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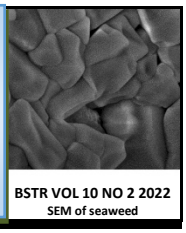

Growth on Sodium Dodecyl Sulphate (SDS) by a Bacterial Consortium Isolated from Volcanic Soil

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ABSTRACT

During biodegradation, microorganisms can directly metabolize surfactants for energy and nutrients or co-metabolize them with other compounds. Maximum growth of the bacterial consortium on SDS was seen between 30 and 35 °C, while the optimal pH range for bacterial consortium growth was between 6.5 and 7.5. As for the nitrogen source, 2 g/L of ammonium sulfate was optimum in supporting the growth of SDS. The greatest growth rate of the bacterial consortium was recorded at a concentration of between 1 and 1.5 g/L of SDS ($p<0.05$). At 2–3, g/L of SDS, the bacterial consortium grew more slowly, and at 5 g/L, growth was severely inhibited. Almost complete degradations of SDS were observed in 3, 5 and 6 days at 0.5, 0.75 and 1 g/L SDS, respectively while higher concentrations showed partial degradation with no degradation observed at 2.5 g/L SDS after 6 days of incubation. In this study, the maximum growth rate, or μ_{max}, K_s, and K_i were 0.517 h^{-1} (95% confidence interval of C.I. from 0.404 to 0.629), 0.132 (g/L) (95% C.I. from 0.073 to 0.191) and 0.909 (g/L) (95% C.I. from 0.544 to 1.273), respectively. Heavy metals like mercury, copper, and chromium can severely stunt growth if they are present in the environment. It was discovered through research into growth kinetics that Haldane substrate inhibition kinetics may be used to model the growth rate. This bacterial consortium has the right properties for the bioremediation of SDS-polluted environments.

INTRODUCTION

Surfactants, which include detergents, are a broad category of compounds designed to facilitate the dissolving or removing substances. About 7.2 million metric tons of synthetic surfactant are produced annually around the world. They typically have a nonpolar hydrocarbon tail that is not simply solvated in water and a polar head group that dissolves readily in water (whether neutral or charged). In this way, surfactants are molecules that incorporate both hydrophilic and hydrophobic characteristics. Foaming agents are often considered to be severe contaminants due to the problems they cause in treatment plants and the direct harmful impact they have on a wide variety of organisms in the environment [1,2]. Many different industries rely on surfactants, including those dealing with food, oil, excavation, soil remediation, water treatment, mining, and textiles. Even though several surfactant types are frequently used in industrial detergents, the most prevalent ones are listed here. Nonionic and anionic surfactants become persistently toxic at concentrations greater than 0.1 mg/L.

It is well-documented [1-3] that detergents have negative effects on marine life. Numerous aquatic creatures are harmed by exposure to anionic surfactants at concentrations between 0.0025 and 300 mg/L, as has been previously shown [4]. Consequently, it caused aquatic organisms' life cycles to shift and their behaviour to alter [5]. Another study found that oysters' digestive glands are more vulnerable to SDS, which negatively disrupts the oyster's nutritional and metabolic activities and reduces the oyster's chances of survival [6]. The hazardous effects on invertebrates and crustaceans will increase as more anionic surfactants are released into water bodies, increasing pollution.

The concentration of detergents in household wastewater is typically between 3 to 21 mg/L, whereas in some types of industrial effluent, it can reach as high as 10,000 mg/L. As a result of the high concentration of surfactants in laundry wastewater, its treatment presents unique challenges. Most laundry detergents use anionic surfactants like sodium dodecyl benzene sulfonate (SDS) or builders like sodium aluminium silicates. Laundry detergent concentrations were observed to be anything from 17 to 1024 mg/L [7]. Therefore, it is absolutely necessary that SDS be remediated. Microorganisms are well-known for their capacity to breakdown organic material, including SDS [8,9], and the utilization of microorganisms as bioremediation agents is economically essential for the removal of xenobiotic pollutants.

The biodegradation of anionic surfactant under aerobic conditions was investigated in one of the earliest reports of an SDS-degrading bacteria, which was for the *Pseudomonas* sp. strain C12B [10]. Since this investigation, numerous SDS-degrading bacteria have been identified [11–14]. Since polluted environments frequently house a wide variety of organic and inorganic contaminants [15], the isolation of bacteria capable of degrading and remediating a wide range of xenobiotics is crucial. In this work, we report on the characterization of a surfactant-degrading bacterial consortium, previously able to grow on acrylamide.

MATERIALS AND METHODS

Growth and maintenance of the bacterial consortium

The bacterial consortium was previously isolated from the topsoil near Mount Marapi in West Sumatera, Indonesia and has the capability to grown on acrylamide and was collected by the late Dr Neni Gusmanizar [17] and stored in the university's culture collection unit. The basal salts (BS) medium (g/L) for bacterial growth contained the followings: KH_2PO_4, (1.36), Na_2HPO_4, (1.39), KNO_3, (0.5), $MgSO_4$ (0.01), $CaCl_2$ (0.01) and $(NH_4)_2SO_4$ (7.7). The medium also contained the following trace elements to the final concentration of 0.01 mg/L: $ZnSO_4.7H_2O$, $MnCl_2.4H_2O$, H_3BO_4, $CoCl_2.6H_2O$, $FeSO_4.2H_2O$, $CuCl_2.2H_2O$ and $Na_2MoO_4.2H_2O$. Sodium dodecyl sulfate, which had been filtered for purity, was added to the medium at a final concentration of 1.0 g/L [13] as the carbon source. Bacteria could live for up to six days on nutritional agar plates treated with the same dose of SDS in an incubator set to 30 °C. The growth of the bacterial consortium was tracked by counting the colony-forming units.

Methylene blue active substance assay (MBAS)

The chloroform extraction method [18] used methylene blue solution read at 652 nm against chloroform blank to calculate SDS residuals. In a nutshell, in a 100 mL separating funnel, we combined 100 L of samples with 9.9 mL of deionized water. Then, 1 mL of chloroform was added, followed by 2.5 mL of methylene blue solution. After violently shaking the funnel for 15 seconds, extraction could begin. If you let the mixture alone for 20 minutes, you'll see that it separates into two distinct layers. The layer of chloroform was removed and transferred to a new funnel. Extractions were performed three times, each time using 1 mL of chloroform.

After adding 5 mL of wash solution to the second funnel, the chloroform extracts were mixed, and the mixture was agitated for 15 seconds. The chloroform from the organic layer was removed and placed in a volumetric flask measuring 10 mL. Two separate extractions, each involving 1 mL of chloroform, were performed. Finally, all the extracts were combined, and 10 mL of chloroform was used to dilute them down to the desired volume.

Kinetic studies

Kinetic parameters can be derived from batch testing by using the profile of several biomass growth rates. Using a plot of dry weight of biomass per liter versus bacterial count, the dry weight of the bacteria was calculated (CFU/mL). In x (bacterial dry weight) against time can be plotted to obtain the specific growth rate coefficient (μ) at each of the starting diesel concentrations. If these data points were plotted against substrate concentration, a nonlinear curve would result. When trying to predict expansion in the presence of substrate inhibition, the Haldane model is preferred over the traditional Monod model. Here are the two models of inhibition:

$$\mu_{\max} \frac{S}{K_s + S} \qquad [19]$$

$$\mu_{\max} \frac{S}{S + K_s + \frac{S^2}{K_i}} \qquad [20]$$

where, μ, $\mu_{\max}$, S, K_s and K_i, are the specific growth rate (h^{-1}), maximum specific growth rate (h^{-1}), substrate concentration (% (v/v) or mg/L), half-saturation constant (mg/L), inhibition constant (mg/L), respectively. The constants were calculated by fitting data to the Michaelis-Menten kinetics of substrate inhibition in GraphPad Prism, and then replacing the kinetic constants with those for SDS degradation.

Statistical analysis

Comparison between groups was performed using a one-way analysis of variance with post hoc analysis by Tukey's test or the Student's t-test [21]. Values are taken as means ± SE for three replicates. $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

One of the ingredients in laundry detergent is sodium dodecyl sulfate (SDS) [22]. It finds extensive use in a variety of commercial and domestic settings [23]. Whenever SDS-laden effluent from factories and homes is released into a waterway, it causes pollution. Surfactants in wastewater have been treated with a variety of methods, some of which make use of microorganisms capable of degrading surfactants [24]. Bacteria's ability to break down SDS was first documented in a 2010 study [10].

Optimization of temperature

Bioremediation could benefit greatly from an understanding of the optimal temperature at which bacteria can grow on xenobiotics. For the purposes of a bioaugmentation experiment, where bacterial growth on a massive scale can be accomplished in controlled, optimal conditions, this is of crucial importance. It was investigated how temperatures between 20 and 50 °C affected the efficiency with which a bacterial consortium degraded SDS. Maximum growth of the bacterial consortium on SDS was seen between 30 and 35 °C, with no statistically significant difference ($p>0.05$) between the two temperatures. Over 40 °C, growth slowed dramatically, and above 50 °C, it was virtually nonexistent (**Fig. 1**). Similar to the results of this study [14,25-35], mesophilic degraders often grow best at temperatures between 25 and 35 °C when degrading or growing SDS.

Here, we look at whether or not a bacterial consortium can break down SDS. Bacteria capable of decomposing SDS have been documented in the literature, and there is a wide range of species. includes *Acinetobacter calcoaceticus* and *Pantoea agglomerans* [36], *Pseudomonas betelli* and *Acinetobacter johnsoni* [37], *Klebsiella oxytoca* [38] as well as *Burkholderia* sp., and *Serratia odorifera* [39,40] and many more [14,25–35]. SDS can be degraded by a psychrotolerant bacterium even at temperatures below 10 degrees Celsius [41].

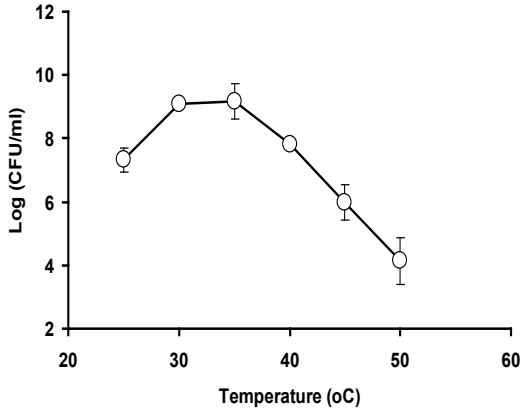

Fig. 1. The effect of temperature on the growth of bacterial consortium. Data is mean ± standard error (n=3).

Optimization of pH

Due to the significant impact of pH on bacterial growth, keeping the medium at a constant pH is essential. Understanding the optimal pH for bacterial growth is a crucial step in developing a successful bioremediation strategy [42]. According to our data, the optimal pH range for bacterial consortium growth was between 6.5 and 7.5 (**Fig. 2**). Similar to the results of this investigation [14,25-35], the literature suggests that neutrophilic degraders prefer a pH range of 6 to 8.0 for SDS degradation or growth. At a pH of 9.5, the growth of the bacterial consortium drastically slowed, likely because of the extremely alkaline circumstances.

Bacteria can survive in a wide pH range because they can adjust the pH of their cytoplasm [43]. However, alterations in the electrical structure of the active site caused by excessively acidic or alkaline circumstances ultimately impede substrate binding. The result is less effort put forward [43]. There are two reasons why it's crucial to learn more about the ideal pH. The first is to cultivate large quantities of the bacteria for use in bioaugmentation experiments, and the second is to determine whether or not the soil pH at polluted areas has to be adjusted to facilitate optimal growth or breakdown of the bacteria.

The effects of nitrogen source on growth

The availability of a nitrogen source is an important factor that affects microbial development. Thus, determining the most effective nitrogen source and the optimal growth concentration for that source could significantly aid in developing a successful bioremediation strategy [44].

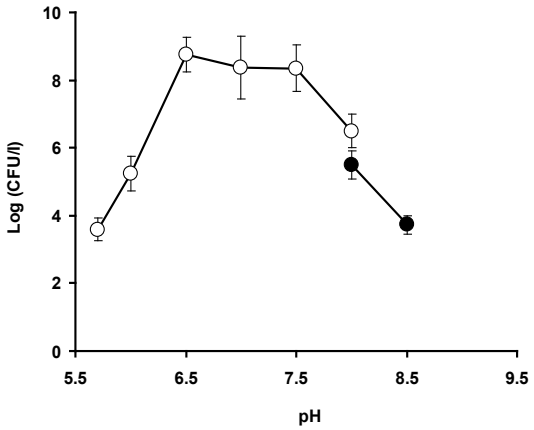

Fig. 2. The effect of pH on the growth of bacterial consortium using an overlapping buffer system consisting of phosphate (○) and carbonate (●). Data is mean ± standard error (n=3).

Bacterial growth was examined by including 0.1 percent (w/v) of various nitrogen sources, including ammonium sulphate, ammonium chloride, potassium nitrite, and potassium nitrate, in BS media supplemented with SDS as the only carbon source. With only ammonium sulfate as a nitrogen supply, we found the bacterial consortium growth rate to be maximum (<p0.05) (**Fig. 3**). 2 g/L of ammonium sulfate was maximum. Nearly all SDS-degraders, including many mesophilic degraders [14,25-35], require a simple nitrogen source like ammonium sulfate to maintain development on SDS.

Dhouib et al. and Shukor et al. [13,38] reported that ammonium sulphate as the best nitrogen source. *Comamonas terrigena* strain N3H demonstrated optimum growth at a higher 5.4 g/L ammonium nitrate [11], while another surfactant degrader *Citrobacter braakii* required and even higher concentration at 7.7 g/L ammonium sulphate for optimum growth on SDS[13].

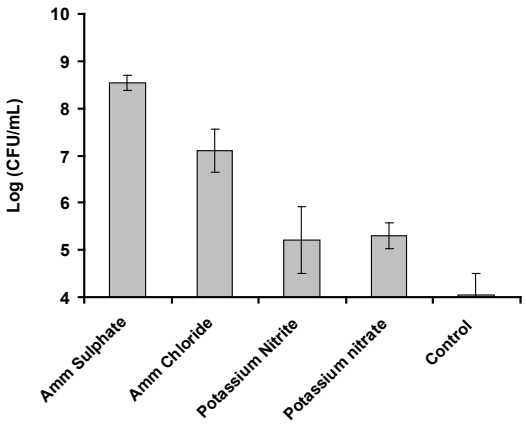

Fig. 3. The impact that a number of different nitrogen sources have on the growthof the bacterial consortium. Data is mean ± standard error (n=3).

Concentrations of sodium dodecyl sulfate and their effects on growth

As carbon is the essential structural unit of all organic molecules, large quantities of sodium dodecyl sulfate are required as the only source of carbon. SDS may also kill the bacteria by removing the outer layer of lipopolysaccharide, a process that is particularly effective against Gram-negative bacteria [1,45].

We demonstrated that a bacterial consortium could use SDS as the only carbon source in their metabolism. The greatest growth rate of the bacterial consortium was recorded at a concentration of between 1 and 1.5 g/L of SDS ($p<0.05$). At 2–3 g/L of SDS, the bacterial consortium grew more slowly, and at 5 g/L, growth was severely inhibited (**Fig. 4**). Although certain degraders can handle >1000 mg/L [14,25-35], many SDS-degraders degrade or grow best at SDS concentrations of less than 500 mg/L.

Bacterial consortia's tolerance for SDS growth falls within the generally accepted SDS concentration range. The critical micelle concentration (CMC) of SDS is 2.34 g/L, which coincides with the limit of maximum degradation capacity by bacteria. Bacterial consortium data demonstrates that after 8 days, at an acceptable dose of 2 g/L, roughly 90% of SDS is digested and cellular growth has reached equilibrium. It took around three days for the bacterial growth to begin to increase in tandem with a decrease in SDS content, suggesting that the bacteria had adapted to a new carbon source. They can decompose 0.5 to 1 g/L SDS in 4 days at 10 °C [41], as reported by Margesin and Schinner. Since *Klebsiella oxytoca* strain DRY14, which was found in a detergent-polluted tropical environment, degrades 2 g/L SDS without a lag phase. When exposed to a detergent such as SDS, it is possible to deduce that the genes that are accountable for the breakdown of detergents are rapidly expressed [38].

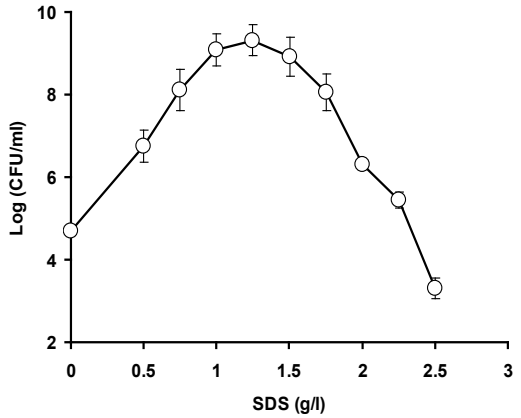

Fig. 4. Concentrations of sodium dodecyl sulfate and their effects on bacterial consortium growth. Data is mean ± standard error (n=3).

Growth on other surfactants

A 1 g/L concentration of other commonly used commercial surfactants were examined for their capacity to sustain the growth of the bacterial consortium. Ethoxylated surfactants, like tergitol, are a kind of surfactant. Fifteen percent of the United States surfactant market [46] was made up of this substance. SDBS is a linear primary alkylbenzene sulfonate that is commonly found in laundry detergent. According to **Table 1**, only the anionic SDBS allowed the bacterial consortium to flourish. As SDBS is a much more resistant substrate than SDS, the ability of the bacterial consortia to break down SDBS together with SDS is an additional benefit. While Tergitol, Witconol, and the cationic detergents benzethonium chloride and benzalkonium chloride have been used as carbon sources, there is very little information on the utilization of SDBS-degrading bacteria [47,48].

Due to the membrane and protein-denaturing features of these strong detergents, many SDS degraders reported in the literature are able to grow on simple linear nonaromatic detergents like SDS while complex detergents including SDBS are either weakly degraded or are strongly hindered [14,25-35].

Table 1. Growth of bacterial consortium on detergents.

Detergent	Type	Growth
Tergitol 15S9	nonionic	-
Benzethonium chloride	cationic	-
Tergitol NP9	nonionic	-
Benzalkonium chloride	cationic	-
Witconol 2301 (methyl oleate)	nonionic	-
Sodium dodecylbenzene sulfonate	anionic	+

SDS-degradation at different initial concentrations

A series of tests were performed on a variety of SDS concentrations to further evaluate the bacterial consortium's degradation capabilities. We demonstrated that 2 g/L of SDS fully inhibited the development of a bacterial consortia (**Fig. 5**). Almost complete degradations of SDS were observed in 3, 5 and 6 days at 0.5, 0.75 and 1 g/L SDS, respectively while higher concentrations showed partial degradation with no degradation was observed at 2.5 g/L SDS after 6 days of incubation. In *Serratia marcescens* strain DRY6, after 6 days, 8 days, and 10 days incubation, SDS concentrations of 0.5, 0.75, and 1 g/L were found to have almost completely degraded the substance, while concentrations of 2.5 g/L and higher showed only partial degradation, with no degradation observed after 10 days [1].

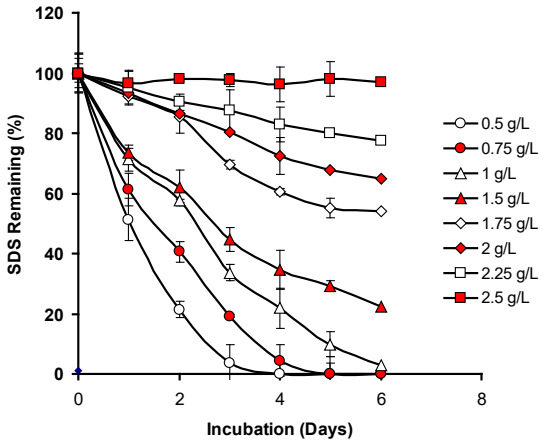

Fig. 5. SDS degradation by the bacterial consortium at various initial concentrations. Data is mean ± standard error (n=3).

Growth kinetics studies

Two growth kinetic models (the Monod and Haldane models) were fitted in CurveExpert Professional (Version 1.6) using a customized equations approach that minimizes sums of squares of residuals. When comparing the Haldane and Monod models, a value of 0.99 for the correlation coefficient between the two

suggests that the Haldane model closely matches the data, while a value of 0.34 shows that the Monod model does not (**Fig. 6**). It was shown that the specific growth rate increased with increasing substrate concentration, but peaked, then gradually decreased, betraying substrate inhibition. In this study, the maximum growth rate, or μ_{max}, K_s, and K_i were 0.517 h^{-1} (95% confidence interval of C.I. from 0.404 to 0.629), 0.132 (g/L) (95% C.I. from 0.073 to 0.191) and 0.909 (g/L) (95% C.I. from 0.544to 1.273), respectively.

In previous work, the maximum growth rate, or μ_{max}, was determined to be 0.13 h^{-1}, and the saturation constant, K_s, was found to be 0.707 g/L SDS. K_i was 11.303 g/L SDS, which is the amount of SDS needed to impede the growth of *Serratia marcescens* strain DRY6 [1]. For example, inhibitory kinetics modelling [14,25-35] is typically overlooked in studies of SDS breakdown or bacterial growth. In the literature, kinetic data on SDS-degradation and -utilization are scarce. Khleifat et al. conclude that Andrew is the best substrate-inhibiting model, with maximum rates (μ_{max}), substrate affinities (K_s), and inhibitory concentrations (K_i) of 0.26 h^{-1}, 0.6 g/L, and 1.5 g/L, respectively [40].

In comparison to values reported for a co-culture combination of numerous SDS-degrading bacteria, including *Burkholderia* sp., *Acinetobacter calcoaceticus, Klebsiella oxytoca,* and *Serratia odorifera*, which range from 0.21 to 0.26 h^{-1} [39,40], the μ_{max} value achieved in this work was much lower. The Haldane model is a strong three-parameter growth rate inhibition kinetics model that has been similarly reported as the best model in the SDS-degraders *Pseudomonas medocina* and *Bacillus consortium* [7].

Microorganisms can either utilise surfactants as a source of energy and nutrients via a process called direct metabolism, or they can co-metabolize the surfactants through a process called secondary metabolism. Both of these processes are involved in biodegradation. As a general rule, the biodegradation efficiency of bacterial strains is superior to that of the isolated strain; this is something that has been demonstrated in a number of experiments that have been published in the past. They stand out due to their capacity to disassemble convoluted chemical compounds, which has led to their identification. Investigating the dynamics of pollutant biodegradation in wastewater has the potential to improve both the process control and the removal effectiveness of contaminants in wastewater treatment plants [11].

These models predict how long it will take for a specific concentration of contaminants to be reached, as well as the necessary time to reduce the chemical concentration to the designed values, the prediction of chemicals that remain at a certain time, the design of ex-situ or in-situ bio-remediation systems to remove toxic contaminant to a desired concentration, and so on. They also estimate the amount of time it will take for a certain concentration of contaminants to be reached. It is feasible to utilize it to estimate the quantity of biocatalyst manufacturing that is achievable at any given moment, as well as provide vital information for research and the prediction of microbial performance. Understanding how microorganisms behave under these circumstances is essential for applying bio-kinetic models to system design and optimization. Wastewater treatment often comes up against substrate restrictions [11,38]. .

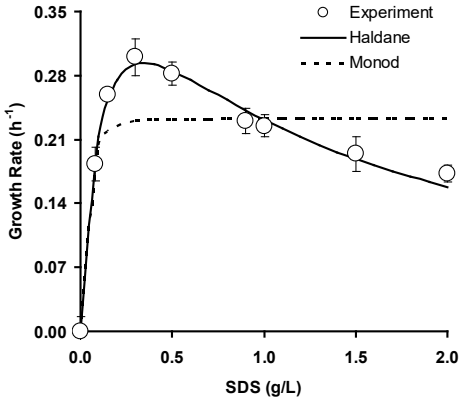

Fig. 6. Growth kinetics of bacterial consortium on SDS. Data represent mean ± SEM (n=3).

Growth of bacterial consortium on heavy metals

Bacterial consortium growth was examined in the presence of zinc (Zn), silver (Ag), nickel (Ni), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), and mercury to ascertain the consortium's potential to utilize these metals (Hg). We demonstrated that a bacterial consortium was strongly inhibited by mercury, copper and chromium (**Fig. 7**). The problem of heavy metals hindering biodegradation can be addressed in a number of ways. Calcium carbonate, manganese oxide, cement, phosphate, and magnesium hydroxide are all examples of treatment additives that can reduce the bioavailability and mobility of metals, making it simpler to clean up metal pollution. Minerals contained in clay can also be used since they have been shown to lessen metals' environmental toxicity and bioavailability [25-27].

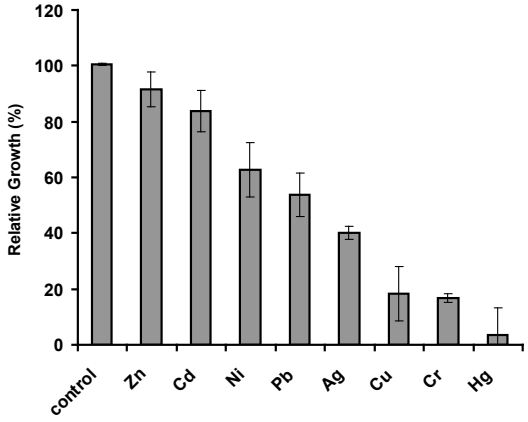

Fig. 7. The effect of various heavy metals on the growth of bacterial consortium. Data is mean ± standard error (n=3).

It has been observed that microbes can thrive on heavy metals when provided with readily assimilable substrates. Heavy metals including Cd, Zn, and Pb can be toxic to some organisms, although Pseudomonas putida has been shown to be resistant [49,50]. *Bacillus thuringeinsis* has been demonstrated to be highly sensitive to Cd and Zn [51], while *Paenibacillus* sp. has been proven to be highly sensitive to Cu. Yet there has been a lack of research on the influence of heavy metals on SDS degradation and of heavy metal-tolerant SDS-degrading bacteria. Therefore, this work provides new information that can be compared to future isolates of SDS-degrading bacteria.

CONCLUSION

During biodegradation, microorganisms can directly metabolize surfactants for energy and nutrients or co-metabolize them with other compounds. Maximum growth of the bacterial consortium on SDS indicates that the bacterial consortium exhibits mesophilic properties while the optimal pH range for bacterial consortium growth was neutral pH. As for nitrogen sources, ammonium sulfate was optimum in supporting the growth of SDS. The greatest growth rate of the bacterial consortium was recorded at a concentration of between 1 and 1.5 g/L of SDS. In this study, the maximum growth rate, or μ_{max}, the saturation constant or K_s, and the inhibition constant or K_i were comparable to several established SDS-degraders. Heavy metals like mercury, copper, and chromium can severely stunt growth if they are present in the environment. It was discovered through research into growth kinetics that Haldane substrate inhibition kinetics may be used to model the growth rate. This bacterial consortium has the right properties for the bioremediation of SDS-polluted environments.

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