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Growth on Sodium Dodecyl Sulphate (SDS) by a Bacterium Isolated from Langkawi UNESCO Kilim Karst Geoforest Park

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ABSTRACT

The breakdown of sodium dodecyl sulphate (SDS) in contaminated environments is crucial for reducing its ecological impact. We examined the influence of various environmental parameters on the growth and SDS degradation efficiency of *Pseudomonas* sp. strain UPM-Langkawi 2. The study explored the effects of temperature, pH, nitrogen sources, SDS concentration, and heavy metal presence on *Pseudomonas* sp. growth and activity. Growth rates were analyzed across temperatures from 20 to 50°C, pH values from 6.5 to 7.5, and various nitrogen sources (ammonium sulphate, ammonium chloride, potassium nitrite, and potassium nitrate) in BS media supplemented with SDS. SDS concentrations ranging from 0.1 to 2.5 g/L and heavy metals at 1 mg/L were also tested. Optimal growth and SDS degradation occurred at temperatures between 25 and 35°C and a pH range of 6.5 to 7.5. Ammonium sulphate at 5 g/L was identified as the most effective nitrogen source for supporting bacterial growth. *Pseudomonas* sp. achieved the highest growth at SDS concentrations between 0.75 and 1.5 g/L. Heavy metals significantly influenced bacterial growth, with mercury showing the most substantial inhibitory effect, followed by silver, copper, and chromium. Environmental parameters critically influence the biodegradation potential of *Pseudomonas* sp. Optimizing these conditions can enhance SDS degradation, offering a viable solution for bioremediation in SDS-polluted sites. Future research should focus on detailed kinetic modeling and field applications to validate these findings under natural conditions.

INTRODUCTION

Detergents are known to have damaging consequences on marine life [1–3]. According to preceding reports, anionic surfactants are harmful to numerous aquatic organisms at levels ranging from 0.0025 to 300 mg/L [4]. It influenced the life cycle of marine organisms and changes in behaviour [5]. Another study reported that the oyster digestive gland is sensitive to exposure to SDS, causing a negative perturbation of the oyster's nutritional and metabolic functions and leading to lower survivability [6]. As more anionic surfactants are released into water bodies, the pollutions caused by these compounds will lead to a rise in the toxic effects on invertebrates and crustaceans.

Anionic surfactants, such as Sodium Dodecylbenzene Sulfonate (SDS), are a major component of laundry detergents and have been detected in varying concentrations in wastewater. The presence of these compounds in water bodies can significantly affect water quality due to their high foaming potential and persistence. Surfactants can disrupt the surface tension of water, affecting the oxygen transfer to aquatic environments and leading to hypoxic conditions. The toxic effects of surfactants on aquatic organisms, particularly

invertebrates and crustaceans, are well-documented. Surfactants can damage cell membranes, leading to increased permeability and leakage of cellular contents [7–9]. Research has highlighted the acute toxicity of SDS to Daphnia magna, a common freshwater invertebrate, where significant mortality was observed at concentrations as low as 5 mg/L [10]. The concentration of anionic surfactants in wastewater varies significantly between domestic and industrial sources. Domestic wastewater typically contains detergent concentrations ranging from 3 to 21 mg/L, while industrial sources, especially those related to textile and laundry services, can exhibit concentrations up to 10,000 mg/L, posing serious challenges to treatment processes. Treating wastewater with high levels of surfactants like SDS is complex due to their resistant chemical structure and ability to interfere with treatment processes [11].

Increased awareness of the effects of surfactants has resulted in more stringent laws and the creation of environmentally friendly, highly biodegradable surfactants. Detergent formulas are being innovated to minimize environmental effects without compromising cleaning performance. A study on the effect of detergent on the local diatom species in the Air Hitam Strait located in the Regency of Meranti Islands revealed that the detergent concentration in these fluids ranged from 0.5714 to 0.8095 mg/l, while the abundance of diatoms ranged from 95.83 to 137.84 cells/l.

A substantial association was found between the concentration of dissolved detergent and the population of diatoms in the water. The authors concluded that detergent in the water might cause environmental issues due to a drop in dissolved oxygen concentration [12]. Detergents can also disturb the microbial equilibrium in the soil, impacting the health of the mangrove plants, resulting in decreased growth and higher mortality rates. Research in India indicates that releasing untreated or poorly treated laundry wastewater into coastal regions has caused damage of the mangrove ecosystems along the Mumbai coast. The elevated concentrations of phosphates and sulfates found in detergents have led to soil and water pollution, negatively impacting plant and animal life [13].

In July 2007, Langkawi became a UNESCO geopark, transforming the island from a quiet Malaysian jewel to a global tourist destination. This UNESCO recognition has driven exceptional growth in its coastal development and tourist industries, boosting its appeal to foreign visitors. For instance, the Kilim Karst Geoforest Park has gone from a peaceful rural location to a bustling tourist destination. The park's distinctive geological formations and natural settings show how geoparks may boost local economies and culture. This tourism boom has caused environmental problems, notably with increased sea traffic along the Kilim River and its ecological effects. An increase in tourism may further damage mangrove ecosystems and erode riverbanks. These habitats protect biodiversity and prevent coastal erosion. Pollutants such as detergents coming from use of household detergents and heavy metals including cadmium (Cd), cobalt (Co), lead (Pb), and zinc (Zn) near the Kilim Karst Geoforest Park possibly from boat emissions and building disruptions, threaten the area's biological balance [14–19].

The isolation of xenobiotics-degrading microorganisms from this region is important for future bioremediation works. In this study, we report on the isolation of an efficient SDS-degrading bacterium from mangrove sediments of the Langkawi UNESCO Kilim Karst Geoforest Park.

MATERIALS AND METHODS

Growth and maintenance of bacterium

The basal salts medium for bacterial growth consisted of the following components per liter: KH_2PO_4, (1.36), KNO_3, (0.5), Na_2HPO_4, (1.39), $CaCl_2$ (0.01) $MgSO_4$ (0.01), and $(NH_4)_2SO_4$ (7.7). Filter-sterilized sodium dodecyl sulphate was added into the medium as a carbon source at the final concentration of 1.0 g/L [20]. Sedimentary soils were taken 5 cm from the topsoil from a mangrove near the Langkawi UNESCO Kilim Karst Geoforest Park. One gram of the soil was mixed with 100 mL of sterile tapwater. About 0.1 mL samples were streaked onto nutrient agar plates containing SDS at the same concentration and then incubated at 30 °C for a maximum of 6 days. Multiple positive colonies were isolated and cultured using serial transfer iterations until pure colonies were achieved. The bacterial growth was measured using the colony count technique.

Morphological, physiological and biochemical characterization of the isolated strain

Various standard methods were utilized to analyze the strain's biochemistry and phenotype, such as colony shape, Gram staining, size and color of agar colonies, motility, oxidase activity (for 24 hours), ONPG (beta-galactosidase), catalase activity (for 24 hours), ornithine decarboxylase (ODC), arginine dihydrolase (ADH), and lysine decarboxylase [21]. The results were interpreted via the ABIS online system [22] as before [23].

Statistical analysis

The data were analyzed using statistical software, namely Graphpad Prism version 5.0. The values are shown as means ± standard error for three replicates. Group comparison was conducted using a one-way analysis of variance followed by post hoc analysis using Tukey's test or the Student's t-test [24]. A p-value less than 0.05 was deemed statistically significant.

RESULTS AND DISCUSSION

Sodium dodecyl sulphate (SDS), or sodium lauryl sulphate, is a common surfactant in various detergent formulas. It is extensively used across a broad spectrum of industrial processes and household products due to its effective cleaning properties and ability to produce foam. As referenced in several studies, SDS is a key ingredient in numerous cleaning and personal care products. The environmental concern with SDS begins when it enters aquatic ecosystems through wastewater discharges from both industrial facilities and residential areas. These discharges can lead to significant environmental pollution if not properly managed [25]. The presence of SDS in natural waters is problematic because it can disrupt aquatic life, potentially causing harm to organisms by interfering with cellular membranes and metabolic processes. Various treatment strategies have been developed and implemented to mitigate SDS's impact on the environment.

One of the most promising approaches involves using specific microbes that can degrade surfactants like SDS [26]. These biological treatments leverage the natural ability of certain bacteria and other microorganisms to break down complex chemical compounds into simpler, less harmful substances. This biodegradation process is seen as an environmentally friendly solution because it utilizes natural biological activity, thus reducing the need for chemical interventions that might themselves pose environmental risks [27].

Preliminary screening of SDS-degrading isolates

Five isolates had the ability to degrade SDS, with Isolate Langkawi 2 exhibiting the maximum efficiency. Further analysis of this isolate included identifying the best conditions for breaking down SDS and doing genetic and enzymatic studies to understand the metabolic pathways responsible for SDS breakdown.

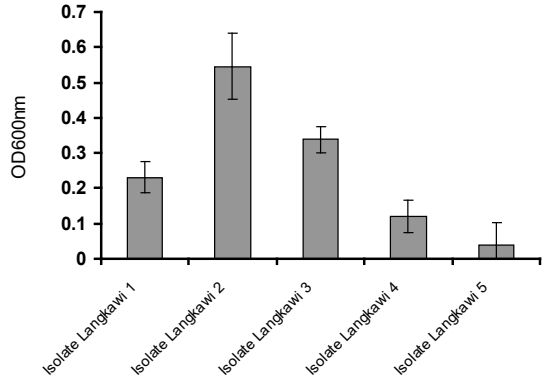

Fig. 1. Growth of SDS-degrading isolates on 1 g/L SDS at room temperature, pH 7, and 1% (w/v) as the nitrogen source. Error bars are mean ± standard deviation of triplicates.

Identification of SDS-degrading bacterium

The bacterium was a Gram-negative, motile, short rod-shaped organism. The bacterium was identified by comparing the findings of culture, morphological, and biochemical tests (Table 1) to Bergey's Manual of Determinative Bacteriology [21] and by utilizing the ABIS online software [22]. The programme provided three choices for the bacterial identification, with *Pseudomonas* aeruginosa having the highest homology (98 percent) and accuracy (89 percent). Molecular identification techniques based on the comparison of the 16srRNA gene will be required in the future to identify this species further.

Table 1. Biochemical tests for *Pseudomonas* sp. strain UPM-Langkawi 2.

Motility	+	*Utilization of:*	
Hemolysis	+	L-Arabinose	–
Growth at 4 ºC	–	Citrate	+
Growth at 41 ºC	+	Fructose	+
Growth on MacConkey agar	+	Glucose	+
Arginine dihydrolase (ADH)	+	meso-Inositol	–
Alkaline phosphatase (PAL)	–	2-Ketogluconate	+
H_2S production	–	Mannose	–
Indole production	–	Mannitol	+
Nitrates reduction	+	Sorbitol	–
Lecithinase	–	Sucrose	–
Lysine decarboxylase (LDC)	–	Trehalose	–
Ornithine decarboxylase (ODC)	–	Xylose	–
ONPG (beta-galactosidase)	–	Starch hydrolysis	–
Esculin hydrolysis	–		
Gelatin hydrolysis	d		
Starch hydrolysis	–		
Oxidase reaction	+		

Note: + positive result, − negative result, d indeterminate result

Sodium dodecyl sulphate (SDS) is a component found in detergent formulas [28]. It is used widely in industrial and household applications [29]. Issues occur when wastewater containing SDS from industrial and residential sources is released into the river, leading to environmental pollution. Various treatments, such as employing microbes capable of breaking down surfactants, have been employed to treat surfactants in wastewater [30]. The initial study detailing bacteria's capability to break down SDS was documented by [31].

Optimization of temperature

Studying the ideal temperature for bacterial growth on xenobiotics would be highly beneficial for bioremediation. Maintaining optimal closed conditions for the large-scale growth of bacteria in bioaugmentation experiments is crucial. The impact of temperature on the breakdown efficiency of SDS by *Pseudomonas* sp. was investigated throughout a temperature range of 20 to 50 °C. Growth rate of *Pseudomonas* sp. on SDS was shown to be the highest between 25 and 35°C with no significant difference ($p>0.05$) was found for growth on SDS at the two temperatures. Growth decreased rapidly at incubation temperature above 40 ºC and almost no growth observed at temperatures higher than 50 ˚C (**Fig. 1**). The optimum temperature for SDS degradation or growth in the literature ranges from 25 to 35 °C similar to the results in this study [32–43], which mesophilic degraders often reflect.

In this study, we investigate the ability of a *Pseudomonas* sp. to degrade SDS. The variety of SDS-degrading bacteria reported in the literature includes *Acinetobacter calcoaceticus* and *Pantoea agglomerans* [44], *Pseudomonas betelli* and *Acinetobacter johnsoni* [45], *Klebsiella oxytoca* [46] as well as *Burkholderia* sp., and *Serratia odorifera* [47,48] and many more [32–43]. In contrast, psychrotolerant SDS-degrading bacteria can carry out degradation at much lower temperatures (less than 10 ˚C) [49].

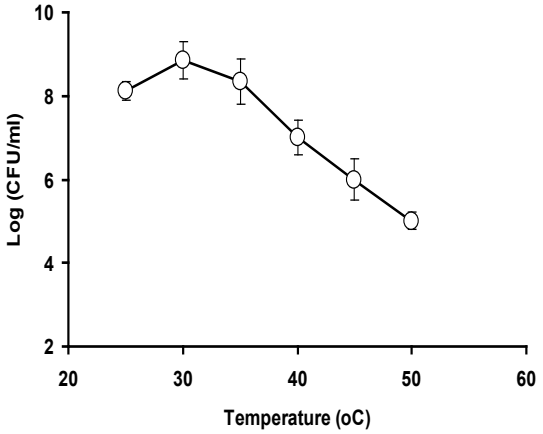

Fig. 1. The effect of temperature on the growth of *Pseudomonas* sp. strain UPM-Langkawi 2. Data is mean ± standard error (n=3).

Optimization of pH

As pH strongly affects bacterial growth, the maintenance of pH in the medium is vital. Once the optimum pH for the bacterial growth is obtained, this can help in designing effective bioremediation strategy [50]. Our results showed that bacterial consortium has the best growth rate in the pH range from 6.5 to 7.5 (**Fig. 2**). The optimum pH for SDS degradation or growth in the literature ranges from 6 to 8.0 similar to the results in this study [32–43], which neutrophilic degraders often reflect. The growth of *Pseudomonas* sp. decreased significantly at pH 9.5, presumably due to extreme alkaline conditions. The ability of bacteria to regulate their cytoplasmic pH allows them to tolerate

a certain range of pH [51]. However, extremely acidic and alkaline conditions affect the state of ionization of active enzyme sites and lead to changes in the active site's electronic configuration, eventually preventing substrate binding. This is translated to a loss of activity [51]. The study of pH optimal is important for two reasons. The first is for mass production of the bacterium in bioaugmentation exercise and the second is to assess whether pH adjustment of soil in polluted sites to match optimal growth or degradation of the bacterium is needed.

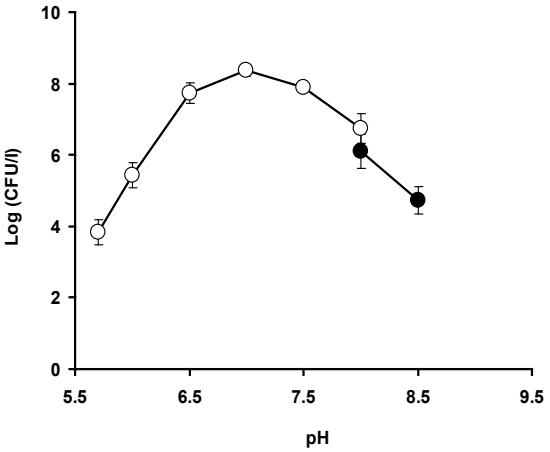

Fig. 2. The effect of pH on the growth of *Pseudomonas* sp. strain UPM-Langkawi 2 using an overlapping buffer system consisting of phosphate (○) and carbonate (●). Data is mean ± standard error (n=3).

The effects of nitrogen source on growth

Nitrogen source is a crucial component that affects the growth of microorganisms, especially when the targeted toxicant; SDS, does not have a nitrogenous compound in the structure. Consequently, detection of the best nitrogen source and its particular optimum concentration for growth could greatly assist in creating successful bioremediation approach [52]. Different nitrogen sources such as ammonium sulphate, ammonium chloride, potassium nitrite, and potassium nitrate were tested at 0.1% (w/v) in BS media supplemented with SDS as the sole carbon source to study their effects on bacterial growth. Our results have shown that *Pseudomonas* sp. growth rate was the highest when ammonium sulphate was the sole nitrogen source ($p<0.05$) (**Fig. 3**).

The optimal concentration of ammonium sulphate was at 5 g/L. Nearly all SDS-degraders require a simple nitrogen source such as ammonium sulphate to support growth on SDS [32–43], which mesophilic degraders often reflect. The use of ammonium sulphate as a nitrogen source is consistent with previous reports by Dhouib et al. and Shukor et al. [20,46]. Other surfactant degraders like *Citrobacter braakii* required 7.7 g/L ammonium sulphate [20] whereas *Comamonas terrigena* strain N3H showed an optimum growth at 5.4 g/L ammonium nitrate [53]. Ammonium sulfate, being a highly absorbable nitrogen form, is also used by *Pseudomonas* species. Under aerobic circumstances, assimilation mechanisms may directly integrate ammonium into organic molecules. This nitrogen source is commonly favored under aerobic settings because it takes less energy for assimilation than nitrate, which must be initially converted to ammonium.

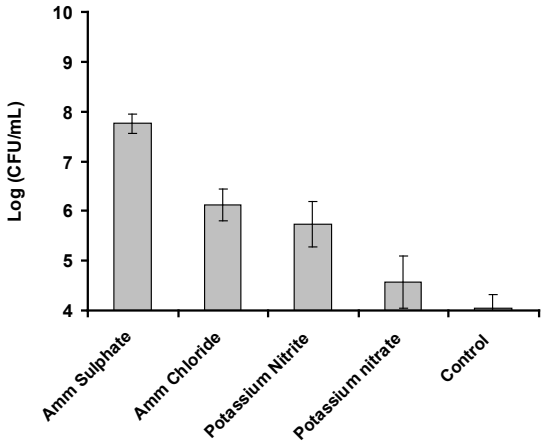

Fig. 3. The effect of various nitrogen sources on the growth of *Pseudomonas* sp. strain UPM-Langkawi 2. Data is mean ± standard error (n=3).

The effects of sodium dodecyl sulphate concentrations on growth

Sodium dodecyl sulphate as the lone carbon supply is required in big amounts as carbon is the fundamental structural unit of all organic substances. The bacteria may also be killed by the stripping of the lipopolysaccharide outer layer by SDS especially in Gram negative bacteria leading to cell death [1,54]. We showed that *Pseudomonas* sp. was able to utilize SDS as a sole carbon source. We observed the growth of *Pseudomonas* sp. on a series of different concentration of SDS and the highest growth was recorded at the concentration between 0.75-1.5 g/L ($p<0.05$) with ANOVA analysis shows no different between these two-temperature range as judged by ANOVA. *Pseudomonas* sp. exhibited lower growth at SDS concentrations higher than 1.5 g/L growth was strongly inhibited at 2.5 g/L (**Fig. 4**). Many SDS-degraders degrade or growth best at SDS concentration of less than 500 mg/L although some degraders can tolerate >1000 mg/L [32–43], which mesophilic degraders often reflect.

The ability of *Pseudomonas* sp. to assimilate SDS for growth falls under common tolerable SDS concentration range reported in the literature. The maximum degradation capacity by bacteria reaches a limit often coinciding with the critical micelle concentration (CMC) of SDS at 2.34 g/L (Singh et al. 2007). *Pseudomonas* sp. shows that at the tolerable concentration of 2 g/L, approximately 90% of SDS was degraded after 8 days and cellular growth had reached equilibrium. However, a longer lag period of approximately three days was observed before the bacterial growth started to increase concomitantly with a reduction in SDS concentration implying that adaptation of the bacteria to different carbon source. Margesin and Schinner reported that their consortia of microbes are able to degrade 0.5 to 1 g/L SDS in 4 days at 10 °C [49]. The tropical isolate *Klebsiella oxytoca* strain DRY14, isolated from a detergent-polluted site, does not exhibit any lag phase during its degradation of 2 g/L SDS, implying that the genes for detergent degradation are quickly expressed upon contact with a detergent such as SDS [46].

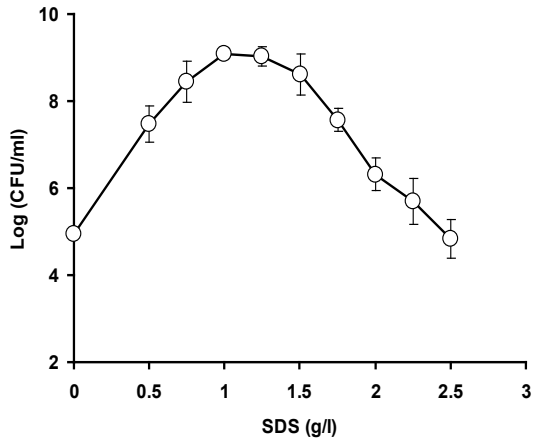

Fig. 4. The effects of sodium dodecyl sulphate concentrations on the growth of *Pseudomonas* sp. strain UPM-Langkawi 2. Data is mean ± standard error (n=3).

Growth of *Pseudomonas* sp. strain UPM-Langkawi 2 on heavy metals

To determine the potential ability of *Pseudomonas* sp. to utilize heavy metals, we tested the growth of *Pseudomonas* sp. on various heavy metals including zinc (Zn), silver (Ag), nickel (Ni), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and mercury (Hg) at 1 mg/L final concentration. We showed that *Pseudomonas* sp. was strongly inhibited by mercury followed by silver, copper and chromium in descending order of inhibition (**Fig. 7**). Growth on Hg was severely inhibited.

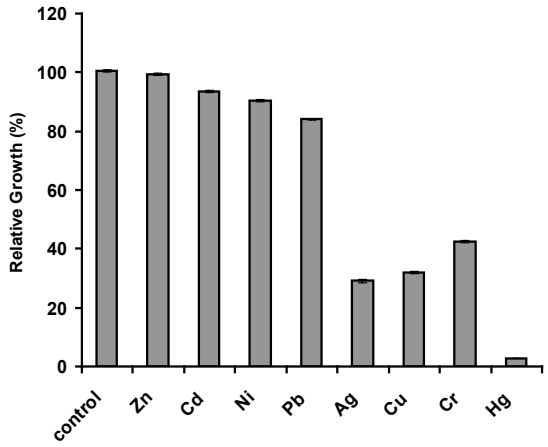

Fig. 7. The effect of various heavy metals on the growth of *Pseudomonas* sp. strain UPM-Langkawi 2. Data is mean ± standard error (n=3).

The ability of microorganisms to grow on heavy metals on easily assimilable substrates has been reported. For instance, *Pseudomonas putida* has been reported to tolerate high concentrations of heavy metals such as Cd, Zn and Pb [55,56]. *Paenibacillus* sp. was shown to have high sensitivity against Cu while *Bacillus thuringeinsis* has a high sensitivity against Cd and Zn [57]. However, heavy metal-tolerant SDS-degrading bacteria or studies on the effect of heavy metals on SDS degradation are limited. Hence, this study offers additional data for comparison on SDS-degrading bacteria isolated in the future. Mercury's interaction with bioremediation on a cellular level primarily influences how mercury affects growth and metabolism, limiting their ability to remove environmental toxins. Mercury is particularly toxic in its mercuric (Hg(II)) form, which can bind to sulfhydryl groups in proteins, causing protein deactivation and disrupting biological activities. Activating the operon is an essential mechanism for bacteria to resist mercury poisoning. This collection of genes allows for the transportation, reduction, and transformation of mercury into a form. Hg(0) is the elemental mercury. This detoxification procedure does not remove mercury. Also helps to eliminate it from the environment [58].

For example, the bacterium Cupriavidus metallidurans is resistant to mercury via mechanisms that work in both oxygen-rich and oxygen-deprived circumstances. It can be used efficiently in certain environments [59]. Mercury is converted into a gas, and it is still removed in anaerobic environments, but with a lower efficiency. The presence of oxygen aids in the reduction and transformation of mercury, hence enhancing the detoxification process. Furthermore, microbes can convert mercury into chemical states by processes such as oxidation, reduction, methylation, and alkylation. These modifications can change the toxicity levels. How quickly it is available for engagement. For example, certain bacteria can convert mercury into less toxic forms, allowing it to be removed from the environment by processes such as volatilization. The complicated link between metabolism and genetic changes demonstrates the complexities of mercury cleaning and the critical molecular pathways required to handle mercury-induced stress [60].

CONCLUSION

In conclusion, the study examined the effects of temperature, pH, nitrogen sources, SDS concentration, and heavy metal exposure on *Pseudomonas* sp. strain UPM-Langkawi 2 biodegradation of sodium dodecyl sulfate (SDS).*Pseudomonas* sp. strain UPM-Langkawi 2 grew well and broke down SDS at 25–35°C. Growth performance did not differ between these temperatures, suggesting a broad thermal tolerance that supports bioremediation in different climates. Bacterial growth and SDS degradation were best at 6.5–7.5 soil pH. Many natural soils have this pH range, so *Pseudomonas* sp. strain UPM-Langkawi 2 could be used in bioremediation without extensive pH adjustments in contaminated sites. The optimal nitrogen source for *Pseudomonas* sp. strain UPM-Langkawi 2 growth was 5 g/L ammonium sulphate. This finding shows that bioremediation strategies must consider nutrient optimization and pollutant removal. SDS was used efficiently by the bacterium at 0.75–1.5 g/L. Concentrations above 1.5 g/L inhibited growth, with severe inhibition at 2.5 g/L. SDS concentrations above this may compromise bioremediation efficiency. Mercury strongly inhibited *Pseudomonas* sp. strain UPM-Langkawi 2 growth. The bacterium was sensitive to silver, copper, and chromium but less than mercury. These findings show that heavy metals may hinder microbial degradation in bioremediation sites with multiple pollutants. This study underscores the need to tailor bioremediation strategies to specific environmental conditions and pollutant types. The ability of *Pseudomonas* sp. strain UPM-Langkawi 2 to adapt to a range of conditions, with the noted exceptions of high SDS concentrations and heavy metal contamination, supports its potential as a versatile agent in bioremediation efforts. Future research should focus on enhancing microbial resistance to heavy metals and optimizing bioremediation protocols to maximize degradation efficiency under varied environmental stresses.

ACKNOWLEDGMENTS

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