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RESEARCH ARTICLE

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Effects of physicochemical factors on PAH degradation by *Planomicrobium alkanoclasticum*

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are environmental pollutants that are mutagenic, carcinogenic, and toxic to living organisms. Here, the ability and effectiveness of selected bacteria isolated from an oil-contaminated area in biodegrading PAHs were evaluated, and the optimal conditions conducive to bacterial PAH biodegradation were determined. Of six bacterial isolates identified based on their 16S rRNA sequences, *Planomicrobium alkanoclasticum* could subsist on and consume nearly all hydrocarbons according to the 2,6-dichlorophenolindophenol assay. The efficacy of this isolate at PAH biodegradation was then empirically confirmed. After 30 days of incubation, *P. alkanoclasticum* degraded 90.8% of the 16 PAH compounds analyzed and fully degraded eight of them. The optimum *P. alkanoclasticum* growth conditions were 35°C, pH 7.5, and $NaNO_3$ as the nitrogen source. Under these biostimulant conditions, *P. alkanoclasticum* degraded 91.4% of the total PAH concentration and completely decomposed seven PAHs after 15 days incubation. Hence, *P. alkanoclasticum* is an apt candidate for the biodegradation of PAHs and the bioremediation of sites contaminated by them.

KEYWORDS
biostimulation, polycyclic aromatic hydrocarbons, oil contamination, biodegradation

1 | INTRODUCTION

Crude oil spillage poses serious threats to human health and the environment and occurs as a result of drilling, transportation, and related processes. It contribute to the greenhouse gas effects, air pollution, groundwater contamination, and so on (Jike, 2004; Wolfe et al., 1994). Polycyclic aromatic hydrocarbons (PAHs) are major crude oil constituents. PAHs are composed of at least two fused aromatic rings and persist in soils and sediments. Anthropogenic activity is the main source of these pollutants in the environment. PAHs may be toxic, mutagenic, and even carcinogenic to humans (Haritash & Kaushik, 2009; Luan et al., 2006; Marini & Frapiccini, 2013). PAHs are ubiquitous in the environment, bioaccumulate, and resist biodegradation. Their environmental fate is affected by chemical oxidation, photo-oxidation, volatilization, adsorption, and, to a far lesser extent, biodegradation (Haritash & Kaushik, 2009; Wild & Jones, 1995). Chemical, physical, and biological remediation methods have been implemented to restore sites contaminated by PAHs. However, one of the most cost-effective, accessible, and efficacious methods is bioremediation. This process involves the use of microorganisms that can eliminate various pollutants including PAHs from the environment (Haritash & Kaushik, 2009). Certain bacteria are able to degrade various pollutants including PAHs in the environment (Haritash & Kaushik, 2009). Certain microbes are enzymatically and catabolically active in PAH biodegradation. They breakdown PAHs via mineralization or biotransformation (Singh & Ward, 2004). Most bacterial species with PAH degradation efficacy have been isolated from contaminated sites that have acquired

Abbreviations: 16 PAHs (ng g^{-1}), sum of results of each compound listed above; BLAST, basic local alignment search tool; HPLC, high-performance liquid chromatography; ND, not detected; PCR, polymerase chain reaction.

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bacteria with a high capacity to degrade PAHs effectively (Riser-Roberts, 1998). However, the rate and extent of PAH biodegradation depend on numerous factors such as pH, nutrient availability, temperature, and bacterial species. Biostimulation provides supplementary nutrients or substrates that promote xenobiotic biodegradation by indigenous microorganisms (Couto et al., 2010). Several studies have attempted to bioremediate PAH-contaminated soils and optimize biostimulation for biodegradation enhancement (Hamzah et al., 2012; Satti et al., 2018; Simarro et al., 2011; Wu et al., 2016). The aims of the present study were to evaluate the potential PAH degradation capacity of bacteria isolated from an oil-contaminated sites and assess the value and impact of biostimulation in activating bacterial PAH biodegradation.

2 | MATERIALS AND METHODS

2.1 | Samples

Eight soil samples from the top 0–15-cm layer were randomly collected from various sites in the Al-Lahis oil fields of western Basra Province, Iraq (30°33'49" N, 47°05'45" E) that had been contaminated by different oil operations for approximately 60 year. The soil texture was sandy. The samples were analyzed for the 16 U.S. Environmental Protection Agency (USEPA) priority pollutant PAHs by USEPA Method 8270 at the (high pressure liquid chromatography laboratories, Marine Science Center, Basra, Iraq). The measured PAH concentrations of the 16 PAHs analyzed are listed in Table 1. The soil samples were stored in tightly sealed sterilized glass bottles and maintained at 4°C until subsequent analyses.

2.2 | Chemicals and media

Basra crude oil was obtained from Southern Oil Co. All other chemicals were purchased from Hi Media Laboratories, Mumbai, India, Bioneer, Seoul, Korea, Geneaid Biotech, New Taipei City, Taiwan, and Thermo Fisher Scientific. The culture media used in this study included Luria-Bertani (LB) broth consisting of 10.0 g peptone, 5.0 g yeast extract, and 5.0 g NaCl per liter distilled water. It was used to enrich the bacterial isolates. Mineral salts medium (MSM) consisted of 5.0 g NaCl, 5.0 g KH_2PO_4, 1.0 g K_2HPO_4, 1.0 g $MgSO_4{\cdot}7H_2O$, 2.0 g NH_4NO_3, 0.02 g $FeCl_2{\cdot}4H_2O$, and 0.02 g $CaCl_2$, per liter distilled water. Its pH was 6.5 and it was used for preliminary isolate screening and the PAH degradation experiments. Mineral salts agar (MSA) had the same composition as the MSM except that it also had 20 g bacteriological agar and 0.1% (w/v) sterile crude oil per liter distilled water. It was used to isolate oil-degrading bacteria. Finally, nutrient agar was used to preserve pure bacterial isolates.

TABLE 1 Polycyclic aromatic hydrocarbon (PAH) concentrations (ng g^{-1}) in soil samples

Location								
PAH compounds	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Naphthalene	ND	ND	7.2	ND	2.3	ND	ND	4.4
Acenaphtylene	11.4	13.6	ND	ND	19.4	18.7	18.0	19.8
Acenaphthene	ND	18.5	ND	ND	ND	ND	13.5	9.5
Fluorene	ND	ND	20.0	ND	ND	ND	9.1	23.3
Phenanthrene	ND	ND	ND	ND	ND	ND	ND	7.5
Anthracene	ND	ND	ND	9.5	ND	ND	5.2	4.6
Fluoranthene	ND	ND	3.4	3.1	6.8	ND	ND	5.0
Pyrene	ND	ND	14.4	4.8	7.5	ND	ND	8.2
Benzo[a] anthracene	6.3	ND	ND	17.5	ND	ND	ND	3.5
Chrysene	9.0	ND	12.7	14.6	5.2	38.9	15.5	14.5
Benzo[b] fluoranthene	10.9	8.1	3.4	9.2	ND	7.3	ND	10.4
Benzo[k] fluoranthene	24.6	5.0	18.7	16.7	ND	8.0	ND	ND
Benzo[a]pyrene	19.1	11.5	24.3	39.0	11.5	21.1	ND	23.3
Dibenzo[a,h] anthracene	9.8	39.6	3.0	31.1	14.0	26.4	8.7	9.4
Benzo[g,h,i] perylene	6.5	13.0	5.1	5.6	16.4	4.3	12.5	4.0
Indeno[1,2,3-c,d]pyrene	10.1	6.8	25.7	10.7	24.4	16.9	35.0	26.6
16 PAHs (ng g^{-1})	107.7	116.1	137.9	161.8	107.5	141.6	117.5	174

2.3 | Isolation of hydrocarbon-utilizing bacteria

PAH-degrading bacteria in soil samples were isolated according to previously reported methods (Prathyusha et al., 2016; Yan et al., 2013). First, 2.5 g of each sample was suspended in 25 ml LB medium to enrich the isolates. The cultures were incubated in a combined rotary shaker incubator at 120 rpm and 30°C for 2 days. Then 1 ml of each culture was added to 10 ml sterile physiological saline, vortexed for 10 min, and diluted up to 10,000-fold. About 0.1 ml of each dilution was inoculated into MSA medium and incubated at 30°C for 7 days. Pure isolates were obtained after several subcultures of each isolate. The pure isolates were stored on nutrient agar slants until the subsequent experiments. A completely randomized experimental design was used throughout.

2.4 | Identification and characterization of oil-degrading bacterial isolates

To identify each isolate, morphological features and Gram reactions of the pure bacterial colonies were compared with Bergey's Manual (Garrity et al., 2005). The bacterial isolates were identified down to the species level by targeting the 16S rRNA gene. Genomic DNA was extracted using a Presto™ Mini bacterial DNA kit (Geneaid Biotech) according to the manufacturer's instructions. The DNA was amplified by polymerase chanig reaction (PCR) using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTA CCTTGTTACGACTT-3′) based on the methods of Sambrook et al. (2001). The PCR reactions were executed in a total volume of 50 μl. An initial denaturation step of 95°C for 5 min was followed by 35 cycles of 95°C for 30 s, an annealing temperature of 55°C for 30 s, an extension at 72°C for 60 s, and a final extension at 72°C for 5 min. The PCR products were separated according to their molecular weight using a 1% (w/v) agarose gel made with TBE (Tris/borate/EDTA) buffer (Thermo Fisher Scientific). The DNA was visualized under UV light using an ethidium bromide DNA stain (Thermo Fisher Scientific). The amplified DNA was purified and sequenced by Macrogen, Inc. Bacterial isolates were identified with a basic local alignment search tool (BLAST) and the NCBI database (National Center for Biotechnology Information, Bethesda, MD, USA).

2.5 | Primary screening with 2,6-dichlorophenolindophenol

Primary crude oil degradation ability for the bacterial isolate was evaluated using 2,6-dichlorophenolindophenol. The use of this method made it possible to determine the capability of microorganisms to utilize crude oil. Pure bacterial cultures were transferred from nutrient agar slants to 100-ml conical flasks each containing 50 ml MSM medium. They were incubated for 24 h at 30°C and 120 rpm rotation. A combination of 0.5% (w/v) 2,6-dichlorophenolindophenol (2,6-DCPIP), 0.1% (v/v) Tween 80, and 3% (v/v) crude oil was introduced into the flasks and the contents were incubated in a combined rotary shaker incubator at 120 rpm and 25°C for 7 days. The cultures were verified daily for color change from blue to colorless. The abiotic control was prepared without inoculum. All treatments were performed in triplicate (Hanson et al., 1993).

2.6 | Aromatic hydrocarbon utilization

The best bacterial candidate which showed the best result during the primary screening with 2,6-dichlorophenolindophenol was selected to assess its ability to degrade aromatic hydrocarbons. The bacterium was activated by subculturing it at 30°C for 24 h in a vial containing 10 ml LB medium. Then 1 ml culture solution (OD_{600} = 0.10) was added to a 250-ml conical flask containing 100 ml MSM medium plus 1% (v/v) crude oil at pH 6.5. Culture flasks were incubated at 30°C in the dark and with constant shaking at 120 rpm for 15 days or 30 days. The abiotic control contained no bacterial inoculum and the experiments were run in triplicate.

2.7 | Effects of certain physicochemical factors on biodegradation

The effects of incubation temperature, pH and nitrogenic source on the ability of the bacteria to degrade aromatic hydrocarbons were evaluated according to the methods of Murthy et al. (2017).

2.7.1 | Incubation temperature

Aromatic hydrocarbon degradation was assayed at 25°C and 35°C using the same medium (MSM) and crude oil concentration (1% v/v). All experiments were conducted in triplicate. The flasks were incubated in a shaker at 120 rpm for 15 days. The abiotic control contained no bacterial inoculum.

2.7.2 | pH

Aromatic hydrocarbon degradation was evaluated at pH 5.5 and pH 7.5 using the same medium (MSM) and crude oil concentration (1% v/v). All experiments were conducted in triplicate. The flasks were incubated in a shaker at 120 rpm for 15 days. The abiotic control contained no bacterial inoculum.

2.7.3 | Nitrogen source

The NH_4NO_3 source in the mineral salts broth was replaced once with inorganic $NaNO_3$ and then with organic peptone. All experiments were conducted in triplicate. The flasks were incubated in a

shaker at 120 rpm for 15 days. The a biotic control contained no bacterial inoculum.

2.8 | Effects of biostimulation on aromatic hydrocarbon biodegradation

To study the effects of biostimulation on aromatic hydrocarbon biodegradation, 100 ml MSM medium was placed in 250-ml conical flasks and subjected to the aforementioned temperatures, pH, and nitrogen sources. Each flask was inoculated with the best bacterial candidate. Uninoculated media served as the a biotic control. The flasks were incubated for 7 days and 15 days in a combined rotary shaker incubator at 120 rpm. All tests were performed in triplicate.

2.9 | Aromatic hydrocarbon extraction and analysis

At the end of each experiment, residual oil was separated and extracted three times with 50 ml dichloromethane in a separating funnel. The organic fractions were decanted into clean glass vials and air-dried (Chettri et al., 2019).

The dried samples were re-dissolved in 5 ml *n*-hexane and the solutions were passed through a silica column. The PAH fractions were separated with 30 ml benzene to identify the aromatic compounds. The samples were evaporated to dryness in a rotary evaporator, re-dissolved in 5 ml solvent, and injected into a Shimadzu HPLC (Shimadzu Corp.) to detect the aromatic compounds. The HPLC conditions were: C18 column (250 mm × 25 cm × 4.6 mm), acetonitrile/water (90:10 v/v) mobile phase, 0.5 ml min^{-1} flow rate, 20 µl injection volume, and 254 nm wavelength.

The PAH degradation percentage was calculated as follows (Li et al., 2008):

$$D\% = 100(MI - MF)MI^{-1}$$

where MF is the final PAH concentration and MI is the initial concentration per treatment at the end of each incubation period.

2.10 | Statistical analysis

Minitab v. 16 (Minitab, LLC) was used to analyze the data by one-way analysis of variance and the relative least significant difference (RLSD) values were calculated to identify significant differences between treatments.

3 | RESULTS AND DISCUSSION

3.1 | Bacterial identification and colorimetric screening

Six different bacterial species were isolated from the soil samples (Table 2). Enrichment generated bacterial species that could both resist PAH toxicity and use PAHs as energy sources (Obi et al., 2016). Morphological characterization and Gram reaction indicated the presence of five gram-positive and one gram-negative bacteria. Of these, bacilli comprised approximately 83.3% while cocci represented approximately 16.6% of the population. Analysis of the partial 16S rRNA sequence showed that the bacterial isolates belonged to the genera *Bacillus*, *Globicatella*, *Planomicrobium*, *Pseudomonas*, and *Staphylococcus*. Similar results were reported in other studies (Engelhardt et al., 2001; Hara et al., 2013; Obi et al., 2016; Vinothi et al., 2015). Some of the aforementioned species can use hydrocarbons as energy sources. The endospores produced by members of the genus *Bacillus* resist unfavorable environmental conditions such as low humidity, high temperature, and high crude oil concentrations in soil. Hence, these bacteria can survive more effectively than other microorganisms in the presence of toxic materials such as crude oil (Obi et al., 2016; Vinothi et al., 2015).

Microorganisms indigenous to crude-oil-contaminated areas more effectively biodegraded PAHs than those isolated from uncontaminated soils. According to the 2,6-DCPIP test, all isolated bacteria tolerated and biodegraded crude oil and positively reacted with the redox indicator 2,6-DCPIP. However, *Planomicrobium alkanoclasticum* reacted with it very intensely and rapidly and completely changed the medium from blue to colorless (Table 3).

TABLE 2 Morphological characterization, Gram reactions, and molecular identification of soil bacterial isolates

Bacterial isolates	Bacterial morphology	Gram reaction test	Molecular identification (most probable taxonomically identical species)	Homology %
1	Bacilli	+	*Planomicrobium alkanoclasticum*	99
2	Bacilli	−	*Pseudomonas otitidis*	100
3	Bacilli	+	*Bacillus cereus*	99
4	Bacilli	+	*B. paramycoides*	100
5	Cocci	+	*Staphylococcus lentus*	100
6	Bacilli	+	*Globicatella sanguinis*	99

Note: −, no color change; +, mild color change, reduction.

TABLE 3 Preliminary screening for bacterial biodegradation ability with 2,6-DCPIP

No.	Bacterium	Degradation intensity
1	*Planomicrobium alkanoclasticum*	++
2	*Pseudomonas otitidis*	+
3	*Bacillus cereus*	+
4	*B. paramycoides*	+
5	*Staphylococcus lentus*	+
6	*Globicatella sanguinis*	+

Note: −, no color change; +, mild color change, reduction; ++, strong color change, reduction.

The 2,6-DCPIP assay tracks color changes from the deep blue oxidized form to the colorless reduced form. This test has been effectively applied to isolate indigenous oil-degrading microorganisms that can degrade PAHs in crude oil-contaminated soil (Bidoia et al., 2010; Hanson et al., 1993; Hara et al., 2013; Milic et al., 2016). Thenmozhi et al. (2012) used the 2,6-DCPIP assay to estimate the PAH degradation capacities of *Bacillus*, *Pseudomonas*, and *Serratia* isolated from PAH-contaminated soil.

3.2 | PAH biodegradation with *Planomicrobium alkanoclasticum*

Based on 1,6-DCPIP screening *Planomicrobium alkanoclasticum* had the highest PAH degradation potential of all bacterial species tested. Hence, it was selected to confirm its PAH degradation capacity. It was first described as a novel member species of the genus *Planococcus* (Engelhardt et al., 2001). It was isolated from a sediment sample and degraded C11–C33 *n*-alkanes. Dai et al. (2005) reassigned this species to the genus *Planomicrobium* based on its 16S rRNA gene sequence. Here, *P. alkanoclasticum* degraded crude oil.

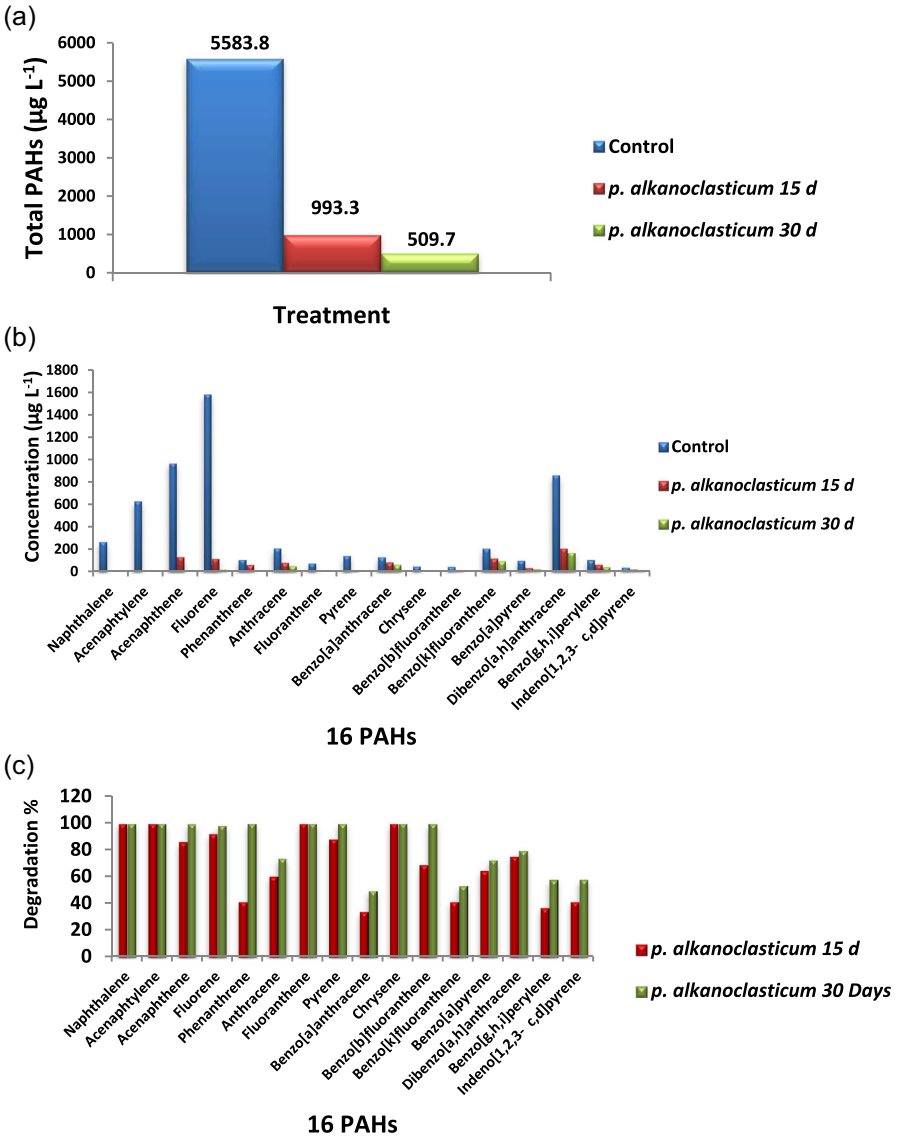

FIGURE 1 Degradation of polycyclic aromatic hydrocarbons (PAHs) by *P. alkanoclasticum* after 15 days and 30 days. (a) Total PAH concentration. (b) Concentrations of various PAH compounds. (c) PAH degradation percentage (%) [Color figure can be viewed at wileyonlinelibrary.com]

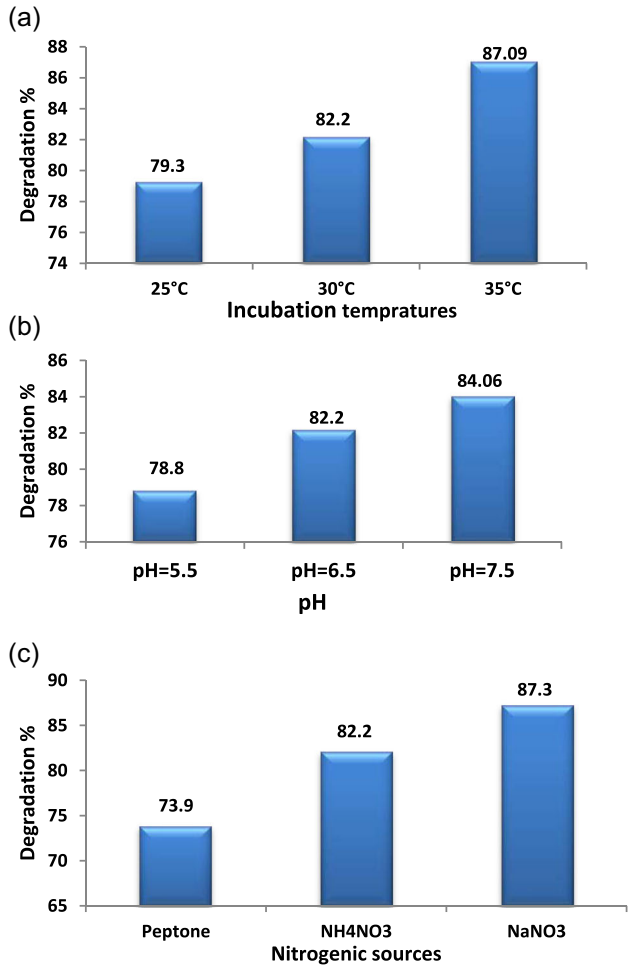

FIGURE 2 Effects of various factors on polycyclic aromatic hydrocarbon degradation. (a) Effects of different temperatures. (b) Effects of different pHs. (c) Effects of different nitrogenic sources [Color figure can be viewed at wileyonlinelibrary.com]

It degraded significantly more total PAHs ($p < .01$) than the abiotic control treatment. HPLC showed that *P. alkanoclasticum* degraded 82.2% of the total PAHs and entirely degraded naphthalene, acenaphthylene, fluoranthene, and chrysene after 15 days incubation. It reduced the total concentration of all 16 PAH compounds to 993.3 $\mu g\,L^{-1}$, compared with the abiotic control, the total PAH concentration was 5,583.8 $\mu g\,L^{-1}$ (Figure 1). After 30 days, the biodegradation efficiency had significantly increased to 90.8% and the bacteria completely degraded naphthalene, acenaphthylene, acenaphthene, phenanthrene, fluoranthene, pyrene, chrysene, and benzo[b]fluoranthene (Figure 1). In most cases bacteria are the most predominant microorganisms involved in the biodegradation of environmental pollutants. Indigenous bacteria isolated from polluted sites are often comparatively more effective than foreign ones at metabolizing PAHs (Li et al., 2008). The biodegradation efficiency depends on the ability of the bacterium to utilize specific substrates as anabolism. Here, *P. alkanoclasticum* exhibited the strongest degradation ability as its growth may have been exponential and its metabolic pathways might have enabled it to metabolize high PAH concentrations. It was assumed that after 30 days incubation, most of the high-molecular-weight PAHs were completely degraded (Figure 1b). In contrast, the low-molecular-weight PAHs were probably degraded earlier as they are relatively more soluble and degradable than high-molecular-weight PAHs. The latter are comparatively more resistant to biodegradation and require more time to be decomposed because these compounds are less soluble and less bioavailable for the microorganisms (Mrozik et al., 2003). These results are consistent with those of other studies that demonstrated the ability of various *Planomicrobium* species to degrade crude oil (Engelhardt et al., 2001; Radwan et al., 2019; Yakimov et al., 2007).

3.3 | Optimization of PAH degradation with *P. alkanoclasticum*

Several factors such as pH, temperature, and nutrient availability play major roles in controlling microbial growth and activity and, by extension, PAH degradation rates. Controlling these factors could increase both microbial growth and biodegradation (Coulon et al., 2007). Temperature markedly affects bacterial growth. In the present study, *P. alkanoclasticum* grew in the range of 25–35°C. However, its biodegradation capacity was optimal near 35°C. At this temperature, the total PAH concentration had declined to 720.4 $\mu g\,L^{-1}$. This level corresponded to 87.09% degradation (Figure 2). Temperature has a positive influence on bacterial growth and metabolic activity and can, therefore, potentially improve microbial PAH biodegradation. Moreover, PAH solubility and, by extension, bioavailability, increase with temperature (Margesin & Schinner, 2001; Sihag et al., 2014). This finding is consistent with those of earlier studies (Sihag et al., 2014; Venosa & Zhu, 2003; Vinothini et al., 2015) reporting that the optimum bacterial growth temperature for their isolates during oil degradation was 35°C.

Enzymatic activity, transport, and nutrient solubility are directly affected by changes in culture medium pH. Hence, the medium pH influences microbial growth (Lin et al., 2010). In this experiment, there was active bacterial biodegradation in the pH 7.5. The maximum degradation efficiency (84.06%) was observed at pH 7.5 (Figure 2). Previous studies reported similar findings (Murthy et al., 2017; Reddy et al., 2010; Sihag et al., 2014). The aforementioned authors stated that the oil biodegradation capacities of most of the bacteria they tested reach their maxima in the pH range of 6–8 and their optima were detected at pH approximately 7.

Effective PAH biodegradation also depends on the availability of nutrients required for growth and metabolism. Most bacteria require nitrogen, phosphorus, and other minerals in addition to a carbon source. Hence, nutrient supplementation is essential for the promotion and maintenance of bacterial growth and the improvement of biodegradation (Atagana et al., 2003). Here, the inorganic nitrogen source $NaNO_3$ enhanced degradation to a greater extent than NH_4NO_3 or peptone. The highest degradation efficiency was recorded for the medium containing $NaNO_3$ (87.3%). In contrast, peptone actually inhibited degradation (-Figure 2). $NaNO_3$ may have been more soluble and readily available to

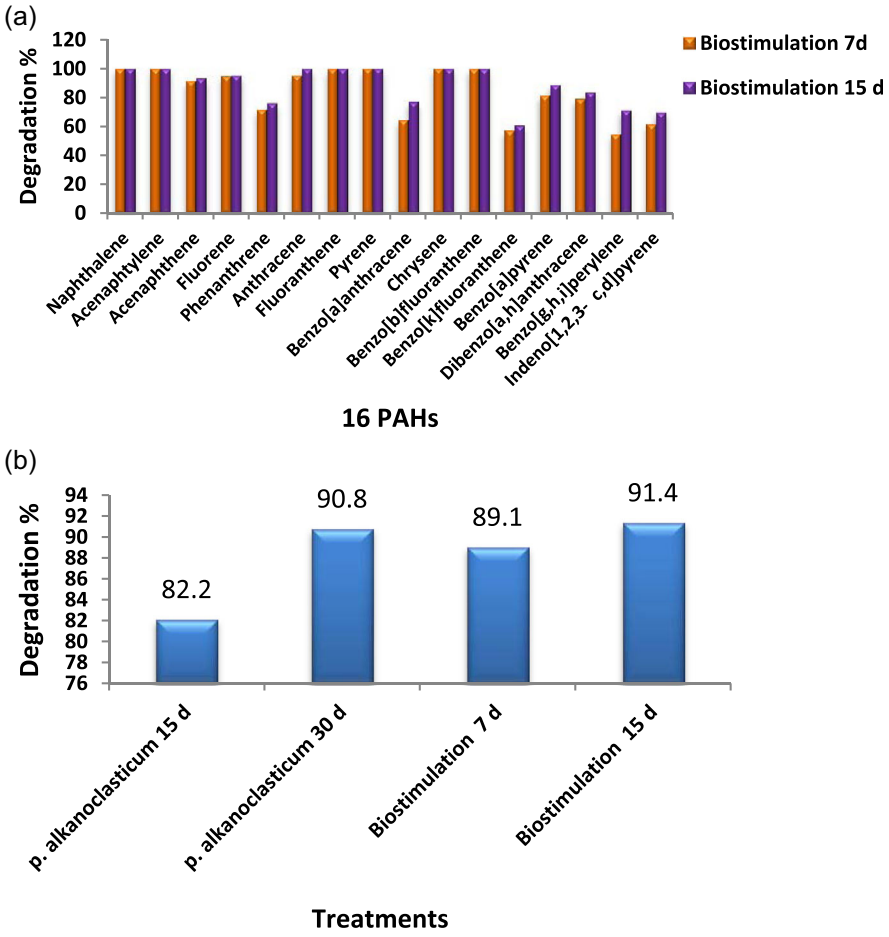

FIGURE 3 Effects of biostimulation on PAH degradation. (a) PAH degradation percentage (%) after 7 days and 15 days. (b) Relative total PAH degradation percentage with and without biostimulation. PAH, polycyclic aromatic hydrocarbon [Color figure can be viewed at wileyonlinelibrary.com]

the bacteria than peptone. The latter is relatively complex and may require metabolism to become bioavailable (Kuappi et al., 2011). Similar results were reported by Simarro et al. (2001). These authors found that $NaNO_3$ was the preferred nitrogen source for bacteria during growth and PAH degradation. Significant differences ($p < .01$) in the PAH concentrations were observed among the treatments differing in temperature, pH, and nitrogen source.

3.4 | Biostimulation of PAH degradation

The optimization of environmental parameters may dramatically improve PAH biodegradation. Biostimulation is the adjustment of environmental conditions so that they stimulate the growth and enzymatic activity of the bacteria present. The effects of 35°C, pH 7.5, and $NaNO_3$ on PAH degradation by *P. alkanoclasticum* were evaluated here. Under biostimulation, the total PAH concentration decreased to 606.8 $\mu g\,L^{-1}$ and the total degradation percentage increased to 89.1% after 7 days (Figure 3). In contrast, the bacterial PAH degradation percentage was only 82.2% after 15 days in the absence of biostimulation. Thus, biostimulation resulted in a high biodegradation efficiency. Therefore, subjecting the bacteria to optimal temperature, pH, and nutrient type increased the relative bacterial growth and enzymatic activity as well as the number of bacterial cells entering into contact with the crude oil, in this manner, the degradation process was stimulated (Adams et al., 2015; Kuappi et al., 2011).

The percentage of PAH degradation increased after 15 days incubation. By that time, the PAH concentrations had decreased to 476.07 $\mu g\,L^{-1}$ and the degradation percentage was 91.4%. In the presence of biostimulation, however, *P. alkanoclasticum* was able to degrade seven PAH compounds after 15 days incubation (Figure 3). There were significant differences ($p < .01$) in degradation percentage between the bacteria subjected to biostimulation and those that did not receive this treatment. PAH degradation efficiency increased with incubation time and this may possibly due to the bacterial biomass in contact with the PAHs and bacterial enzyme secretion both increased (Atagana et al., 2003; Kuappi et al., 2011). Our discoveries were consistent with those of several earlier studies and confirmed that biostimulation improved bacterial PAH biodegradation (Atagana et al., 2003; Kuappi et al., 2011; Murthy et al., 2017; Wu et al., 2016).

4 | CONCLUSION

Biodegradation and microbial PAH removal are currently applied at site contaminated by PAH compounds. This study showed isolating bacteria indigenous to oil-contaminated area and providing favorable

growth conditions improved degradation efficacy. The results of this study showed that sites heavily contaminated with PAHs harbor several bacteria capable of rapidly and effectively biodegrading these xenobiotic compounds. This strain of *Planomicrobium alkanoclasticum* which we isolated during this study proved to be an efficient PAH degrader. It was highly efficacious under laboratory conditions and degraded 90.8% of all 16 PAHs after 30 days incubation. Biostimulation under optimal pH, temperature, and nitrogen source conditions further promoted PAH degradation by *P. alkanoclasticum*. After biostimulation, about approximately 91.4% of all PAHs were degraded after only 15 days incubation. Hence, *P. alkanoclasticum* is a promising PAH biodegrading agent and could enhance bioremediation at PAH-contaminated sites.

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CONFLICT OF INTERESTS

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.

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