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Analysis of diesel hydrocarbon decomposition using efficient indigenous bacterial isolate: Bacterial growth and biodegradation kinetics

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Abstract−Industrialization and urbanization have increased the demand for petroleum hydrocarbons; hence the likelihood of contamination of air, soil, and water bodies increases. The survival and biodegradation capabilities of fifteen bacterial isolates were tested in a harsh diesel environment. The bacteria were isolated from soil samples and identified by 16S rRNA gene sequencing. The biodegradation capability of isolates was performed in batch experiments, and diesel degradation analyses were conducted on gas chromatography-mass spectroscopy (GC-MS). The results revealed that only two bacterial isolates (A_1 and E_5) sufficiently consumed diesel hydrocarbons as a carbon-based energy source. 16S rRNA sequencing identified both isolates as *Bacillus* genera. An average of 60% of 3% (v/v) diesel was degraded in about 16 hours. *Bacillus* sp. E_5 strain could degrade about 72% and 68% heavier compounds of C_{24} and C_{26}. The Monod kinetic model for *Bacillus* sp. E_5 utilizing diesel as a substrate showed maximum specific bacterial growth rate (μ_{max}) as 0.1131 hr^{-1} at 1%, while 0.1287 hr^{-1} for 3% diesel. Results suggest that the isolated bacterial strain *Bacillus* sp. E_5 has bioremediation potential and can be used as an alternative method for cleaning contaminated petroleum hydrocarbon field sites for sustainable development.

Keywords: Biodegradation, Indigenous Isolate, Diesel Hydrocarbons, Monod Kinetics, Wastewater

INTRODUCTION

Diesel (C_{10}-C_{25}) is a complex fuel consisting of hundreds of organic compounds [1]. It is sparingly soluble in water, has an excellent adsorption coefficient, and has a recalcitrant aromatic ring structure. Diesel's chemical constituents negatively affect biotic and abiotic life when introduced into the environment [2,3]. Petroleum products cause pollution at every stage of their life cycle, including exploration/drilling, refining, transportation, and the actual burning of the fuel [4]. Contaminants are globally classified based on environmental impacts, with petroleum hydrocarbons (HCs) being on the top of the list due to the intensity of damage caused by them [5]. HCs contamination has increased owning to industrial and accidental releases with severe environmental consequences, especially in developing countries. According to a comprehensive review by Mojiri and team (2019), polyaromatic hydrocarbons (PAHs) range from 0.03 $ng\cdot L^{-1}$ as found in Southeastern Japan Sea, Japan, to 8,310,000 $ng\cdot L^{-1}$ from a wastewater treatment plant at Siloam, South Africa [6]. Petroleum HCs badly affect air, soil, surface, and groundwater with excessive biological and chemical oxygen demands (BOD and COD) measured in effluent water [7-10].

Remediation of contaminated sites has remained a prime objective of scientists around the globe. Physical and chemical technologies, like boom, skimmers, sorbents, separators, dispersants, and emulsions, have been used for effective remediation. However, conventional methods are not universally applicable, cost-effective, and eco-friendly. Biological treatment methods may provide an environmentally sustainable approach [9]. This promising technology uses biological machinery like microorganisms (bacteria, fungi) and plants to remove contamination from the environment [10-13]. The rate and the extent of biological degradation depend upon the metabolic ability of the organisms involved [13]. For bioremediation, utilizing suitable indigenous microorganisms is the key to effective removal. The indigenous microbial community demonstrates metabolic capability for using a variety of petroleum hydrocarbons by aerobic or anaerobic pathways as an energy source [14]. Various researchers have used individual or combinations of microbial species to achieve biodegradation in a liquid medium and confirmed better degradation potential of indigenous isolates due to their acclimatized nature [15-17]. Their community flourishes due to the variety of substrates and metabolites provided by hydrocarbon degradation. Thus, isolating and identifying indigenous differential microbes for hydrocarbon degradation is a long-recognized method.

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A typical bioremediation process can take weeks to months to successfully remove the desired pollutants [18]. The application of indigenous isolates often provides us with the best results in reducing harmful hydrocarbon contamination [16]. In a pilot study for bioremediation of hydrocarbon contaminated soil, the indigenous isolates degraded 94% of the contamination after 191 days of the experiment [19]. However, the process relies upon selecting efficient microbes since poor selection can significantly impact the results [20]. Hydrocarbon degrading microorganisms could be divided into two broad categories, i.e., first, highly specialized in hydrocarbon degradation, and second, capable of thriving on a wide range of carbon sources. Accustomed microorganisms can increase the decomposing efficiency while decreasing the time required to reach the optimum rate. Fu et al. [21] suggested that surface adsorption of hydrocarbons is crucial before internal uptake and biodegradation. Surface adsorption dominates the rate of biodegradation in the early stage. However, the biodegradation rate supersedes in the later stages, where specific bacterial catabolic enzymes (oxygenases and hydroxylases) degrade the complex hydrocarbons. Kinetic models, such as the Monod model, have been applied widely in environmental engineering, especially in bioremediation, representing and explaining bacterial growth in engineered systems [22]. The application of kinetics provides a theoretical framework to design [23] and optimize conditions in the pilot-scale microbial study [24]. Monod kinetics is an effective modal to quantify bacterial efficacy with kinetic parameters such as half-saturation rate constant (K_s) and maximum specific growth rate (μ_{max}) [17]. Effective hydrocarbon degraders, including members of *Pseudomonas*, *Arthrobacter*, *Acinetobacter*, *Nocardia*, *Corynebacterium*, *Bacillus*, and *Mycobacterium* genera, have been reported in previous studies showing up to 98% degradation [14]. The degradation capability of microbes (bacterial and fungal) is also linked with the type of hydrocarbon with ease of degradation as linear alkanes<branched alkanes<small aromatics<cyclic alkanes. Compounds, for instance, PAH, are generally recalcitrant [25].

The current study deals with isolating and identifying indigenous bacteria and assessing their metabolic capacity to degrade petroleum HCs faster. We hypothesized that isolation from the hydrocarbon-contaminated site would provide us with bacteria with high tolerance and degradation ability due to their acclimatized nature. These pre-acclimatized bacteria would be effective at a higher dose with rapid degradation, even for recalcitrant hydrocarbons found in diesel fuel. Monod model would be used for growth kinetics for comparative assessment between best degraders. Using bacterial isolates to treat HCs contaminated water can provide a low-cost system while reusing wastewater without freshwater consumption.

MATERIALS AND METHODS

1. Sample Collection and Chemicals

All solution and media preparation were done with autoclaved distilled water. Chemicals used for experimental analysis were procured from Sigma Aldrich Chemical®. Soil and wastewater samples were collected from Attock Oil refinery, Rawalpindi (33.552215 -73.068799; 33.536363 -73.079141). Soil samples were locked in Ziploc bags, while wastewater samples were collected in sterilized bottles, placed on ice, and stored at 4 °C before further analysis (EC, pH, COD, BOD, etc).

2. Isolation and Biochemical Characterization

For bacterial isolation, soil samples were prepared by serial dilution in autoclaved distilled water and ultimately incubated for 24 h on nutrient agar at 36 °C. After 24 h, the bacterial cultures were separated, and 15 bacterial isolates were selected based on their distinct morphological features such as shape, size, and color [26]. Bacterial isolates were sub-cultured thrice to ensure the purity of each strain, and biochemical tests were performed to differentiate among the isolates [27].

3. Identification through 16S rRNA Gene Sequencing

To identify the most efficient bacterial species, isolates were streaked using the plate streak method onto the nutrient agar and incubated for 24 hrs [28]. Genomic DNA was extracted from isolates using standard molecular techniques. For molecular characterization, PCR amplification was performed with 0.2 μM each of forwarding primer 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) and reversed primer 1492R (5′-TAC GGY TAC CTT GTT ACG ACT T-3′). The remaining procedure was followed as described in the previous study [26]. The obtained gene sequence was subjected to phylogenetic analysis. The evolutionary history was inferred using the maximum likelihood method based on the Jukes-Cantor model [29]. The tree with the highest log-likelihood was prepared, and evolutionary analyses were conducted in MEGA7 [30].

4. Screening for Diesel Tolerant Species

For screening experiments, 15 morphologically distinct isolates were selected and tested for their tolerance on a solid media compromising of simple agar plates without any additional nutrient/carbon source. Sterile filter papers (Whatman No 1) soaked with filter-sterile diesel were placed on the lid of each inverted Petri plate and incubated for seven days. The saturated filter papers supplied HCs through the vapor phase at 36 °C, acting as the sole carbon source. Un-inoculated control was also set up along with inoculated control with no diesel substrate.

5. Diesel Biodegradation and Bacterial Growth Kinetics

M.S.M. liquid media (4 g of $NaNO_3$, 1.5 g of KH_2PO_4, 0.5 g of Na_2HPO_4, 0.0011 g of $FeSO_4 \cdot 7H_2O$, 0.2 g of $MgSO_4 \cdot 7H_2O$, and 0.01 g of $CaCl_2$ per liter of water) was used in all degradation batch experiments [31]. Each bacterial isolate was trialed with controls (positive and negative) with 1% inoculum after adjusting pH to 7.0 [26]. Those isolates with high diesel tolerance were subjected to 1% (v/v) and 3% (v/v) diesel. The final experiment of the bacterial consortium was carried out with inoculates mixed at a 1 : 1 ratio with a 1% diesel dosage. Aluminum foils were used to cover flasks to avoid photodegradation through light exposure [26]. Samples were withdrawn from experimental and control groups to determine bacterial growth. Using the spread plate technique, CFU mL^{-1} was determined at predefined time intervals. The diesel degradation involved using gas chromatography (GC-MS).

To investigate the exponential growth of the isolates on diesel HCs, the Monod equation (Eq. (1)) was applied:

$$\mu = (\mu_{max} \cdot S)/(K_s + S) \quad (1)$$

where μ defines specific growth rate (hr^{-1}), μ_{max} is maximum specific growth rate (hr^{-1}), S is known as substrate concentration (mmol·

L^{-1}) at time t, and K_s denotes half-saturation coefficient (mmol·L^{-1}) [32]. μ is calculated by Eq. (2):

$$dX/dT = \mu X \quad (2)$$

Doubling time (td) is calculated by Eq. (3) by plotting ln (dX) vs. time [23].

$$td = (\ln 2)/\mu \quad (3)$$

6. Sample Extraction and Analysis of Residual Diesel Oil Using GC-MS

2 mL samples were taken at specified time intervals to study the diesel degradation trend. The culture present in the medium was removed by centrifugation, followed by extracting the liquid oil with n-hexane (1 : 1) [14,33]. Ultimately, excess water was removed by using Na_2SO_4 [8,9]. Degradation was assessed using GC-MS by injecting 1 mL of the sample. M.S. capillary column dimensions were 30 m*25 mm*0.25 μm with an injector split ratio of 40 : 1. The initial oven temperature of 40 °C was programmed to rise to 270 °C [34] at the rate of 8 °C, where it was held for 5 min [35]. The injection temperature was 120 °C, while the ion source and interface temperature were 235 °C. The carrier gas used for diesel determination was Helium (He). The diesel degradation ratio was calculated using Eq. (4):

$$n = \left(\frac{C_o - C_1}{C_0}\right) * 100 \quad (4)$$

The relationship describes the degradation ratio where C_0 and C_1 represent the diesel oil in the control and test samples [36].

RESULTS

1. Sample Analysis and Isolation

The soil microbial community is influenced by soil physio-chemical characteristics, including pH and temperature, which govern microbial activity and diversity [20]. Samples collected for this study from diesel polluted soil and water were analyzed for physio-chemical characteristics (EC, pH, COD, BOD). The pH of water and soil was found to be 7.6 and 7.9, while electrical conductivity was about 391 and 449 μS, respectively. Often, contaminated soil with diesel, the pH values were ≤8 [37]. The water sample's COD was 7,384 mg L^{-1}, while the BOD level was 4,430 mg L^{-1}. Microbial growth was obtained after enrichment from soil and water medium. Changes in bacterial communities in soil and water medium as compared to controls have been reported as a result of contamination. These changes resulted from the reduction of certain microbes that were incompetent to some hydrocarbon fractions. Thus, only those who survived could consume these fractions [38,39]. After enrichment, fifteen surviving bacterial isolates were selected for biochemical analysis. Their characteristics, along with morphological features, are provided in Table 1.

2. Screening Test

Indigenous strains are found to be more efficient degraders in comparison with exogenous strains for removing hydrocarbons. Higher efficiency owns to their exposure and adaptation to the contaminants [20]. After 48 hrs incubation, only 7 out of the 15 isolated indigenous bacterial strains showed significant growth on agar amended with diesel. Those flourishing with distinct colonies included A1, C3, E5, H8, J10, N14, and O15. E_5 showed abundant growth in a period of 48 h. Although J_{10} had developed by 48 hrs, it was not selected for the further experiment as only relatively weak growth was recorded. No growth was monitored during the investigation for the un-inoculated (control group).

3. Identification

The 16s rRNA gene sequencing of the best bio-degraders revealed that the isolated strains belonged to the *Bacillus* genera (Fig. 1).

4. Diesel Biodegradation and Bacterial Growth Kinetics

Diesel was used as a sole substrate on an incubator shaker with

Table 1. Morphological features and biochemical test results of bacterial isolates

Isolation media	Samples	Shape	Size	Color	Margin	Elevation	Texture	Appearance	Gram +/-	Oxidase +/-	Catalase +/-
Water	A_1	Circular	Small	Yellow	Entire	Pulvinate	Smooth	Dull	+	-	+
	B_2	Circular	Moderate	Yellow	Entire	Convex	Rough	Dull	+	-	+
	C_3	Irregular	Moderate	White	Undulate	Umbonate	Rough	Shinny inner-outer dull	+	-	+
	D_4	Irregular	Large	White	Undulate	Raised	Rough	Dull	+	-	+
	E_5	Circular	Small	White	Entire	Convex	Smooth	Shiny	+	+	+
	F_6	Irregular	Moderate	Off white	Lobate	Raised	Rough	Shiny	+	+	+
	G_7	Filamentous	Moderate	White	Rhizoid	Raised	Rough	Shiny	+	+	+
Soil	H_8	Filamentous	Moderate	Cream	Lobate	Pulvinate	Smooth	Shiny	+	+	+
	I_9	Irregular	Small	Tan	Undulate	Umbonate	Moist	Dull	+	+	+
	J_{10}	Circular	Small	Cream	Entire	Pulvinate	Smooth	Shiny	+	+	+
	K_{11}	Filamentous	Large	White	Rhizoid	Raised	Smooth	Shiny	+	+	+
	L_{12}	Filamentous	Moderate	Light pink	Rhizoid	Convex	Smooth	Shiny	+	+	+
	M_{13}	Rhizoid	Small	Light pink	Filamentous	Convex	Smooth	Shiny	+	+	+
	N_{14}	Irregular	Moderate	White	Undulate	Convex	Smooth	Shiny	+	-	+
	O_{15}	Irregular	Moderate	White	Lobate	Convex	Smooth	Shiny	+	-	+

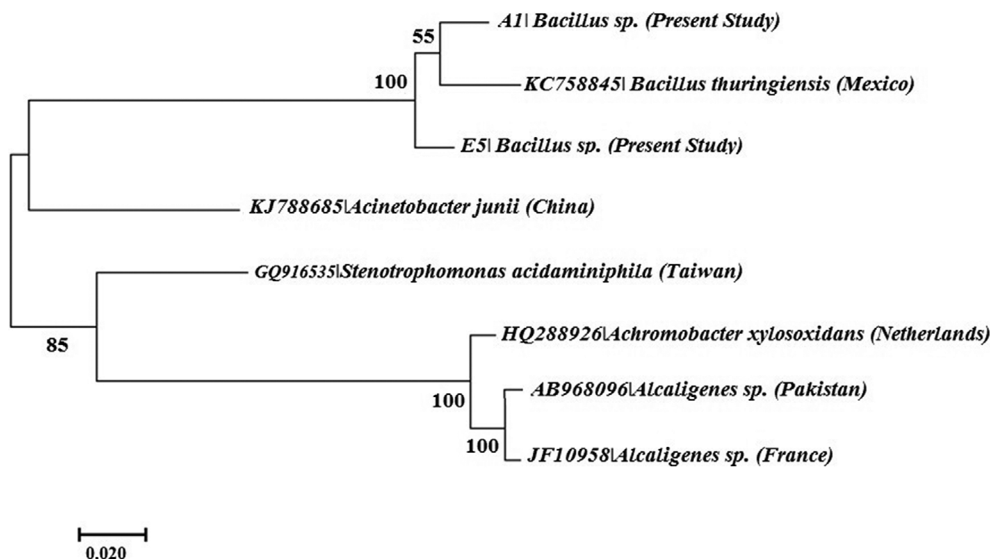

Fig. 1. Molecular phylogenetic analysis by maximum likelihood method.

M.S.M. for biodegradation studies. Upon exposure to diesel supplemented M.S.M., only two bacterial isolates substantially rose in CFU mL^{-1}. N_{14} died instantaneously within the next six hours, which could be attributed to changes in pH, oxygen levels in the M.S.M., etc. Bacterial strains capable of surviving and growing in higher petroleum concentrations are limited since only particular microbes have the degradation capability. Thus, it is feasible to obtain strains from diesel contaminated sites that potentially degrade hydrocarbons [40].

Moreover, these strains take days to consume the desired contaminant. In a study conducted on *Pseudomonas aeruginosa*, with optimum conditions provided (pH 7 and 30 °C temperature), the isolate degraded up to 60% of the provided diesel substrate (8,500 mg/kg) over 13 days [41,42]. Valer and Loana [43] observed 83% degradation for petroleum hydrocarbons (PHCs) over 12 weeks after inoculation with *Pseudomonas* and *Bacillus* spp.

Further experiments were carried out on the two prosperous isolates, Bacillus E_5 (MK387180) and Bacillus A_1 (MK583607). When investigated to survive in 1% (Fig. 2(a)) and 3% (Fig. 2(b)) diesel environment, *Bacillus* A_1 failed to survive at 3% (v/v) diesel concentration. However, in the case of Bacillus E_5, its CFU mL^{-1} increased by about 30% upon exposure to more carbon sources. In soil contaminated with petroleum hydrocarbons, *Bacillus* strains often increase as increased utilization activates the growth, as reported by in-situ bioremediation studies. The *Bacillus* species are advantageous microbial populations due to their degradation ability and are also found at different depths in the soil. In recent experiments, *B. thuringiensis* has been reported to degrade Phenanthrene in batch flasks culture experiment, and almost complete (97.3%) removal was achieved in approximately 10 days [44]. In another study, *B. cereus* and *B. subtilis* were able to degrade more than 70% and 80%, respectively, within 15 days at pH 7 and 37 °C temperature [45].

Four phases can describe the bacterial growth pattern: lag, log, stationary, and death. Microorganisms exhibit different growth curves depending upon species and environmental conditions [46]. The growth curve of the *Bacillus* isolates (A_1 and E_5) on diesel mimicked the bacterial growth curve consisting of a lag, log, and stationary phases followed by a decline in growth for the dose experiment. A shorter lag phase was observed until 4 hr for 1% and 5 hr for 3% for the E_5 isolate. As the isolates were previously exposed to diesel and thus, they may have instantly adjusted to the new environment and grew exponentially. During this period, no significant increase in microbial number was observed. The log phase, which lasted until 15 hr for 3% diesel, demonstrated an exponen-

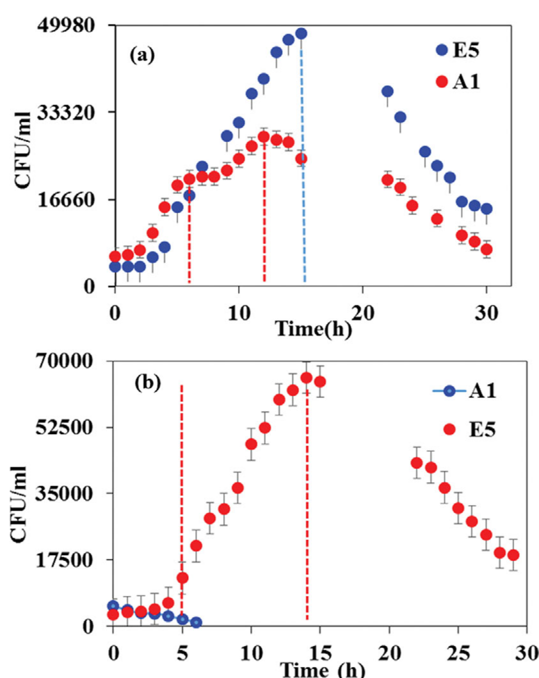

Fig. 2. (a) Shows 1% diesel dose of strain E_5 and A_1; (b) shows the growth of strain E_5 in CFU mL^{-1} at 3% diesel concentration.

tial increase in growth with doubling time up to 5.39 hr for *Bacillus* E_5, while time was found to be 7.77 hr with μ_{max}, 0.129 hr^{-1}. Both isolates observed a short lag phase followed by a short stationary phase leading to the death phase. Lag phase for *B. subtilis* (InaCC-B289) has been reported between 4 and 24 hrs on nutrient agar incubation at 37 °C [47]. In a study by Iqbal and team [27] on 1.0% diesel dose, no significant increase in biomass was observed during the lag phase because this phase may have acted as a period of acclimatization for the bacterial strain during induction of enzymes for diesel as a substrate. Short lag phases up to 5 hr before logarithmic growth have been observed for *Bacillus* species by Ismail and coworkers [48]. It was observed that bacteria growing at higher temperatures show fast growth rates and short lag phases [49]. Also, bacteria isolated from the harsh environment tend to be versatile in substrate consumption, thus taking less time to adjust and showing higher degradation rates [50].

In the consortium experiment, neither of the two species, *Bacillus* A_1 and E_5, was able to multiply exponentially under the given conditions, and eventually, both were wiped out. In a consortium, members of the same population tend to compete to acquire similar nutrients, and nutrition is the major point for microbial competition. The theory predicts that competing microorganisms can be completely wiped out under specific ratios of nutrient concentrations over time [51], as observed in our study. The increase in bacterial populations is linked to the concentration of contaminants [44,52]. Higher diesel concentration means more available carbon sources for the isolates to grow on. Although elevating diesel concentrations provides more carbon, it also leads to toxicity; thus, cellular growth drastically drops for most microbes [53]. The loss of degradation activity can be deduced that a higher concentration of diesel is toxic for microorganisms due to the solvent effect as it destroys the cell membrane. It may also damage cells or disrupt the integrity of the membrane at higher concentrations [45]. Moreover, some bacteria are not capable enough to compete for required nutrients; thus, a decline in growth is observed [20].

Monod kinetic model was applied to investigate *Bacillus* E_5 and A_1 growth kinetics by plotting the graph ln (CFU mL^{-1}) vs. incubation period. As shown in Fig. 3, for *Bacillus* E_5 at 1% dose, μ_{max} was 0.1133 hr^{-1} and 0.0522 hr^{-1} for *Bacillus* A_1, while the doubling time was 6.1178 hrs and 13.2787 hrs, respectively. A study on crude

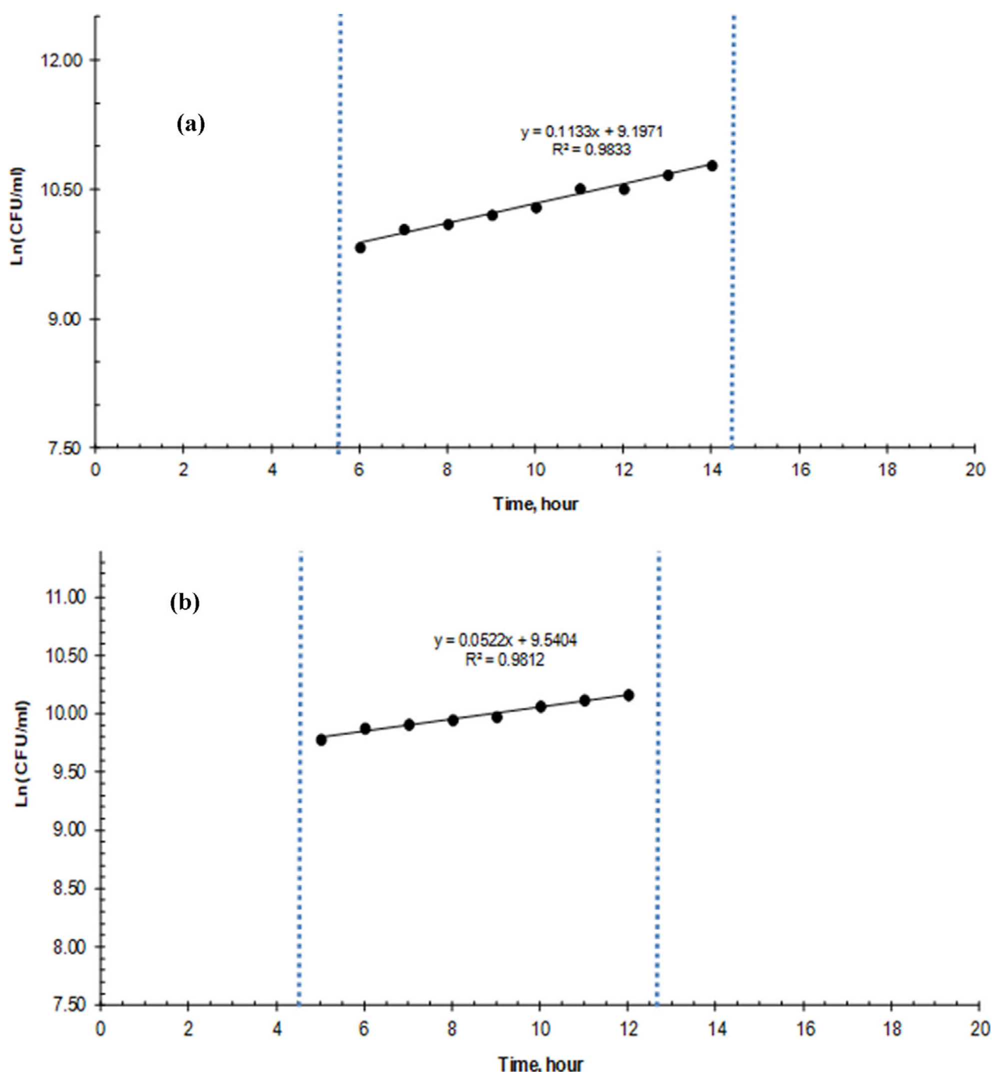

Fig. 3. Growth kinetic results of (a) strain E_5 and (b) A_1 over 1% diesel dose.

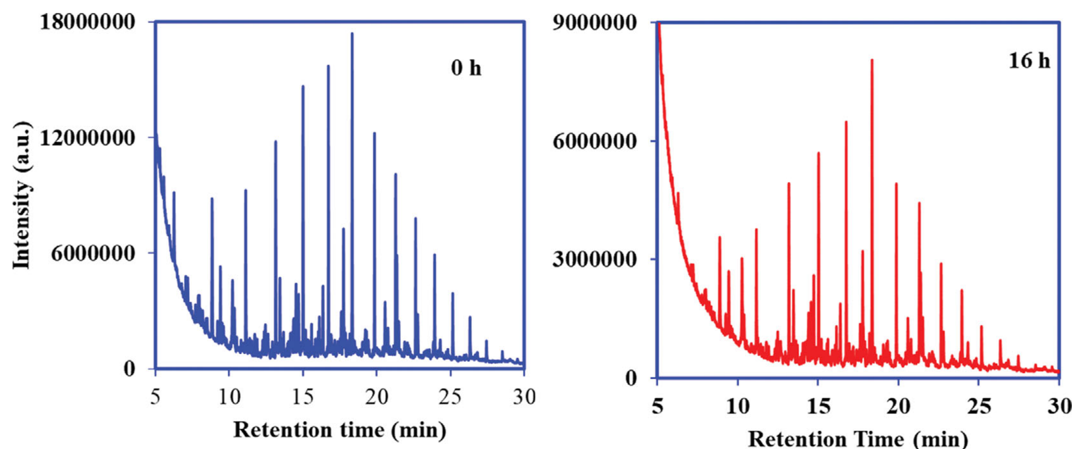

Fig. 4. GCMS profiles of initial and residual concentration of 3% diesel for Bacillus E_5.

Table 2. Diesel degradation by *Bacillus* E_5 in M.S.M. (3% diesel dose)

Carbon. No	Name of compounds	Chemical structure	Percentage degradation
C_{11}	Undecane		48%
C_{12}	Dodecane		73%
C_{13}	Tridecane		50%
C_{14}	Tetradecane		88%
C_{16}	Hexadecane		75%
C_{18}	Octadecane		45%
C_{20}	Eicosane		55%
C_{21}	Hexadecane 2,6,11,15-tetramethyl		49%
C_{24}	Tetracosane		72%
C_{26}	1-Hexacosanol		68%
C_{27}	Heptacosane		32%

oil using *Bacillus* species by Sakthipriya and team [23] demonstrated specific growth rates up to 0.075±0.0016 hr^{-1} at 35 °C. Similarly, doubling time of 9.25±0.12 hr^{-1}, μ_{max} of 0.388 hr^{-1}, and R^2 of 0.9764 were reported for *Pseudomonas putida* (MTCC 1194) [54]. Also, bacterial-specific growth for initial diesel concentrations of 1.0% (v/v) was found to be 0.0665 hr^{-1} with a cell doubling time of 10.42 h by Iqbal and coworkers [27]. Results from our study illustrate that the Monod model efficiently represents bacterial efficiency. Rivera and team [55] reported that *B. thuringiensis* species have complications due to cellular variations in batch experiments. Therefore, the Monod kinetic model could best describe the bacterial growth kinetics.

5. Degradation Analysis of 3% (v/v) Diesel with E_5 Strain

GC-MS analysis of diesel oil extracted during the experimentation revealed its composition, which ranged from C_{10} to C_{27} as shown in Fig. 4. The lighter compounds were found at initial retention time, followed by heavier compounds with increased retention time. The substrate exposed to *Bacillus* E_5 showed a decline in the intensity of characteristic peaks revealing a significant degradation of diesel. Within the short period of 16 hours, an average of 60% of 3% (v/v) diesel was degraded. *Bacillus* E_5 could degrade heavier compounds of C24 and C26 to about 72% and 68%. While CFU mL^{-1} coincides with these results as it had substantially increased to sixty-four thousand by the 14th hour. The peaks obtained at different retention times at 0 and 16th h were analyzed, shown in Table 2, with their proposed chemical structure. However, maximum degradation was achieved for C_{11} to C_{16}. A significant rise in the bacterial population was observed during the same period of 4-16 hrs (see Fig. 2), indicating that the acclimatized bacterial strain instantly adjusted themselves to a higher dose, multiplied, and consumed the substrate present very quickly. Their fast growth correlated with the degradation ability and consuming diesel as a food source.

As shown in Fig. 5, all the compounds ranging from C_{11} to C_{27} were degraded by the *Bacillus* isolate E_5 within 16 hours. It was observed that C_{14} was the most consumed compound among all, with 86% degradation, while C_{16} was removed at around 75%. However, C_{27} is the least degraded among all compounds. Diesel hydrocarbons, including $C_{11\text{-}14}$, C_{16}, C_{20}, C_{24}, C_{26} were best degraded (50-88%) by cultures of *Bacillus* E_5, while others C_{18}, C_{21}, C_{27} (32-48%) were least degraded. None of the microbes could consume every hydrocarbon as biodegradation extents rely upon the enzymatic activity. Since enzymes are microbe specific, the ability to

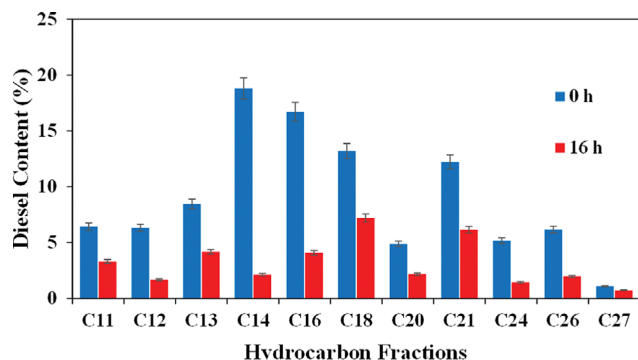

Fig. 5. Degradation profile of 3% diesel by *Bacillus* E_5.

biodegrade hydrocarbon depends upon the type of enzymes [26]. Various investigations reported that simpler fractions found in diesel-like alkanes and aromatics are easier to degrade and thus are degraded before the complex fractions [27,56-59]. Although different hydrocarbons had variable removal rates, lower compounds (C_7-C_{11}) were often biodegraded completely [27,60,61]. In this study, it was observed that the overall degradation of lighter alkanes (C_{12-23}) was more significant than heavier alkanes (C_{24-29}), as reported by [14,61]. The substantial biodegradation was achieved in the initial period of 15 hrs, emphasizing the importance of utilizing this bacterial species as a step forward in biodegradation studies.

The incomplete consumption of smaller compounds may be attributed to lower bioavailability and miscibility over bacterial membranes, as reported by various researchers [27,14]. The heavier compounds were consumed less since degradation of more complex fractions can be a little problematic [27]. Toxic intermediates can also be produced that can have a negative impact on the degradation process. Consequently, it contributes to the lowest degradation rates for complex fractions [14]. The inability to degrade and consume aromatic structures indicates that the degradation of these compounds is thermodynamically not favorable [62].

The biodegradation rates can be linked to different hydrocarbon structures and chain lengths for which microbes require different degradative enzymes; this can also explain the difference in biodegradation rates [24,27]. It has been reported that bacteria degrade carbon compounds ranging from C_{14-20} as an energy source [63]. *Pseudomonas* sp. J10 degraded about 69% diesel in 48 hrs with a maximum specific bacterial growth rate of about 0.0644 hr^{-1} and half velocity constant (K_s) as 4,570 mg L^{-1} [27]. *Bacillus* sp. is known to degrade hydrocarbon with high capability. *B. subtilis* [53] could biodegrade diesel compounds between C_9-C_{12} to about 80% and above, with the highest removal of 87% for C_{10}. While for C_{14-19}, between 60-80% biodegradation took place while heavier molecular weight compounds, more significant than C_{19} removal rates, ranged from 40-60%. Overall, the removal efficiency for 3% (v/v) diesel fuel for *Bacillus* E_5 was about 50% in our experiment. Analysis of degraded oil samples through gas chromatography confirmed degradation rate up to 66% in 21 days, with *Bacillus* sp. on an orbital shaker at 120 RPM and 22 °C [38]. *Bacillus* A_1 was able to degrade compounds between C_{15-19} to 97%, indicating capability for consumption of diverse ranges of HCs found in crude oil [63]. The lowest degradation was observed for heavier compounds such as C_{28} (15.46%) with the highest removal rates (81.87-89.48%) for C_{12-17} hydrocarbons (from 49.88-76.47%) [60]. Mid-chain n-alkanes (C_{13-19}) found in diesel fuel were favored in degradation as up to 90% was consumed by *Pseudomonas* sp. in eight days. The lighter fraction found at initial retention time was less consumed with a degradation rate of C_{9-10} degraded by 30%, while C_{11} degraded by more than 40% [62]. *Acinetobacter calcoaceticus* CA16 degraded about 82-92% of long chain HCs (C_nH_{n+2}; where n=12-18) in four weeks. A study reports that about 13-43% degradation was visualized for C_{12}-C_{18}, respectively, as compared to controls after two weeks, while after four weeks, 82-93% degradation was achieved against 88% abiotic control groups [64].

Table 3. Comparison of hydrocarbon degradation assessments

Carbon No.	Alkane	*Bacillus* E_5 (This study)	*Corynebacterium variable* [65]	Mixed consortium [66]	*E. aurantiacum* [67]	*B. cepacia* [67]	*Cellulosimicrobium cellulans* [14]	*Acinetobacter baumannii* [14]
C_{11}	Undecane	48%	43%	>70%	69%	84%	90%	88.9%
C_{12}	Dodecane	73%	89%	68%	68%	85%	91.4%	90%
C_{13}	Tridecane	50%	94%	>65%	-	-	90.4%	88.2%
C_{14}	Tetradecane	88%	100%	70%	81%	91%	24.6%	18.5%
C_{16}	Hexadecane	75%	88%	>65%	65%	73%	20.3%	0.2%
C_{18}	Octadecane	45%	73%	-	100%	100%	93.3%	91.7%
C_{20}	Eicosane	55%	47%	-	58%	63%	17.9 %	7.9%
C_{21}	Hexadecane 2,6,11,15-tetramethyl	49%	35%	-	-	-	-	-
C_{24}	Tetracosane	72%	12%	-	57%	56%	11.8%	88.8%
C_{26}	1-Hexacosanol	68%	-	-	-	-	41.3%	30%
C_{27}	Heptacosane	32%	-	-	-	-	46.7%	35.9%
Conditions		3%, 180 RPM, 37 °C in 16 h	1%, 180 RPM, 30 °C in 7 days	10%, 300 RPM, 25 °C in 7 days	1%, 120 RPM, 30 °C in 15 days		2%, 120 RPM 32 °C in 10 days	

This study observed that crucial compounds were either mostly degraded or, to a certain extent, within a short period compared to other studies, which took days to achieve the same task as represented in Table 3.

CONCLUSION

MK583607 and MK387180 identified as *Bacillus* genera survived in harsh diesel conditions, suggesting bacterial selectivity for a carbon source. GC-MS analysis revealed that about 72% and 68% heavier compounds of C_{24} and C_{26} were consumed by *Bacillus* sp. E_5 (MK387180). While consumption of C_{14} (Tetradecane) was 88%, C_{16} (Hexadecane) was 75%, suggesting that it can also thrive on a broad spectrum of hydrocarbons. E_5 (MK387180) confirmed bacterial stability in the media compared to control. Many hydrocarbon-degrading microbes fail to consume the available carbon source in such a short time and thus show slow degradation. This can be due to a strong inability to utilize available petroleum hydrocarbon and failure to withstand high concentrations. Generally, isolates require at least seven days to achieve an overall sufficient degradation rate. However, these indigenous isolate strains which have been pre-exposed can act as a breakthrough to reduce the time required to consume and degrade the contaminant.

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AUTHOR CONTRIBUTIONS

Shazra Khalid: Conceptualization, Methodology, Investigation, Writing-Original Draft Preparation. **Aneela Iqbal:** Data curation, Methodology, Writing-Original draft preparation. **Asif Javed:** Visualization, Investigation. **Jamshaid Rashid:** Supervision, Writing-Reviewing and Editing. **Ihsan-ul-Haq:** Resource provision. **Rajeev Kumar:** Resource provision, Writing- Reviewing and Editing. **M. A. Barakat:** Writing-Reviewing and Editing.

Declaration of interest: None

Scientific Nomenclature: BOD: Biological Oxygen Demend; **COD:** Chemical Oxygen demand; **EC:** Electrical Conductivity; GC-MS; Gas Chromatography-Mass Spectrophotometer; **HCs:** Hydrocarbons; **MSM:** Mineral Salts Medium; **PAHs:** Polyaromatic hydrocarbons; **pH:** degree of acidity or alkalinity.

REFERENCES

1. H. I. Mustapha, P. K. Gupta, B. K. Yadav, J. Van Bruggen and P. Lens, *Chemosphere*, **205**, 166 (2018).
2. H. Li, T. Shen, T. Song, M. Bao and P. Sun, *J. Taiwan Institute Chem. Eng.*, **93**, 519 (2018).
3. D. Cerqueda-García, J. Q. García-Maldonado, L. Aguirre-Macedo and U. García-Cruz, *Mar. Pollut. Bull.*, **150**, 110775 (2020).
4. T. Lambert, *Cantaurus*, **25**, 29 (2017).
5. M. J. Kennish, *Practical handbook of estuarine and marine pollution*, C.R.C. Press (2017).
6. A. Mojiri, J. L. Zhou, A. Ohashi, N. Ozaki and T. Kindaichi, *Sci. Total Environ.*, **696**, 133971 (2019).
7. T.-C. Lin, F.-T. Shen, J.-S. Chang, C.-C. Young, A. B. Arun, S.-Y. Lin and T.-L. Chen, *J. Taiwan Institute Chem. Eng.*, **40**, 580 (2009).
8. H. N. M. E. Mahmud, A. O. Huq and R. Bint-Yahya, *RSC Adv.*, **6**, 14778 (2016).
9. M.-Y. Choo, L. E. Oi, P. L. Show, J.-S. Chang, T. C. Ling, E.-P. Ng, S. M. Phang and J. C. Juan, *J. Taiwan Institute Chem. Eng.*, **79**, 116 (2017).
10. A. J. Pete, B. Bharti and M. G. Benton, *ACS EST Eng.*, **1**, 928 (2021).
11. P. Sharma, S. Kumar and A. Pandey, *J. Environ. Chem. Eng.*, **2**, 105684 (2021).
12. M. Jain, S. A. Khan, K. Sharma, P. R. Jadhao, K. K. Pant, Z. M. Ziora and M. A. T. Blaskovich, *Bioresour. Technol.*, **344**, 126305 (2022).
13. C. Marchand, M. St-Arnaud, W. Hogland, T. H. Bell and M. Hijri, *Int. Biodeter. Biodegr.*, **116**, 48 (2017).
14. B. M. Nkem, N. Halimoon, F. M. Yusoff, W. L. W. Johari, M. P. Zakaria, S. R. Medipally and N. Kannan, *Mar. Pollut. Bull.*, **107**, 261 (2016).
15. M. L. Bôto, C. Magalhães, R. Perdigão, D. A. M. Alexandrino, J. P. Fernandes, A. M. Bernabeu, S. Ramos, M. F. Carvalho, M. Semedo, J. LaRoche, C. M. R. Almeida and A. P. Mucha, *Front. Microbiol.*, **12**, 633659 (2021).
16. Y. Wang, J. Liang, J. Wang and S. Gao, *Environ. Sci. Pollut. Res.*, **25**, 2830 (2018).
17. M. K. Kureel, S. R. Geed, B. S. Giri, B. N. Rai and R. S. Singh, *Bioresour. Technol.*, **242**, 92 (2017).
18. C. W. Liu and H. S. Liu, *Process Biochem.*, **46**, 202 (2011).
19. A. K. Karamalidis, A. C. Evangelou, E. Karabika, A. I. Koukkou, C. Drainas and E. A. Voudrias, *Bioresour. Technol.*, **101**, 6545 (2010).
20. A. A. Mansur, M. Taha, E. Shahsavari, N. Haleyur, E. M. Adetutu and A. S. Ball, *Int. Biodeter. Biodegr.*, **115**, 179 (2016).
21. X. Fu, H. Wang, Y. Bai, J. Xue, Y. Gao, S. Hu, T. Wu and J. Sun, *Environ. Sci. Ecotechnol.*, **2**, 100028 (2020).
22. D. C. Manheim and R. Detwiler, *MethodsX*, **6**, 1398 (2019).
23. N. Sakthipriya, M. Doble and J. S. Sangwai, *J. Petrol. Sci. Eng.*, **160**, 412 (2018).
24. M. Bhattacharya, S. Guchhait, D. Biswas and S. Datta, *Bioproc. Biosyst. Eng.*, **38**, 2095 (2015).
25. N. Das and P. Chandran, *Biotechnol. Res. Int.*, **2011**, 1 (2011).
26. K. S. M. Rahman, T. Rahman, P. Lakshmanaperumalsamy and I. M. Banat, *J. Basic Microbiol.*, **42**, 284 (2002).
27. A. Iqbal, M. Arshad, R. Karthikeyan, T. J. Gentry, J. Rashid, I. Ahmed and A. P. Schwab, *3 Biotech.*, **9**, 35 (2019).
28. M. A. Islam, T. Thorstensen and J. L. Clarke, *Agrobacterium protocols*, Vol. 2, Springer, New York, NY (2015).
29. T. H. Jukes and C. R. Cantor, *Mammalian protein metabolism*, Vol. 3, Academic Press, New York (1969).
30. S. Kumar, G. Stecher and K. Tamura, *Mol. Biol. Evol.*, **33**, 1870 (2016).
31. Y. Shen, L. G. Stehmeier and G. Voordouw, *Appl. Environ. Microbiol.*, **64**, 637 (1998).

32. S. A. Hasan and S. Jabeen, *Biotechnol. Biotec. Eq.*, **29**, 45 (2015).
33. A. O. Adeniji, O. O. Okoh and A. I. Okoh, *J. Chem.*, **2017**, 1 (2017).
34. G. F. Da Cruz, E. V. Dos Santos-Neto and A. J. Marsaioli, *Brazil. Org. Geochem.*, **39**, 1204 (2008).
35. N. Palanisamy, J. Ramya, S. Kumar, N. Vasanthi, P. Chandran and S. Khan, *J. Environ. Health Sci. Eng.*, **12**, 142 (2014).
36. Q. Luo, J. G. Zhang, X. R. Shen, Z. Q. Fan, Y. He and D. Y. Hou, *Ann. Microbiol.*, **63**, 633 (2013).
37. R. G. Lacalle, M. T. Gómez-Sagasti, U. Artetxe, C. Garbisu and J. M. Becerril, *Sci. Total Environ.*, **618**, 347 (2018).
38. M. Udgire, N. Shah and M. Jadhav, *Int. J. Curr. Microbio. Appl. Sci.*, **4**, 708 (2015).
39. T. H. Makadia, E. M. Adetutu, K. L. Simons, D. Jardine, P. J. Sheppard and A. S. Ball, *J. Environ. Manage.*, **92**, 866 (2011).
40. K. Ramadass, M. Megharaj, K. Venkateswarlu and R. Naidu, *Int. J. Environ. Sci. Technol.*, **13**, 2863 (2016).
41. M. N. Raju, R. Leo, S. S. Herminia, R. E. B. Morán, K. Venkateswarlu and S. Laura, *Bull. Environ. Conta. Toxicol.*, **98**, 698 (2017).
42. J. H. Hong, J. Kim, O. K. Choi, K. S. Cho and H. W. Ryu, *World J. Microbiol. Biotechnol.*, **21**, 381 (2005).
43. M. Valer and S. Ioana, *Sustainability*, **13**, 8165 (2021).
44. X. Zhao, F. Fan, H. Zhou, P. Zhang and G. Zhao, *Bioproc. Biosyst. Eng.*, **41**, 871 (2018).
45. H. Dixit, M. Lowry, U. Mohsin, M. Moond, S. Kumar, A. Chokriwal and S. Gupta, *Int. J. Recent. Sci. Res.*, **9**, 24730 (2018).
46. O. I. Odola, C. Owabor and M. N. Odola, *Int. J. Atmos. Oceanic. Sci.*, **3**, 8 (2019).
47. R. M. Safitri, W. Mangunwardoyo and H. Ambarsari, *AIP conference proceedings*, Vol. 2021, American Institute of Physics, New York, USA (2018).
48. W. Ismail, I. S. Al-Rowaihi, A. A. Al-Humam, R. Y. Hamza, A. M. El Nayal and M. Bououdina, *Int. Biodeter. Biodegr.*, **84**, 168 (2013).
49. M. A. Kacena, G. Merrell, B. Manfredi, E. Smith, D. M. Klaus and P. Todd, *Appl. Microbiol. Biot.*, **51**, 229 (1999).
50. F. O. Kehinde and S. A. Isaac, *Afr. J. Bioethanol.*, **16**, 1242 (2017).
51. M. E. Hibbing, C. Fuqua, M. R. Parsek and S. B. Peterson, *Nat. Rev. Microbiol.*, **8**, 15 (2010).
52. A. F. A. Roslee, C. Gomez-Fuentes, N. N. Zakaria, N. A. Shaharuddin, A. Zulkharnain, K. A. Khalil, P. Convey and S. A. Ahmad, *Biology (Basel)*, **10**, 493 (2021).
53. M. S. Safdari, H. R. Kariminia, Z. G. Nejad and T. H. Fletcher, *Water, Air, Soil Poll.*, **228**, 1 (2017).
54. A. Kumar, S. Kumar and S. Kumar, *Biochem. Eng. J.*, **22**, 151 (2005).
55. D. Rivera, A. Margaritis and H. De Lasa, *Can. J. Chem. Eng.*, **77**, 903 (1999).
56. K. M. Ghanem, S. M. Al-Garni and M. A. Al-Zahrani, *Pol. J. Environ. Stud.*, **25**, 97 (2016).
57. P. Singh, P. Dipali and P. Ajit, *Bull. Environ. Pharma. Life Sci.*, **4**, 91 (2015).
58. M. Santhakumar, S. Sivalingam, D. Dharmapal and S. R. Sadras, *J. Microbiol.*, **48**, 637 (2017).
59. Q. Chen, J. Li, M. Liu, H. Sun and M. Bao, *Plots ONE*, **12**, e0174445 (2017).
60. W. Sun, W. Cao, M. Jiang, G. Saren, J. Liu, J. Cao, I. Ali, X. Yu, C. Peng and I. Naz, *RSC Adv.*, **8**, 39710 (2018).
61. M. T. Alnuaimi, T. A. Taher, Z. Z. Aljanabi and M. M. Adel, *Res. Crops*, **21**, 650 (2020).
62. R. C. Striebich, C. E. Smart, T. S. Gunasekera, S. S. Mueller, E. M. Strobel, B. W. Mcnichols and O. N. Ruiz, *Int. Biodeter. Biodegr.*, **93**, 33 (2014).
63. P. Parthipan, E. Preetham, L. L. Machuca, P. K. Rahman, K. Murugan and A. Rajasekar, *Front Microbiol.*, **9**, 193 (2017).
64. M. T. Ho, M. S. M. Li, T. McDowell, J. Macdonald and Z. C. Yuan, *BMC Biotechnol.*, **20**, 39 (2020).
65. M. Hassanshahian, M. S. Zeynalipour and F. H. Musa, *Mar. Pollut. Bull.*, **82**, 39 (2014).
66. D. D. S. P. Silva, D. De Lima Cavalcanti, E. J. V. De Melo, P. N. F. Dos Santos, E. L. P. Da Luz, N. B. De Gusmão and M. D. F. V. De Queiroz, *Int. Biodeter. Biodegr.*, **97**, 85 (2015).
67. G. Mohanty and S. Mukherji, *Int. Biodeter. Biodegrade.*, **61**, 240 (2008).