 500-mL Erlenmeyer flask containing 360 mL of MSM supplemented with 10% diesel fuel. A total of 40 mL of *P. aeruginosa* B031 culture that had been acclimatized with 5.5×10^5 CFU/mL was inoculated into the system.

The biodegradation using the batch system was performed for 62 days from 31 °C-37 °C. In the second method, biodegradation was conducted in a continuous system in a column bioreactor. The inlet basin was filled with 3,617 mL of bacterial culture that had been acclimatized with 5.5×10^5 CFU/mL and 10% diesel fuel. The flow rate from the inlet basin to the bioreactor column was set at 0.63 mL/min. The continuous system was performed for 38 days with a temperature range of 31 °C-37 °C.

The biodegradation of diesel fuel by *P. aeruginosa* B031 in both batch and continuous systems was analyzed based on changes in the chemical and biological parameters of the reactor during the processes. The biodegradation of diesel fuel by bacteria in the batch system was observed on days 0 and 62, while that in the continuous system was observed on days 0, 4, 8, 14, 22, 30, and 38. At the end of the continuous system, the degradation by *P. aeruginosa* bacteria was also analyzed based on the expression of the *alkB* gene via real-time quantitative PCR (RT-qPCR).

Chemical characterization of reactor

The chemical characterization of the reactor included monitoring the temperature, pH, oxidation-reduction potential (ORP), phenolic content, biological oxygen demands (BOD), chemical oxygen demands (COD), total organic carbon (TOC), and compounds resulting from the degradation of diesel fuel by bacteria.

The pH and ORP tests were conducted using the SNI 06-6989.11-2004 method using the Eutech™ pH 700 Meter type ECPH70042GS (platinum electrodes). The phenolic content was tested using the Folin–Ciocalteu reagent according to SNI 06-6989.21-2004 to determine the total amount of phenolic compounds that were toxic to the sample.

The BOD testing was conducted by calculating the difference between DO_0 and DO_5 according to SNI 6,989.72-2009. The DO (dissolved oxygen) test referred to SNI 06-6989.14-2004 using the Winkler method. The COD testing was conducted by measuring the amount of oxygen needed to degrade the organic pollutants contained in the sample according to SNI 06-6989.2-2004 using Thermo Fisher Scientific GENESYS™ 180 UV-Vis Spectrophotometer type 840-309000.

The TOC was tested using the Walkley–Black method (Walkley and Black, 1934). The compounds resulting from the degradation of hydrocarbons were analyzed using Thermo Scientific™ Nicolet™ iS10 FTIR spectrometer to identify and characterize the chemical compounds contained in the sample through the absorption of infrared spectra (Pathak et al., 2017).

Biological characterization of reactor

The biological characterization was conducted based on the number of bacteria and the production of bacterial exopolysaccharides (EPS) during the degradation of diesel fuel. The number of bacteria was analyzed using the total plate count method. The amount of EPS was measured using the phenol-sulfuric method with a glucose standard solution (Dubois et al., 1956; Celik et al., 2008; Onbasli and Aslim, 2009).

Expression of alkane 1-monooxygenases (alkB) gene

Cultures of *P. aeruginosa* B031 grown in MSM with and without the addition of 10% diesel after reaching 1.5×10^7 CFU/mL were harvested. Bacterial RNA was isolated using the Zymo Research-YeaStar RNA Kit R1002. The quality of total bacterial RNA was confirmed using an RNA 6,000 Nano LabChip in an Agilent 2,100 bioanalyzer. A total of 10 µL of RNA was synthesized into cDNA using ExcelRT™ Reverse Transcription Kit II 100 Rxn type RP1400.

The cDNA synthesis was verified using nanodrop to determine the concentration and purity. Afterward, the gene expression was evaluated using a thermal cycler qPCR CFX96 machine (Bio-Rad). The qPCR mix used was SensiFAST SYBR No-ROX Kit (Bioline). The reaction started with predenaturation process (95 °C for 1 min), 40 cycles of amplification (denaturation at 95 °C for 10 s, annealing at 57 °C–58.8 °C for 10 s, and extension at 72 °C for 30 s).

The primers used, and the qPCR thermal cycle reaction are shown in Table 1. The fluorescence of the qPCR results was read at the end of the extension stage, followed by melt curve analysis. The gene expression was calculated relative to the sample with the cycle threshold comparison method ($2^{-\Delta\Delta CT}$).

Table 1. Oligonucleotide primer sequence for qPCR.

Gene	Sequence 5’ → 3’	Tm	Length of qPCR fragment	References
alkB	F-ACCATGTACACGTCTCGACCC R-CGTACCACCAGATCAGCTCGT	61.82 61.88	183 bp	In silico
16S rRNA	F-AGAGTTTGATCCTGGCTCAG R-CGGTTACCTTGTTACGACTT	57.00 57.00	1450 bp	Husain and Aziz (2022)

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Results and Discussion

Acclimatization of P. aeruginosa in a medium containing varying concentrations of diesel fuel

During the 14-day acclimatization, *P. aeruginosa* B031 showed adaptation and growth at diesel fuel concentrations of 1%, 2%, 4%, 6%, 8%, and 10%. Figure 1 shows the ability of *P. aeruginosa* B031 to adapt and grow at the lowest and highest diesel fuel concentration, i.e., 1% and 10%, respectively. Although diesel fuel is toxic to bacterial cells, *P. aeruginosa* B031 could adapt to the presence of this. Bacteria can degrade hydrocarbons and utilize them as carbon sources and cell biomass. The degradation process of diesel fuel by bacteria passes through several stages, including contact with hydrocarbons, transmembrane transport, and intracellular transformation. The degradation pathways of these pollutants include aerobic and anaerobic pathways, such as oxidation, reduction, hydroxylation, and dehydrogenation (Ganesan et al., 2022).

Figure 2. Adaptation and growth of *P. aeruginosa* B031 in 1% diesel fuel (A) and in 10% diesel fuel (B).

Biodegradation of diesel fuel by P. aeruginosa in batch system

In the batch system, significant differences ($p<0.05$) were present between the chemical and biological parameters of the reactor on day 0 (beginning of incubation) and day 62 (end of incubation) (Table 2). At the end of incubation, the pH of the reactor decreased to 6.38 because of bacterial activity in the degradation of diesel fuel. *P. aeruginosa* used diesel as a carbon source and degraded this into monomers, organic acids (acetic and formic acids), carbon dioxide (CO_2), methane (CH_4), and ions that are weakly acidic (Tamnou et al., 2021). The decrease in pH was also followed by decreases in other parameters, including ORP, phenol concentrations, TOC, BOD, and COD. At the end of incubation (day 62), the concentrations of phenols, TOC, BOD, and COD decreased by 46%, 49%, 60%, and 72%, respectively. These decreases indicate a decrease in the amount of diesel fuel pollutants due to their degradation by bacteria.

Table 2. Changes in chemical and biological characterization during biodegradation of diesel fuel by *P. aeruginosa* B031 in batch system during the 62-day incubation.

Characteristics	Incubation time (days)	
	0	62
Chemical characteristics		
Suhu (ºC)	35	34
pH	7.00	6.38
ORP (mV)	101.3	82.1
Phenol (mg/L)	6.0954	3.3208
Total organic carbon (%)	3.55	1.82
Biological Oxygen Demands (mg/L)	88	35
Chemical Oxygen Demands (mg/L)	195	55
Biological characteristics		
Cells number of *P. aeruginosa* (CFU/mL)	5.5×10^5	2.3×10^7
Exopolysaccharide concentration of *P. aeruginosa* (mg/L)	9.529	22.892

Hashim et al. (2016) used the BOD/COD ratio of a bioreactor to determine the biodegradability index of a pollutant in a system. A BOD/COD ratio >0.6 indicates that a pollutant has a high biodegradability, while a ratio ranging between 0.3-0.6 indicates a low biodegradability. A BOD/COD ratio <0.3 indicates that the pollutant is nonbiodegradable, including by bacteria. The BOD/COD ratio of the bioreactor on days 0 and 62 significantly differed ($p <0.05$) at 0.45 and 0.42, respectively, so the diesel fuel pollutants were categorized as ones requiring treatment. The biodegradability index of diesel fuel pollutants agrees with the results of previous studies that demonstrate that diesel fuel contains various hazardous components with high toxicity and low biodegradability (Al-Wasify and Hamed, 2014; El-Borai et al., 2016; Liu and Zhang, 2019). After the 62-day incubation, the bacteria had a high ability to degrade the diesel fuel pollutants, as shown by an increase in BOD/COD to 0.64.

The number of bacterial cells and level of EPS increased during the 62-day incubation, indicating that the bacteria used the degradation of diesel pollutants to grow and build cell biomass. The EPS layer is a collection of immobilized cellular polymers comprising polysaccharides, proteins, nucleic acids, lipids, and humus. The layer enables bacterial cells to

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be more resistant to pollutants (Li et al., 2022). The EPS layer will interact with pollutants through various mechanisms, including emulsification, dissolution, adsorption, binding, precipitation, complexation, ion exchange, and an increase in the bioavailability of bacterial cells. The interaction will further enhance enzyme catalysis in degrading diesel fuel into harmless compounds such as CO_2, H_2O, and CH_4. The EPS layer produced by bacteria enables bacterial cells to attach to and communicate with each other to form a biofilm community. Premnath et al. (2021) reported similar results, i.e., soil and sediments contaminated with polycyclic aromatic hydrocarbons can be efficiently removed by inoculating bacteria that can produce EPS. Other researchers also reported that biofilm communities can effectively be used in the bioremediation process of pollutants, including hydrocarbon pollutants (Retnaningrum and Wilopo, 2016; Catania et al., 2020; Mahto and Das, 2020; Bouteh et al., 2021).

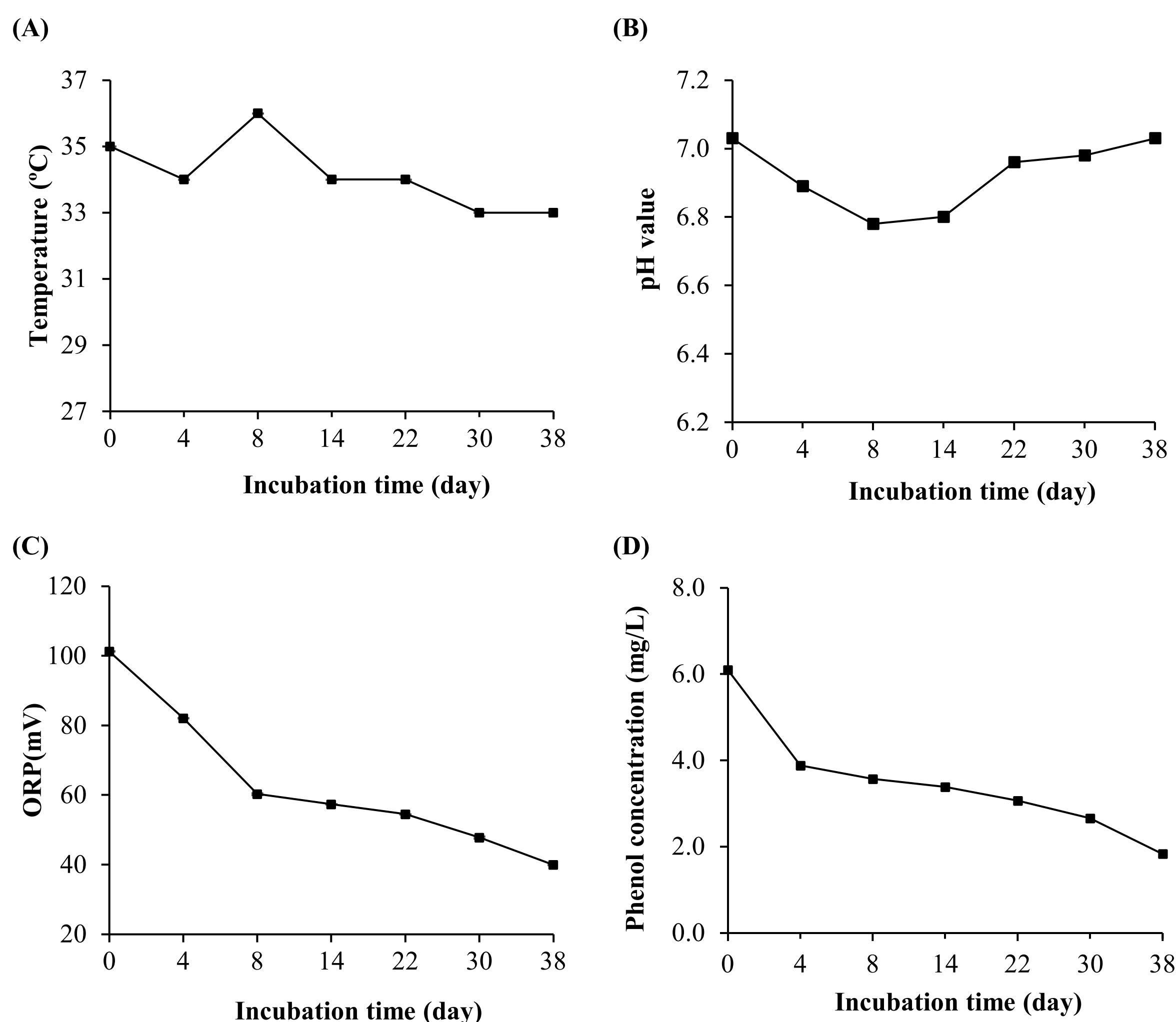

Figure 3. Changes in temperature (A), pH (B), ORP (C), and phenolic content (D) during the biodegradation of diesel fuel by *P. aeruginosa* B031 in a column bioreactor in a continuous system.

Biodegradation of diesel fuel by P. aeruginosa in a column bioreactor in a continuous system

Changes in the chemical characterization of the column bioreactor

The chemical parameters, including pH, ORP, and phenolic content, of the continuous system in a column bioreactor changed significantly ($p<0.05$) at days 0, 4, 8, 14, 22, 30, and 38 (Figure 3). However, the reactor temperature did not significantly change ($p<0.05$). After 4 to 14 days of incubation, the pH decreased due to the degradation of hydrocarbons. In the bioreactor column, the volume of culture in the column was maintained by adjusting the flow rate from the inlet basin into the column and adjusting the flow rate of the biodegradation products. The flow rate adjustment resulted in neutral pH during observation on day 22 until the end of incubation on day 38.

During the incubation, the ORP of the bioreactor column also decreased. This decrease was due to the electron transfer that took place in the *P. aeruginosa* cells through oxidation-reduction reactions from diesel

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compounds into simpler compounds. During the electron transfer process, oxygen serves as the main acceptor, thus affecting the intracellular metabolism of the bacteria through oxidoreductase reactions on the cell membrane. The two primary cofactors (i.e., NAD+ and NADP+) form NADH and NADPH after receiving electrons from other compounds, causing them to be dominant in the intracellular metabolism. The more dominant the intracellular metabolism, the higher the oxygen consumption by bacteria (Frangipani et al., 2008; Wu and Li, 2014; Roussel et al., 2022). In addition, a decrease in ORP indicates a decrease in the diesel fuel compounds. The more negative the ORP, the lower the oxidation of complex organic compounds. This is shown by the decrease (by 70%) in the concentration of phenolic compounds in the column bioreactor during the incubation. This decrease in phenolic contents in the column bioreactor was due to the degradation of diesel fuel by *P. aeruginosa* as a source of carbon and energy for bacterial growth. Wang et al. (2011) reported similar results where *Pseudomonas* sp. strains under aerobic conditions can degrade phenol into pyruvic acid and citric acid, which can be used as carbon sources. The degradation mechanism of phenolic compounds by bacteria is reported to pass through several stages, namely transportation, hydroxylation, oxygenation, ring cleavage, and compound conversion (Figure 4).

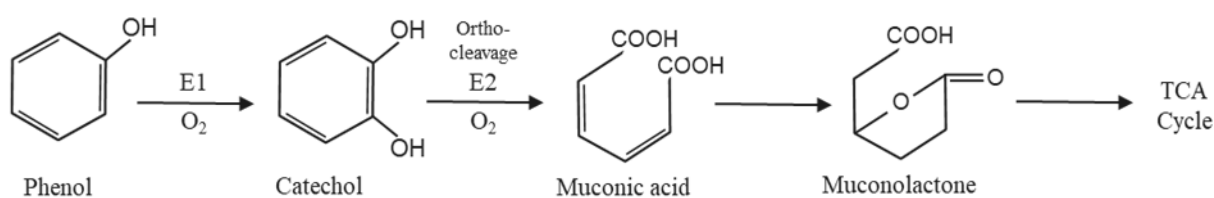

Figure 4. Pathway of phenolic degradation by *P. aeruginosa* under aerobic conditions, E1: phenol hydroxylase; E2: catechol 1,2-dioxygenase) (Li et al., 2019).

In the transportation stage, phenolic compounds enter *P. aeruginosa* cells through passive diffusion or using protein permease. After the phenol enters *P. aeruginosa* cells, phenol hydroxylase produced by the cell will catalyze the reaction of phenol into hydroquinone. Afterward, the hydroquinone will undergo an oxygenation reaction catalyzed by hydroquinone oxygenase to produce catechol. Then, through ortho-cleavage, catechol is degraded into muconic acid and muconolactone catalyzed by catechol 1,2-dioxygenase, causing the degradation of carbon bonds in the ring. The resulting muconolactone compound undergoes a series of reactions, producing simple inorganic compounds such as acetyl-CoA and succinyl-CoA. These simple inorganic compounds will enter the tricarboxylate cycle, causing *P. aeruginosa* to produce ATP as an energy source (Wang et al., 2011; Chanif et al., 2017; Li et al., 2019; Panigrahy et al., 2022). During the incubation, the TOC, COD, and BOD of the column bioreactor decreased significantly ($p<0.05$; Figure 5). At the end of the 38-day incubation, the TOC concentration decreased by 85% because *P. aeruginosa* consumed organic carbons that constitute diesel fuel by degrading complex hydrocarbons into cell constituents and energy (Kadri et al., 2021). El-Borai et al. (2016) stated that the degradation of hydrocarbons can occur because of the bacterial oxygenase enzymes, so the hydrophobic interaction between pollutants and bacterial cell surfaces is optimized.

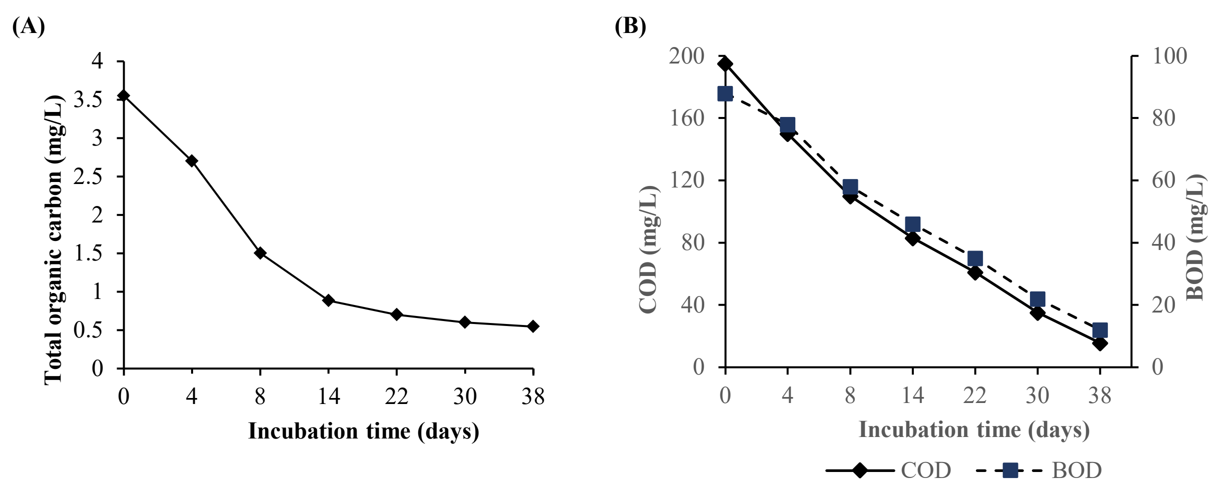

Figure 5. Changes in TOC (A), COD and BOD (B) during the biodegradation of diesel fuel by *P. aeruginosa* B031 in a column bioreactor in a continuous system.

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Consequently, the interfacial tension of the hydrocarbon pollutants decreases, thus increasing the surface contact between pollutants and bacteria. After the surface contact between pollutants and bacteria, hydrocarbons are degraded into soluble simple molecules, which can then enter the bacterial cells (Al-Wasify and Hamed, 2014; Chanif et al., 2017).

The BOD and COD concentrations in the column bioreactor in the continuous system decreased by 86% and 92%, respectively. The efficiency of diesel fuel biodegradation by *P. aeruginosa* in the continuous system was higher than that in the batch system. Two factors that can increase the efficiency of the continuous system include the continuous flow of nutrients to the inlet basin and the use of carrier material in the form of gravel and sand. The continuous flow of nutrients to the inlet basin will affect the growth of bacteria, allowing optimal conditions to be achieved. The use of the carrier materials allows bacteria to adhere to the surface where the diesel degradation occurs, thus preventing bacterial leaching and reducing the lag phase time in its growth. Previous researchers reported similar results, where the addition of carrier material in the form of zeolite to a down-flow fluidized-bed reactor can increase the activity of sulfate-reducing bacteria and decrease the concentration of manganese in synthetic wastewater (Retnaningrum and Wilopo, 2018; Zhang et al., 2021). Based on the BOD/COD ratio of the column bioreactor, the longer the incubation time, the more significant the increase in the BOD/COD ratio of the bioreactor ($p<0.05$; Table 3). The highest BOD/COD ratio was measured at the end of the incubation (0.77). The BOD/COD ratio was also greater than that in the bioreactor in the batch system, indicating better degradation of diesel fuel by bacteria in the continuous system than in the batch system.

Table 3. Biodegradability index of *P. aeruginosa* B031 in bioreactor in continuous system during incubation.

No	Incubation time	Biodegradability Index
1	0	0.45
2	4	0.52
3	8	0.53
4	14	0.55
5	22	0.57
6	30	0.63
7	38	0.77

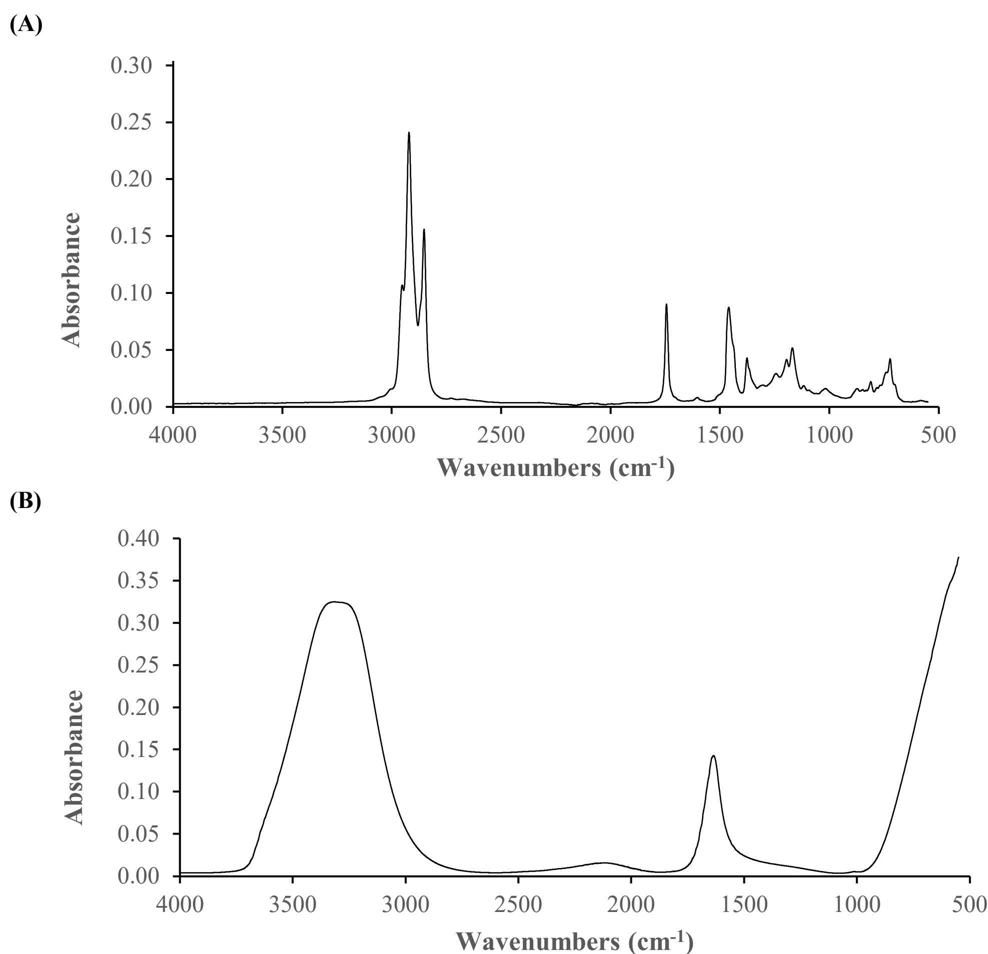

Figure 6. Fourier transform infrared (FT-IR) spectra of diesel fuel (control sample) and biodegradation of diesel fuel by *P. aeruginosa* B031(sample).

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Table 4. Fourier transform infrared (FT-IR) peaks of diesel fuel (control sample) and biodegradation of diesel fuel by *P. aeruginosa* B031 (sample).

Sample	No	Wavenumber (cm^{-1})	Type of Vibration	Functional class
Diesel fuel (control sample)				
	1	2952, 2921	Asymmetrical stretch of CH_3	Alkanes
	3	2852	Symmetric stretch of CH_3	Alkanes
	4	1744	Stretch of the carbonyl group bond C=O	Carboxylic acids
	5	1459	Asymmetric deformation of the bonds CH_3	Alkanes
	6	1303	Symmetric stretch of N–O	Nitro compounds
	7	1118	Stretching of bond C–O–C	Ether
	8	848	Bending of =C–H groups	Alkanes
	9	722	Bending (rocking) of $–(CH_2)n–$	Alkanes
Biodegradation of diesel fuel by *P. aeruginosa* B031 (sample)				
	1	3318	H-bonded O-H stretch, hydroxyl group	Alcohol
	2	2129	Triple bond of C≡C, C≡N stretch	Alkynes, nitriles
	3	1635	Stretching of C=O, carbonyl group	Ketones, aldehydes

Changes in the biological characterization of the column bioreactor

The number of bacteria and level of EPS in the column bioreactor increased by 4-fold and 2.3-fold more than those in the batch system, although the incubation time required for bacteria was shorter (Figure 7). This is possibly because the diesel fuel waste in the column bioreactor was continuously flowing, resulting in increased bacterial growth and EPS synthesis. EPS compounds could change the adhesion and physicochemical properties of microbial cells and increase cellular self-defense from harmful environmental stresses when exposed to hydrocarbon contaminants (Shiu et al., 2020). In addition, EPS also acts as an emulsifier and offers adsorption sites for the binding of diesel fuel and bacterial cells, thus improving the degradation of diesel fuel by bacteria (Meng et al., 2019; Premnath et al., 2021). An increase in the number of bacteria and levels of EPS in the column bioreactor is consistent with FTIR results, indicating the bacterial ability to degrade diesel fuel.

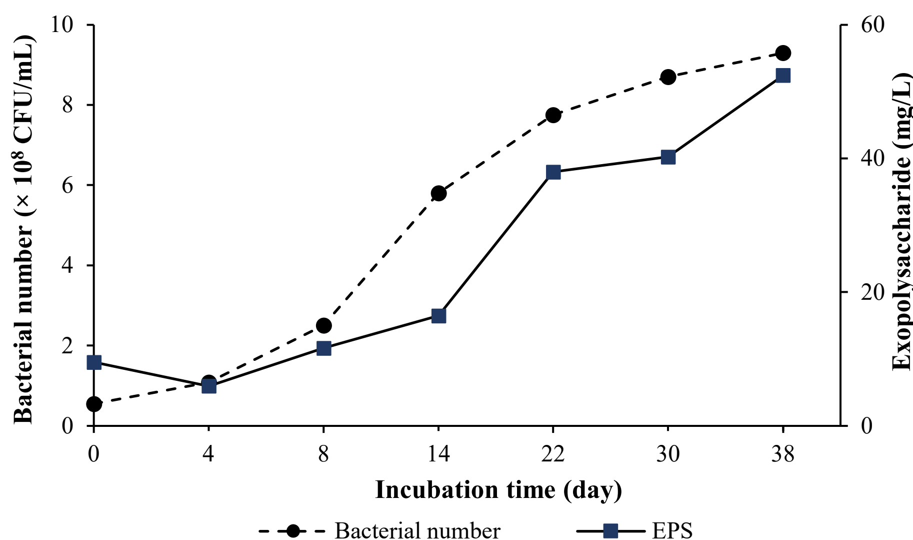

Figure 7. Changes in bacterial number and exopolysaccharide levels during the biodegradation of diesel fuel by *P. aeruginosa* B031 in a column bioreactor in a continuous system.

Expression of alkane 1-monooxygenases (alkB) gene

Based on the qPCR results of *alkB* gene of *P. aeruginosa* B031 in two conditions, namely with and without treatment (exposure to diesel fuel), the levels of gene expression were relatively significantly different ($p<0.05$) by 1-fold and 239.69-fold (Figure 8). The increase in gene expression led to the synthesis of alkane hydroxylase by *P. aeruginosa* B031, which acted as a catalyst for degrading diesel fuel. Exposing *P. aeruginosa* B031 to diesel fuel in the column bioreactor induced the *alkB* expression of *P. aeruginosa* B031and led to higher synthesis of the alkane hydroxylase enzymes, resulting in an optimal hydrocarbon degradation (Muthukumar et al., 2022; Shapiro et al., 2022). The results of this study agree with those by Chen et al. (2020), who tested the *alkB*

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gene expression of the bacterial consortium on the reformation of alkanes with medium chain lengths of C_{20}, C_{24}, and C_{26}, which increased by 79.9-fold, 93.5-fold, and 6.4-fold, respectively. An increase in the *alkB* gene expression of *P. aeruginosa* B031 in the column bioreactor was followed by a decrease in pH, ORP, phenolic content, TOC, COD, and BOD. Furthermore, the increase in the gene expression was also supported by changes in functional groups, as shown by FTIR analysis, followed by an increase in the number of bacteria and level of EPS. These results represent the activity of *P. aeruginosa* bacteria in degrading diesel fuel through the synthesis of alkane 1-monooxygenase encoded by *alkB* gene.

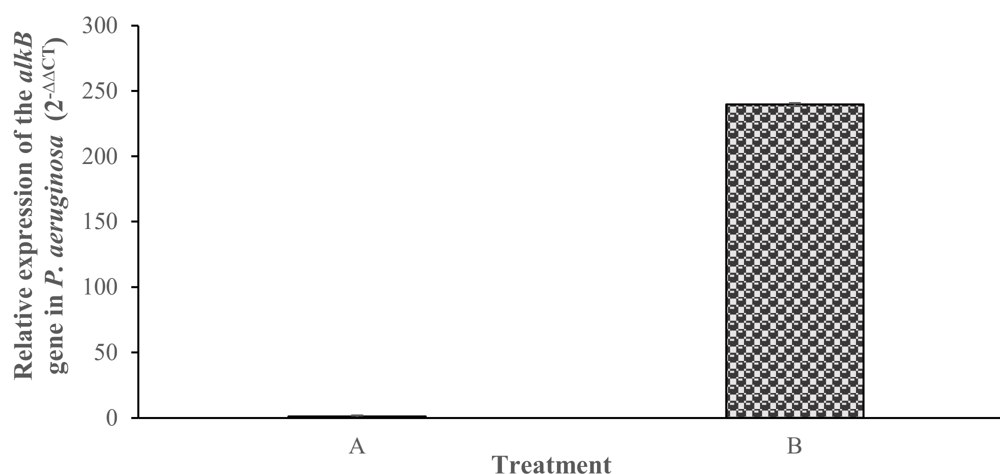

Figure 8. Relative expression of *alkB* genes of *P. aeruginosa* bacteria in column bioreactor without treatment (A) and with exposure to diesel fuel (B)

Conclusion

This study showed that *P. aeruginosa* B031 had a significant ability to biodegrade diesel fuel. Compared to the batch system method, the column bioreactor was more efficient in biodegrading diesel fuel. The concentrations of phenol, TOC, COD, and BOD in the column bioreactor experienced a greater decrease than those in the batch system by 1.5-fold, 1.7-fold, 1.4-fold, and 1.3-fold, respectively. The decrease in the concentrations was followed by changes in functional groups, as shown in the FTIR results. CH_3 groups, carbonyls, nitro compounds, ethers, and alkenes were detected in diesel fuel, while the molecules produced by *P. aeruginosa* B031 contained alcohol groups of alkynes, nitriles, ketones, and aldehydes. In addition, bacterial activity was also higher in the column bioreactor, as seen from the number of cells and production of EPS, which was 4-fold and 2.3-fold greater than those in the batch system, respectively. Exposing *P. aeruginosa* B031 to diesel fuel in the column bioreactor induced the expression of *alkB* gene by 239.69-fold, increasing the level of alkane hydroxylase and thereby optimizing the degradation of hydrocarbons. Consequently, the use of column bioreactor is recommended for diesel bioremediation processes.

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