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Biodegradation of Anthracene by *Proteus* sp. strain BTE_BCH isolated from oil-spill contaminated soil

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Abstract

Biodegradation of harmful chemicals is of paramount importance for keeping a clean environment. Anthracene is a polycyclic aromatic hydrocarbons (PAHs) with a significant pollution potential and health risk. Because of its relative toxicity it has been utilized as a model for PAHs degradation investigations. Endophytic and rhizospheric bacteria can degrade PAHs like anthracene, which is a time/cost-effective method for eco-restoration. Therefore, techniques to eradicate this contaminant from the environment are urgently needed. In this study, the possible degrading ability of anthracene by *Proteus* sp. Strain, BTE_BCH from Nigeria was assessed using the one-factor-at-a-time (OFAT) technique. Mineral Salt Medium was used to culture the bacterium augmented with anthracene as its only energy and carbon source, at an optimum temperature of 35°C, pH 7.5, inoculum size of 400 µL and 400 mg/L substrate concentration, after 72 h incubation time. In addition, the bacterium can tolerate 2 mg/L Cu, Hg, Fe, Cr, Zn, Cd, Ni, Pb and As. The bacterium was able to degrade 97.5% anthracene after 72 h incubation. This data could be beneficial for optimizing anthracene biodegradation environmental factors; most importantly in the cleanup of anthracene-polluted areas. Furthermore, this study revealed that *Proteus* sp. strain BTE_BCH might be used to biodegrade anthracene-contaminated soil since it is less expensive, easier, eco-friendly and non-pathogenic.

Keywords: Anthracene, Bioremediation, Enrichment, Heavy metals, OFAT, *Proteus* sp.

1. Introduction

Incomplete combustion of organic waste may result in the formation of polycyclic aromatic hydrocarbons (PAHs), a category of environmental contaminants with a wide range of chemical properties [1]. Forest fires, volcanic eruptions, and plant and bacterial responses are all examples of how PAHs are created in the natural world [2]. According to recent studies, chronic exposure to PAHs has been linked to aquatic animal cancers and increased sediment mutagenicity [3]. Because of their carcinogenicity, toxicity and extensive distribution, the US Environmental Protection Agency has itemized 16 PAHs as primacy contaminants [4]. The low-molecular-weight (LMW) PAHs, which include two or three aromatic rings are highly hazardous. Still, the high-molecular-weight (HMW) PAHs, which contain four or more rings are usually thought to be genotoxic [5]. Although it is well known that PAHs can covalently bind to proteins, ribonucleic acid (RNA), and deoxyribonucleic acid (DNA), the amount of covalent interaction between DNA and PAHs is the most closely related to cancer risk. A significant variety of microorganisms that degrade PAHs have been discovered in recent years, with potential to eradicate PAHs from the environment via bioremediation. Several papers have been written on isolation and

characterization of bacteria that can thrive only on PAHs, such as naphthalene (NAP), pyrene (PYR), fluoranthene (FLU) and phenanthrene (PHE), as their only source of energy and carbon [1-3].

Because of its relative toxicity, anthracene was chosen as one of more than 100 PAHs to examine PAH degradation. These compounds have a carbon skeleton and hydrogen atoms organized into two or more low-solubility aromatic rings that protect them from nucleophilic assault [6]. PAHs are extremely lipid-soluble and easily absorbed by the gastro-intestinal tract of animals, and they can be exposed by inhalation, ingestion, or skin contact. In the human body, PAH metabolism generates epoxide chemicals that are carcinogenic and mutagenic. PAHs are extensively spread in the environment and can be found in different places such as soil, water and air. Many human operations, like oil refining and petrochemical industry produce them naturally or as a result of incomplete combustion of organic waste. Anthracene, being a tricyclic aromatic hydrocarbon that may be found in large quantities throughout the natural world. They have been utilized as model substrates in research on the environmental degradation of PAHs since both structures are present in carcinogenic PAHs, such as benzo[a]pyrene, benzo[a]anthracene, and 3-methylcholanthrene, among others [7]. Bacterial cultures from freshwater and marine environments can metabolize anthracene and phenanthrene as the only source of carbon, showing that they are capable of doing so [6]. Studies on the utilization of microbes for anthracene degradation was reported across the globe, but there has been no report from northern Nigeria. Similarly, microbial degradation of anthracene have been well considered in fungal and bacterial strains, mostly in the genus of *Serratia* sp., *Pseudomonas* sp., *Beijerinckia* sp., *Rhodococcus* sp*., Bacillus* sp*., Paenibacillus, Acinetobacter* and *Mycobacterium,* among other species [6,8,9].This study characterized the role of an indigenous, anthracene-degrading bacteria, producing evidence that this species can be used as a cost-effective method of removing harmful PAH from the environment.

2. Materials and methods

2.1 Chemicals

Analytical grade anthracene was obtained from Sigma Aldrich USA. Anthracene was dissolved in an analytical grade acetone as a stock solution (100 g/L). The stock solutions were sterilized using membrane filtration (0.45 µm). All other solvents and chemicals were of analytical grade and purchased from recognized manufacturers, e.g., Merck (Darmstadt, Germany) and Fisher (Malaysia).

2.2 Sample collection and isolation anthracene degrading bacteria

Oil-spilled contaminated soil was collected from Nigeria National Petroleum Corporation (NNPC) depot, Kano (12.0022N°, 8.5920°E). The samples (3) at a depth of 1-10 cm below the topsoil were collected using a soil auger, transferred into sterile polyethene bags, and transported to the Microbiology laboratory, Bayero University Kano for analyses. The liquid medium used to supplement the anthracene-degrading bacterium was prepared according to the techniques of Abdelhaleem et al. [9] with some little alterations except otherwise stated. The following conditions were used in the experiment: 10 g contaminated soil samples were suspended in 90 mL of distilled water, and 10 mL of this suspension was transferred into a 250 mL conical flask containing 90 mL of mineral salt media (MSM). The MSM used comprise (in g/L): 3.0 NH_4NO_3, 1.5 K_2HPO_4, 0.05 $CaCl_2$, 0.25 KH_2PO_4, 0.02 Fe SO_4, pH 7.0 [9]. The medium was supplemented with 0.1 g/L of anthracene. For solid medium, 15 g/L nutrient agar (NA) was supplemented for the anthracene medium in 1L of distilled water prior to autoclaving at a temperature of 121°C for 15 min and 115 kPa pressure. The bacterium was sustained on the above medium and sub-cultured every fourth night in solid media containing anthracene.

2.3 Screening of isolates with high anthracene tolerance

A single colony was injected into a freshly prepared liquid mineral salt medium (devoid of nutrient agar) using a sterilized wire loop. Anthracene (1000 mg/L) was added as the only energy and carbon source and the medium incubated at 37°C, pH 7 for 120 h. The media was refrigerated and used subsequently used for inoculation of the subsequent experiment in sterile airtight bottles. Turbidity in the medium at 600 nm was an indicator of the capacity of each isolate to use anthracene, as determined using a Ultra violet-Visible (UV-Vis) spectrophotometer [10].

2.4 Identification of isolated bacteria

Pure cultures of the heterotrophic bacterial isolates were identified morphologically and biologically using t biochemical method described by Oyeleke et al. [11]. Bacteria were identified to species level based on 16S ribosomal RNA (rRNA) gene sequencing, as explained below.

2.4.1 Molecular identification and phylogenetic analysis

Gram staining and 16 S rRNA gene sequencing were used to identify the bacteria to species level. Gram staining was determined using Cappucino and Sherman's [12] standard procedure. Genomic DNA was extracted using the Wizard® Genomic DNA (gDNA) purification kitTM (Qiagen, Germany) according to the company's instructions. The extracted DNA obtained was detected using agarose gel electrophoresis (1.0% (w/v). Quantitatively, the concentration and purity of the extracted genomic DNA was assessed using a NanoDrop® (ND)-1000 UV-Vis spectrophotometer prior to sequencing process. The concentration of the DNA was assessed by measuring the absorbance at 260 nm and the purity was determined at $A_{260/280}$ ratio. For the amplification of the 16S rRNA region, the extracted gDNA was amplified using eubacterial universal primers, 27F (5'AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACGGGTTACCTTGTTACGCTT-3'), respectively, both of which were synthesized by MyTACG Bioscience Enterprise (Kuala Lumpur, Malaysia) [13]. The polymerase chain reaction (PCR) mixture (50 µL volume) comprised of 0.4 µM forward each of forward and reverse primers, 2 µL gDNA template, 25 µL of 2× Bestaq PCR master mix (Applied Biological Materials, Canada) and 19 µL deionised water. The PCR was carried out using Hercuvan GS-96 Thermal cycler (Sdn, Bhd, Malaysia) with an initial denaturation at 94°C for 3 min; followed by 34 cycles each of 94°C for 60 s, annealing at 58°C for 60 s, and extension at 72°C for 2 min; and a final elongation for 10 min at 72°C., and 4°C. PCR amplification was confirmed by amplicon was separating 5 µL of amplicon on 1% agarose gel [14] and amplicons sequenced on both strands using the above primers. Nucleotide Basic Local Alignment Search Tool (BLAST) search (http://www.ncbi.nlm.nih.gov/BLAST/) was carried out to identify the bacterium to species level and the sequences were submitted to GenBank under the accession number: MW766369.

2.4.2 Phylogenetic analysis

The relationships between the *Proteus* sp. strain BTE_BCH and other *Proteus* strains were compared using a phylogenetic tree. Sequences from 20 closely related 16S rRNA gene sequences were retrieved from the GenBank and used for analysis, alongside those from with *Proteus* sp. The phylogenetic tree was constructed using Molecular Evolutionary Genetics Analysis (MEGA) version 7 [15].

2.5 Characterization of Anthracene-degrading Bacteria

The *Proteus* sp. was optimized to establish optimal conditions to growth and mineralize anthracene. Per se, the bacterial growth parameters were optimized using one-factor-at-a-time (OFAT) technique [6]. *Proteus* sp. cultures were prepared by inoculating bacterial colony on NA into 100 mL of nutrient broth (NB) and then incubated for 24 h at 37°C, on a rotary shaker set to 150 rpm. Following incubation, bacterial pellets were harvested by centrifugation at 12,000 rpm for 10 min. The resulting bacterial pellets were re-adjusted to an optical density of 0.9-1.0 before being inoculated on anthracene liquid media. The effects of different environmental factors, for example pH, inoculum size, anthracene concentration and temperature were optimized. For each OFAT analysis the optimal parameter was used to replace the previously used for the subsequent experiment. All the experiments were conducted in triplicates under same settings and the data are presented in mean ± standard deviation.

2.5.1 Effect of initial pH

The influence of the initial pH of the medium was exploited to determine the optimal pH for anthracene degradation by the bacterial strain. pH ranging from 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0, respectively, was optimised with no difference in units. The adjustment of the pH was done by the addition of 1 M HCl or 1 M NaOH.

2.5.2 Effect of inoculum size

The effect of inoculum size on bacterial growth was studied by varying the concentration of the inoculum from 50-1000 µL (0.50-10 %.). The optical density of the various concentrations was determined using a spectrophotometer and the optimum inoculum size concentration of the bacterium that enhances the anthracene degradation was established.

2.5.3 Effect of substrates (anthracene) concentration

For bacterial growth, anthracene was used as the only source of carbon and energy, with optimization of concentration, ranging from 50 - 1000 mg/L. At high concentrations, anthracene has been shown to be toxic to microorganisms [16], due to the inability to withstand and mineralize anthracene efficiently.

2.5.4 Effect of temperature

The effect of temperature on cellular growth of *Proteus* sp. strain BTE_BCH was studied, by varying temperature between 25 to 50°C at 5°C intervals. All experiments were carried out in triplicates. To exclude any abiotic degrading affection, non-inoculated controls were used throughout the analysis under the identical conditions.

2.6 Effect of heavy metals ions

Heavy metals are the most common kind of inorganic pollutant, and a significant amount of land has been polluted with them due to mining, manufacturing, agricultural, and military operations [16], the majority of which are related to PAHs. Therefore, assessing the interaction of heavy metals with the growth of bacteria is of paramount importance. The effects of different interacting heavy metals such as chromium (Cr), iron (Fe), arsenic (As), mercury (Hg), lead (Pb), copper (Cu), cadmium (Cd), nickel (Ni) and zinc (Zn) were examined on the growth of *Proteus* sp. strain BTE_BCH. Each heavy metal was added at a concentration of 2 mg/L to the MSM media containing anthracene. NB medium (1 mL containing the bacteria) was transferred to 100 mL of anthracene media containing the above heavy metals, individually. The cultures were incubated for 72 h at 37°C on a rotary shaker, at 150 rpm. The anthracene-degrading activity of the culture was monitored, and bacterial growth was measured at 600 nm.

2.7 Analytical assays

A Gas Chromatography-Mass Spectrometry (GC-MS) study of the degraded anthracene metabolites was carried out to quantify the percentage degradation. 5 mL of the cultures were extracted three times using 2.5 mL of ethyl acetate as the solvent. The cellular material was removed by utilizing separating funnels after the incubation period. The vials were maintained at 4°C prior to analysis. To assess abiotic losses of the substrate, an uninoculated control was incubated in parallel with the inoculated control. A GC-MS (Thermo MS DSQ II) coupled with a non-polar DB 35-MS Capillary Standard Non-polar column (30 mx0.25mmx0.25mm) to determine the degradation impact of naphthalene and anthracene. Organic phase (1 µL) was subjected to GC-MS analysis. A GC with a split-splitless injector (50:1) and a split-less injector was used for the GC-MS analysis. The oven temperature was set to 40°C at first, then gradually increased to 270°C at an 8°C/min rate for 5 minutes. Temperatures of 250°C were reached in the injector, transfer line, and ionization source. The carrier gas was helium, and the electron impact ionization was adjusted to 1.122 X 10^{-17} J 70 electron volts (eV) with an average linear velocity of one milliliter per minute.

2.8 Statistical analyses

The Statistical Package for the Social Science (SPSS) statistics Version 25 software tool (SPSS Inc., Chicago, Illinois, USA) was used to compare data among treatment groups, and Tukey's test was employed to conduct *post hoc* pairwise testing if significant differences were identified. The analyses were carried out in triplicate, and the results are reported as a mean ± standard deviation.

3. Results and discussion

3.1 Morphological, biochemical and molecular identification of the isolate

The bacterial isolate was physically motile and creamy in color. Upon microscopic inspection, the isolate was identified as Gram-negative, short rod, non-spore producing, motile, non-pigmented and was capable of degrading anthracene. The biochemical test revealed that it was positive for urease, indole, motility, catalase, and methyl red, and negative for citrate and oxidase (Table 1). The parameters were compared with Bergey's manual, which showed that it belongs to the genus of *Proteus*. This result was in accordance with the result reported by Abdelhaleem et al [9], except that the difference is based on the biochemical test (urease and indole) who reported that the urease and indole test were negative, which confirmed it to be *Bacillus* spp, while this study found that the isolate was positive for both urease and indole, which conformed with that of *Proteus* sp. strain BTE_BCH. The bacterium was identified through phylogenetic analysis of the 16S rRNA ribosomal gene sequence, with NCBI BLAST showing 99% similarity to the corresponding sequences of *Proteus* sp., (Figure 1). The strain is named *Proteus* sp. strain BTE_BCH.

Table 1 Biochemical tests for the candidate isolate.

Tests	Characteristics
Methyl Red	+
Catalase production	+
Oxidase production	-
Urease production	+
Motility test	+
Indole production	+
Citrate production	-

Key = + = positive test, - = negative test.

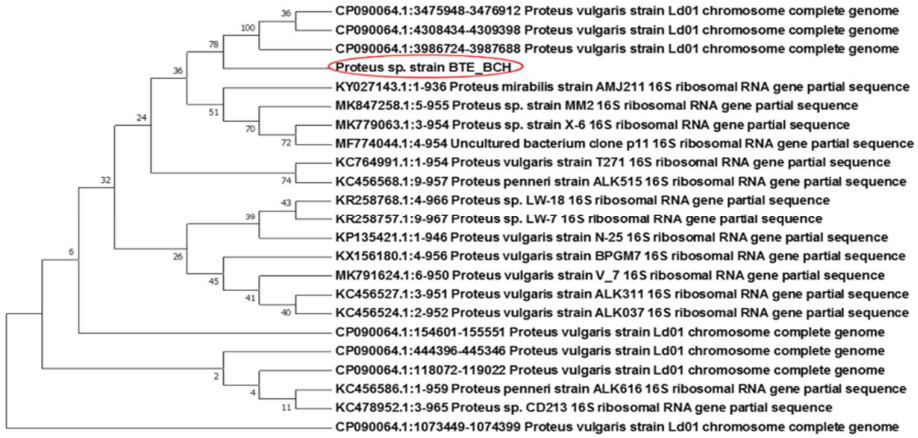

Figure 1 A maximum likelihood phylogenetic tree indicating the evolutionary relationship between *Proteus* sp. strain BTE_BCH and referenced related microorganisms, based on 16S rRNA gene sequences.

3.2 Effect of initial pH

pH is a crucial element that influences the capacity of bacteria capable of detoxification to degrade substances Ibrahim et al. [17]. Enzymes are in charge of controlling microbial metabolic activity, and a certain pH range is essential to maintain metabolism in bacterial cells. Extreme pH also inhibits an enzyme's activities and functions, producing conformational changes in its structure [18]. As a result, identifying the optimal pH for microbial development is critical for successful bioremediation [19]. The effect of pH on bacterial growth was investigated at various initial pH values ranging from pH 5.5 to 8.0, with an optimal pH of 7.5 obtained (Figure 2). Increased medium pH, above 7.5 resulted in a decrease in bacterial growth and hence degradation, which could be attributed to the reduced bacterial activity. Analysis of variance indicated an overall significant difference amongst the incubation time in terms of bacterial growth [$F(5,12) = 897.61$, $p<0.001$]. A significant difference in growth was observed between the optimum pH of 7.5 and the other pH values ($p<0.05$) based on *post hoc* comparison. Furthermore, there was a significant difference between and within all groups tested ($p<0.05$).

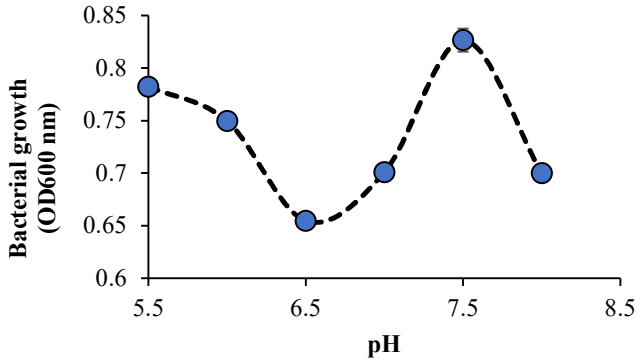

Figure 2 Effect of initial pH on the anthracene degradation by *Proteus* sp. BTE_BCH after 72 hours incubation. Data represents mean ± standard deviation, n =3.

This result is similar to the observation by Abdulrasheed et al. [20] who reported the maximum degradation percentage of diesel oil at an initial pH of 7.5 by Antarctic bacteria, *Arthrobacter* sp. strains AQ5-06 and AQ5-

05. In contrast, Bibi et al. [21] and Jacques et al. [6] reported a maximum degradation percentage of pH 7.0 for anthracene biodegradation by *Bacillus cereaus* S_{13} and *Pseudomonas* sp, respectively. Similarly, in a separate finding for the anthracene biodegradation and different PAHs by a yellow laccase from *Leucoagaricus gongylophorus*, Ike et al. [22] revealed an optimum pH of 6.0. These findings revealed that anthracene degradation was likely to occur at alkaline and neutral states. It might be because acidic conditions improve the stability of anthracene and its resistance to chemical bacterial degradation. Other researchers have found that, neutral pH is the best for hydrocarbon mineralization in both single and mixed colonies [19,23]. Furthermore, studies have shown that the optimal pH range for bioremediation in a controlled setting and in the field is between 6.5 and 7.5 [19], with a pH of about 7 supporting the development of hydrocarbon-degrading bacteria, which is consistent with the findings of this study.

3.3 Effect of inoculum size

Inoculum size is a paramount parameter in determining the degradation of activity as appropriate concentration of cells facilitates the degradation rate of anthracene. The quantity of bacteria in the medium has an impact on the cell's acclimatization and the degree of enzymes synthesized to support cell metabolism [24]. Because the size of the bacterium inoculum determines the amount of nutrients intake, the bacterial population should be kept under control [19]. The effect of inoculum size on anthracene degradation by *Proteus* sp. BTE_BCH was studied ranging from 50 - 1000 µL. The optimal inoculum size concentration was found to be at 400 µL (Figure 3). Analysis of variance showed significant differences [$F(5,12) = 10.18$, $p<0.001$]. However, *post hoc* comparison tests identified significant difference between the optimum, 400 µL and all other inoculum size tested ($p<0.05$) but there was no difference in terms of growth between the optimal volume (400 µL), compared with 200, 800 and 1000 µL ($p>0.05$). Furthermore, inoculum size greater than 600 µL was found to greatly retard anthracene degradation. A decrease in degradation may be associated to the competition of the inoculated cells with adverse metabolic constituents inhibition that are produced by the inherent microbe [25]. The growth of anthracene-degrading *Proteus* sp. BTE_BCH increased with increasing inoculum. The initial population drop may be compensated for by a higher initial inoculum, and survivors could reproduce and metabolize anthracene. Another cause for increased degradation with increased inoculum quantity might be because of the fact that the surviving microbes could use dead microbial structures as a nutrient source [19].

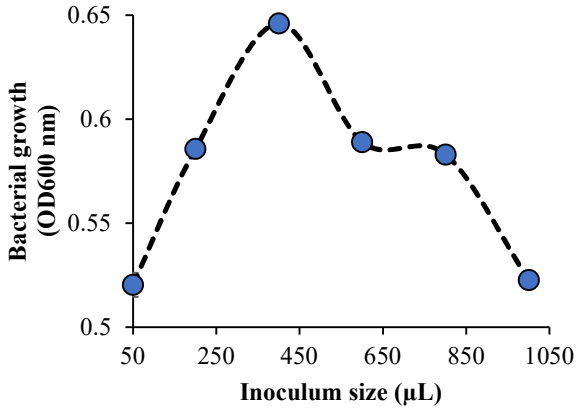

Figure 3 Effect of inoculum size on the anthracene degradation by *Proteus* sp. BTE_BCH after 72 h incubation. Data represents mean ± standard deviation, n =3.

3.4 Effect of substrate concentration

Some substrates can be lethal to microbes at high concentrations. Anthracene was added to a bacterial growth medium to serve as the microorganism's only energy and nutrients source for survival, growth and metabolic processes. The effects of different initial concentrations of anthracene ranging from 50-1000 mg/L on bacterial growth rate were investigated. Figure 4 displays the influence of initial anthracene on *Proteus* sp. BTE_BCH indicating that 400 mg/L supported the optimum growth for the strain following 72 h incubation. One-way ANOVA identified a significant overall difference between the concentration treatment groups [$F(6, 14) = 1173.76$, $p<0.001$]. Furthermore, there was significant difference between the bacterial growth attained with the optimum substrate concentration of 400 mg/L and all other concentration tested ($p<0.05$) as predicted by *post hoc* comparison, while no differences were obtained between 100, 800 and 1000 mg/L ($p>0.05$). The data obtained showed that at high anthracene concentration above 400 mg/L is toxic to the bacterial strain. This is contrary with

the result of Bibi et al. [21] and Jacques et al. [6] who reported 1000 mg/L and 500 mg/L as the maximum anthracene degradation concentration by *Bacillus cereus* S13 and *Pseudomonas* sp., respectively. Shukor et al. [26] and Abdulrasheed et al. [20] have also reported a diesel-degrading *Pseudomonas* strain and *Arthrobacter* sp. isolated from Antarctic region which grew optimally at a diesel concentration of 3.5 and 3.0% (v/v), respectively.

The rate of microbial activity was faster at low anthracene concentrations than at higher concentrations. This might be because, bacterial degradation starts slowly and requires a period of acclimatization before rapid degradation occurs at higher concentrations [27,28]. Furthermore, a comparatively higher anthracene concentration may be a key factor influencing bacterial growth, since the higher the anthracene concentration, the slower the rate of degradation with or without a lag phase.

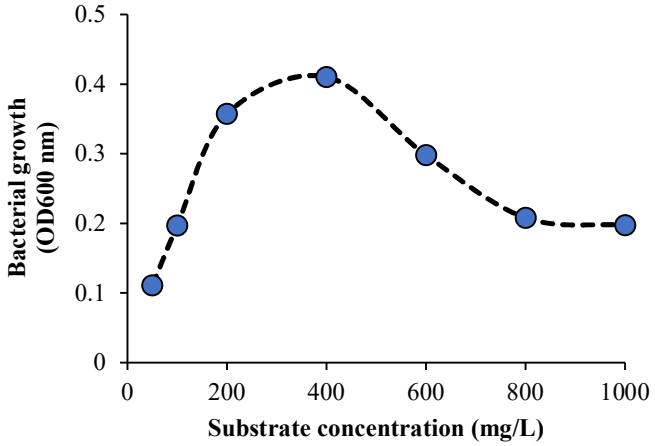

Figure 4 Effect of substrate concentration on the anthracene degradation by *Proteus* sp. BTE_BCH after 72 h incubation. Data represents mean ± standard deviation, n =3.

3.5 Effect of temperature

Temperature, has a considerable influence on the enzyme activities involved in anthracene degradation, is a critical determinant for achieving effective anthracene degradation by the bacteria [29]. As such, it is an important parameter for microbial growth and activity. In this study, the effect of temperature on anthracene degradation by *Proteus* sp. BTE_BCH was investigated over an incubation temperature ranging from 25 - 50°C with 35°C being the optimal temperature (Figure 5). One-way ANOVA a significant difference in terms of overall bacterial growth [$F(6, 14) = 99.72$, $p < 0.001$]. There was a significant difference between the optimum temperature 35°C and all other temperatures tested ($p < 0.05$), except for 30°C, which shows no significant difference ($p > 0.05$) based on *post hoc* comparison. More so, there were no significant differences between 37 and 25, 40°C and 45°C. This is contrary to the findings of Jacques et al. [6] and Ike et al. [22] who reported maximum anthracene degradation by *Pseudomonas* sp. and *Leucoagaricus gongylophorus* at 30°C. Similarly, Bibi et al. [21] had reported 30°C to be the optimum temperature for degradation of anthracene, for newly isolated rhizospheric bacteria *Bacillus cereus* S_{13}. The *Proteus* sp. BTE_BCH recorded low degradation at a very low temperatures which suggests that the temperature is insufficient for enzyme activity. These findings revealed that temperature variations greatly influence the degradation of anthracene. Other studies have made similar observations [30].

3.6 Effect of interacting heavy metals

Heavy metals are required by microorganisms because they are used as trace elements. Heavy metals are naturally found in Earth's crust [16]. Because they cannot be broken down like other forms of contaminants, they pose a major environmental concern if accumulated. At high concentration some heavy metals become toxic for microorganism [27,31]. The effects of different heavy metals (2 mg/L) on bacterial growth and anthracene degradation were investigated. Nine (9) heavy metal salts, namely Zn, Cd, Pb, Cr, Fe, Ni, As, Cu and Hg were used for this study. Figure 6 showed that Cd, Pb, Cu, As and Cr had negative effects on the anthracene degradation, while Zn has the least inhibitory effect on anthracene degrading potential of *Proteus* sp. BTE_BCH. Heavy metal concentrations over a certain threshold reduce microbial activity by inhibiting microbial enzymes and inducing DNA mutation [32]. Heavy metals toxicity at high concentrations may be responsible for the quick decline in the population of *Proteus* sp. BTE_BCH that occurs when the concentration of heavy metals increases, which is consistent with the results of Darma et al. [33].

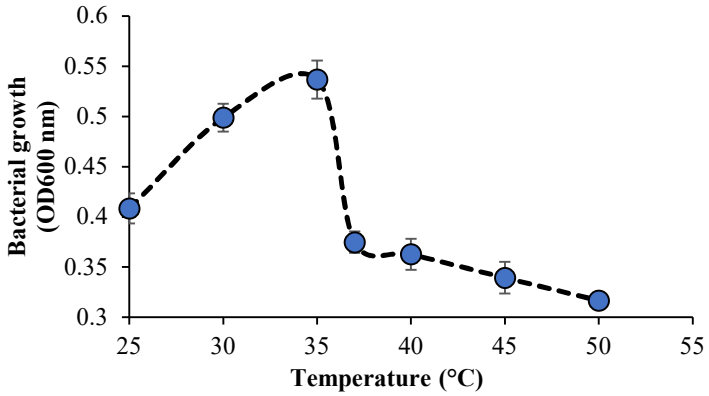

Figure 5 Effect of temperature on the anthracene degradation by *Proteus* sp. BTE_BCH after 72 h incubation. Data represents mean ± standard, n =3.

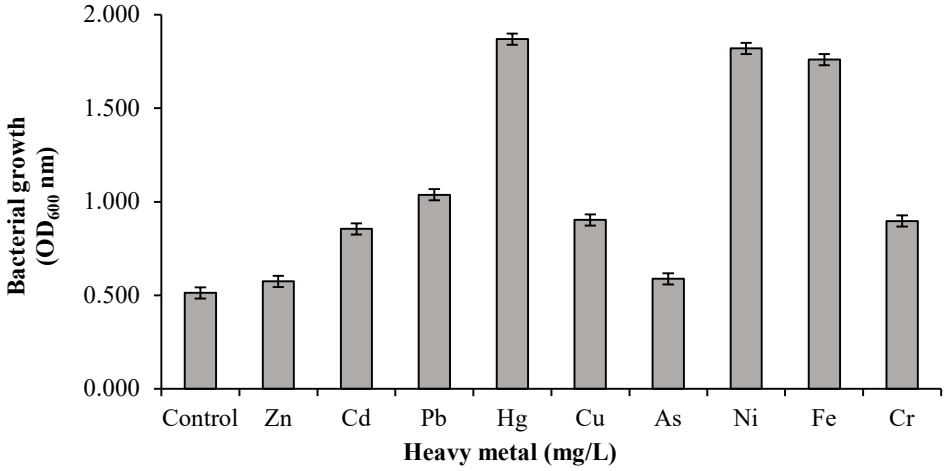

Figure 6 Effect of heavy metals at 2 mg/L on the growth of *Proteus* sp. BTE_BCH supplemented with 400 mg/L of anthracene on MSM media for 72h at 37°C. Data represents mean ± standard deviation, n =3.

3.7 GC-MS Analysis

The results of this investigation were utilized to quantify the percentage of substrate (anthracene) degradation by the isolate based on the retention time and area under the curve (AUC) values obtained from the experiment. The isolate was subjected to GC-MS analysis, which demonstrated that the percentages of degradation increased as the substrate concentration increased up to 400 mg/L of the substrate tested. However, there was a drop in the isolate's growth before it reached the steady phase. Thus, the degradation process was optimum at 400 mg/L of substrate or a steady-state for anthracene. At this point, the degrading isolate has adequate nutrient for growth and energy has high efficiency to mineralize naphthalene and anthracene. Therefore, the percentage degradation of anthracene was 97.5% (Figure 7) at a concentration of 400 mg/L following 72 h of incubation at 35°. Gupte et al. [34] reported similar findings in a study on the degradation of anthracene but the different isolated species of bacteria. It was discovered that high concentrations of anthracene were favorable to the development of isolated bacteria since they may function as the only carbon source for the bacterium's metabolism. Increased anthracene concentrations result in a rising trend in anthracene degradation due to the increased affinity of the substrate for product production caused by the increased concentration. Therefore, low concentrations would represent a growth constraint for the bacterial culture as the concentrations decreased. The metabolites of anthracene degradation found in this study compared to the data available on the national institute of standards and technology (NIST) library based on mass spectrum and fragmentation pattern were identified as; 1,4-naphthoquinone cis 1,2-dihydroxy naphthalene, 1,2-naphthalene diol, and cis-cis muconic acid. These findings were similar to Wu et al. [35] and Abdelhaleem et al. [9].

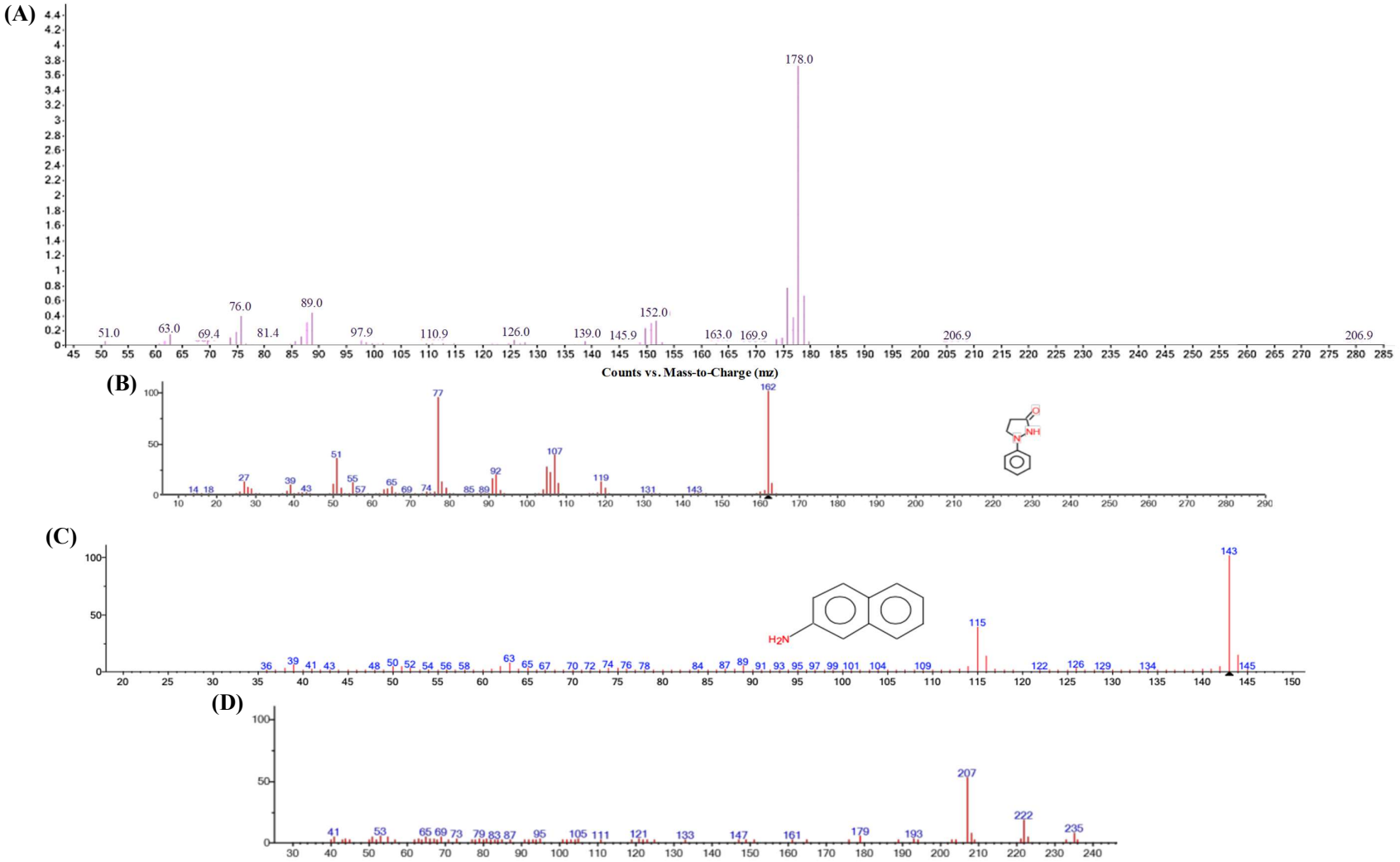

Figure 7 Mass spectrum of the degraded anthracene product; (A) Anthracene control, (B) Cis-cis muconic acid, (C) anthracene cis 1,2 dihydro diol, and (D) 9-10 anthraquinone.

4. Conclusion

A bacterium with a strong capacity to degrade anthracene has been identified. Under 72 hours of incubation at a pH of 7.5 and a temperature of 35°C, *Proteus* sp. BTE_BCH was shown to be capable of utilizing 400 mg/L of anthracene as the only carbon and energy source. When the growth media was treated to aeration and mechanical agitation, the rate of degradation of anthracene by *Proteus* sp. BTE_BCH was stimulated. The rate of degradation was best stimulated at an inoculum size of 400 L. The bacterium can endure 2 mg/L Cu, Hg, Fe, Cr, Zn, Cd, Ni, Pb, and As, which might help it survive in anthracene-polluted environments where these heavy metals are also present. This isolate is potentially a candidate for future anthracene bioremediation.

5. Acknowledgements

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6. References

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