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SDS-degrading Bacterium Isolated from a Paddy Field

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ABSTRACT

A bacterium capable of degrading sodium dodecyl sulphate (SDS) isolated from a paddy field water is characterized. In this report, we showed that almost complete degradation of SDS was observed in 6 to 10 days when the bacterium was grown on medium supplemented with SDS ranging from 0.75 to 1.75 g/L while higher concentrations showed partial degradation with no degradation was observed at concentrations higher than 2.0 g/L. The SDS-degrading bacterium was partially identified and provisionally named *Pseudomonas* sp. strain Maninjau1. We also showed that the presence of metal ions such as silver, copper, cadmium, chromium, lead and mercury inhibit the ability of the bacterium to degrade SDS by 50%. Growth kinetic studies show a correlation coefficient value of 0.99 for the Haldane model indicates it fits the curve while a low correlation coefficient value of 0.67 for the Monod model indicates poor fitting. The specific growth rate μ was discovered to rise as the substrate concentration was increased but it reached a peak value followed by a slow decrease indicating substrate inhibition. The calculated q_{max} or maximum degradation rate was 0.917 h^{-1} (95% confidence interval or C.I. from 0.664 to 1.171) while the saturation constant K_s or half velocity constant was 0.178 g/L SDS (95% C.I. from 0.089 to 0.266). The inhibition constant K_i was 0.605 g/L SDS (95% C.I. from 0.358 to 0.941). The very high maximum degradation rate obtained in this study indicates that this bacterium can be an efficient agent for bioremediation of SDS especially in soils.

INTRODUCTION

The diverse group of compounds known as surfactants, which also includes detergents, are designed to have the ability to either dissolve or clean everything they come into contact with. It is estimated that around 7.2 million tons of synthetic surfactant are produced each year across the globe. They typically consist of a polar head group, which may or may not be charged, and a nonpolar hydrocarbon tail, which may or may not be simply solvated in water. Additionally, they may or may not be well dissolved in water. As a result, surfactants are molecules that combine both hydrophilic and hydrophobic characteristics in a single molecule. As a result of their high capacity for producing foam, which, in addition to having a direct toxic effect on a wide variety of organisms in the environment, is one of the primary reasons why they are considered to be among the most serious contaminants, they can cause many problems in treatment plants. The mining industry, the textile industry, the food industry, the oil industry, the field of soil remediation, the field of water treatment, and excavation all employ surfactants. Despite the fact that there are a number of different types of surfactants that are frequently used in commercial detergent applications. In most cases, a concentration of nonionic and anionic surfactants higher than 0.1 mg/L is required for the occurrence of chronic deadliness. It is a well-known fact that detergents have negative effects on marine life [1–3]. According to the findings of previous studies, anionic surfactants pose a risk to the health of a wide variety of aquatic creatures at concentrations ranging from 0.0025 to 300 mg/L [4]. It had an effect on the aquatic species' reproductive cycles as well as their behavioral patterns [5]. According to the findings of another study, the digestive gland of oysters is sensitive to the effects of exposure to SDS, which results in a disruption of the oyster's dietary and metabolic processes, which in turn results in a reduced likelihood that the oyster will survive [6]. As more anionic surfactants are discharged into bodies of water, the pollution generated by these

chemicals will lead to an increase in the number of invertebrate and crustacean species that are adversely affected by their toxicity.

The concentration of detergents in home wastewater can range anywhere from 3 to 21 mg/L, whereas the concentration of detergents in certain industrial effluent can reach up to 10,000 mg/L. The high concentration of surfactants in the wastewater from a washing facility makes treatment extremely challenging. In the production of laundry detergents, the anionic surfactants and builders that are utilized the most frequently are sodium dodecylbenzene sulfonate (SDS) and sodium aluminum silicates, respectively. According to the reports, the content of detergents in wastewater originating from laundry ranged from 17 mg/L all the way up to 1024 mg/L [7].

As a consequence of this, the remediation of SDS is of the utmost significance. Microorganisms are famous for their capacity to decompose organic substances, including SDS, and this trait is well documented [8,9], In addition, their application as bioremediation agents is financially necessary for the removal of xenobiotic pollutants. One of the early reports of SDS-degrading bacteria was the biodegradation of anionic surfactant under aerobic circumstances by the bacterium *Pseudomonas* sp. strain C12B [10]. Since the publication of this work, a significant number of bacteria that degrade SDS have been found [11–21]. Rarely are studies conducted on numerous xenobiotics-degrading or -remediating microorganisms, and since polluted areas frequently contain a wide variety of organic and inorganic contaminants, these studies are especially important [22], isolation of such unique microorganisms is important. In this work we report on the characterization of a surfactant-degrading bacterium isolated from a paddy field from soils of the Maninjau Lake in West Sumatra, Indonesia.

MATERIALS AND METHODS

Isolation and maintenance of SDS-degrading bacterium

A paddy field's water sample was taken in 2017 from near the Maninjau Lake West Sumatra, Indonesia. The basal salts (BS) enrichment medium (g/L) 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 pH was set at 7.0. Filter-sterilized sodium dodecyl sulphate was added into the medium as a carbon source at the final concentration of 1.0 g/L [23]. For maintenance of the pure culture of the bacterium was maintained on slant nutrient agar plate supplemented with SDS and incubated at 30 °C for up to 6 days and can be stored for a maximum of two months in the refrigerator. For longer maintenance, an 80% glycerol stock is stored in liquid nitrogen. Measurement of the bacterial growth was carried out using the colony count method (CFU/mL) with suitable dilutions in autoclaved tap water.

Partial identification of the bacterium

The strain was biochemically and phenotypically characterized according to the Bergey's Manual of Determinative Bacteriology [24]. Interpretation of the results was carried out via the ABIS online system [25].

Methylene blue active substance assay (MBAS)

The determination of SDS residuals was based on the measurement of the color of methylene blue using the chloroform extraction procedure, with the blue solution being measured at 652 nm against the chloroform blank. Briefly, 0.1 mL of separating funnels were filled with 9.9 mL of deionized water and 0.1 mL of samples, and then the mixture was stirred. After that, 2.5 mL of methylene blue solution and 1 mL of chloroform were both added to the mixture, respectively. To begin the extraction process, the funnel was violently agitated for a period of 15 seconds. After allowing the mixture to sit for twenty minutes, it will separate into two layers.

The chloroform layer was transferred to a different funnel. The extraction procedure was carried out a total of three times, with 1 mL of chloroform being used for each extraction. After that, the chloroform extracts were mixed together in the second funnel, and then the second funnel was rapidly shaken for 15 seconds after 5 mL of wash solution was added to it. The organic chloroform layer was separated out into a volumetric flask that held 10 mL. The extraction procedure was carried out twice more with a total volume of 1 mL of chloroform. In the end, all of the extracts were combined, and the volume was brought down to 10 mL by adding more chloroform [26]

Kinetic studies

The inhibitory effect of substrate to the growth rate (μ) or the degradation rate (q) can be utilized in obtaining kinetic parameters from batch works. In this study, the degradation rate was studied. The specific degradation rate coefficient (q) can be found at each of the initial diesel concentration by plotting In x (bacterial dry weight) vs. time. A nonlinear curve will be obtained when these values were plotted against substrate concentration. As opposed to the classical Monod [27] model (Eqn. 1) the Haldane [28] model (Eqn. 2) is the model of choice in modelling growth under substrate inhibition conditions. The inhibition models are as follows;

$$q = q_{max}\frac{S}{K_S+S} \qquad \textbf{(Eqn. 1)}$$

$$q = q\frac{S}{K_S+S+\frac{S^2}{K_i}} \qquad \textbf{(Eqn. 2)}$$

where, q, q_{max}, S, K_s and K_i, are the specific degradation rate (h^{-1}), maximum specific degradation rate (h^{-1}), substrate concentration (% (v/v) g/L or mg/L), half-saturation constant (g/L), inhibition constant (g/L), respectively. The constants were obtained by running data on GraphPad Prism using the Michaelis-Menten substrate inhibition kinetics and replacing the resultant enzyme kinetic constants with SDS degradation kinetics constants.

Statistical analysis

In order to conduct an analysis of the findings, statistical software Graphpad Prism version 3.0 was utilized. The values are shown as the mean standard error for three separate trials. A one-way analysis of variance followed by a post hoc analysis using either Tukey's test or the Student's t-test was utilized in order to compare the results obtained from the various study groups [29]. $P < 0.05$ is statistically significant.

RESULTS AND DISCUSSION

Partial identification of the bacterium

The bacterium had the shape of a short rod and was Gram-negative. It also had the ability to move or motile. Comparing the outcomes of culture, morphological, and a number of other biochemical tests allowed for the bacterium's identification (**Table 1**). *Pseudomonas putida* was identified as the bacterium with the highest level of homology (81 percent), and the level of accuracy was 85 percent. The ABIS online software software supplied three possibilities for the bacterial identity. To further

identify this species, however, additional efforts in the future, particularly molecular identification techniques based on comparisons of the 16srRNA gene, are required. At this point in time, however, the bacterium is just being provisionally identified as *Pseudomonas* sp. strain Maninjau1.

Table 1. Biochemical tests for the bacterium.

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	+
Indole production	–	Mannose	+
Nitrates reduction	–	Mannitol	–
Lecithinase	–	Sorbitol	–
Lysine decarboxylase (LDC)	–	Sucrose	+
Ornithine decarboxylase (ODC)	–	Trehalose	–
ONPG (beta-galactosidase)	–	Xylose	–
Esculin hydrolysis	–		
Gelatin hydrolysis	–		
Starch hydrolysis	–		
Urea hydrolysis	+		
Oxidase reaction	+		

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

One of the ingredients that goes into making detergent is called sodium dodecyl sulphate, or SDS for short [30]. It finds widespread application in both commercial and domestic settings [31]. Among the first work describing the ability of bacteria to degrade SDS was reported by [10]. *Pseudomonas* spp are among the dominant SDS-degrading bacteria reported in the literature [13,15,16,19,20,32–34]

Optimization of temperature

For the objectives of bioremediation, the investigation of the temperature at which bacteria thrive on xenobiotics at their optimal rate would be highly valuable. This is especially significant when it comes to the bioaugmentation experiment since it allows for the cultivation of huge quantities of bacteria in controlled environments that are optimized for their growth. The influence that temperature has on the amount of SDS that bacteria can degrade per unit of time was investigated at temperatures ranging from 20 to 50 °C.

It was determined that the highest growth rate of bacteria on SDS occurred between 25 and 30 °C, and there was not a significant difference ($p>0.05$) detected between the two temperatures for growth on SDS at either temperature. When the incubation temperature was increased above 40 °C, there was a significant slowdown in growth, and at 50 °C, almost no growth was seen.(**Fig. 1**). The optimum temperature for SDS degradation or growth in the literature ranges from 25 to 35 ºC similar to the results in this study [13,16–20,35–40], which are often reflected by mesophilic degraders.

In this work, we evaluate whether or not bacteria have the potential to break down SDS. The diversity of bacteria that can degrade SDS that has been documented in the scientific literature includes *Acinetobacter calcoaceticus* and *Pantoea agglomerans* [41], *Pseudomonas betelli* and *Acinetobacter johnsoni* [42], *Klebsiella oxytoca* [43] as well as *Burkholderia* sp., and *Serratia odorifera* [44,45] and many more [13,16–20,35–40]. In contrast, psychrotolerant SDS-degrading bacteria can carry out degradation at much lower temperatures (less than 10 ˚C) [46].

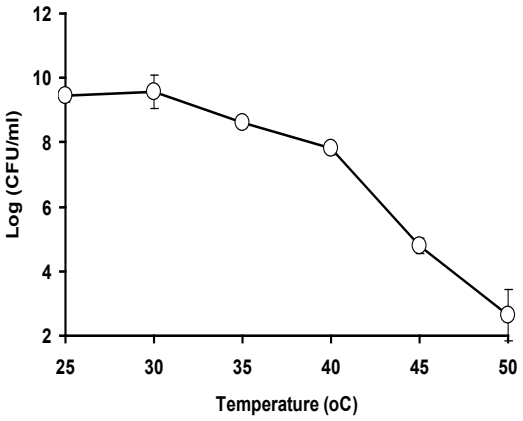

Fig. 1. The effect of temperature on the growth of bacterium on 1 g/L SDS. Data is mean ± standard error (n=3).

Optimization of pH

The pH of the medium is extremely important to consider, as it has a significant impact on the development of bacteria. Once the optimal pH for bacterial growth has been determined, using that information to build an efficient bioremediation plan can be of great assistance [47]. Our results showed that bacterium 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 [13,16–20,35–40], which are often reflected by neutrophilic degraders. At a pH of 9.5, the growth of the bacterium drastically slowed down, most likely as a result of the extremely alkaline environment.

Because bacteria are able to control the pH level inside their cytoplasm, they can survive a certain range of pH levels [48]. However, conditions that are excessively acidic or alkaline have the potential to alter the state of ionization of an enzyme's active site, which in turn might cause changes in the electronic configuration of the active site and, in the end, prohibit substrate binding. This translates to a reduction in overall activity [48]. The investigation of the ideal pH is significant for two different reasons. The first objective is to produce large quantities of the bacterium as part of a bioaugmentation exercise. The second objective is to determine whether or not the pH of the soil at polluted sites needs to be adjusted in order to match the optimal conditions for the growth or degradation of the bacterium.

The effects of nitrogen source on growth

A microorganism's rate of growth can be affected by a number of factors, one of which is its nitrogen source. Therefore, determining the most effective source of nitrogen and the optimal concentration of that source for growth could be of considerable assistance in the development of a successful bioremediation strategy [49].

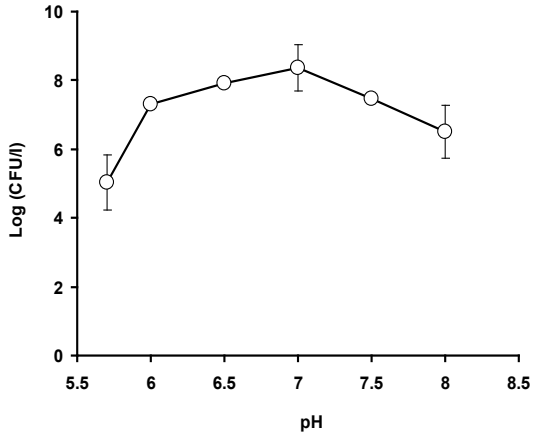

Fig. 2. The effect of pH on the growth of bacterium using an overlapping buffer system consisting of phosphate (○) and carbonate (●). Data is mean ± standard error (n=3).

In order to investigate the effects that various nitrogen sources have on the growth of bacteria, BS media with SDS as the only carbon source was supplemented with 0.1 percent (w/v) of various nitrogen sources. These nitrogen sources included ammonium sulphate, ammonium chloride, potassium nitrite, and potassium nitrate. According to the findings that we obtained; the growth rate of the bacterium was the highest when ammonium sulphate was the only source of nitrogen ($p<0.05$) (**Fig. 3**). The optimal concentration of ammonium sulphate was at 2 g/L. Nearly all SDS-degraders require a simple nitrogen source such as ammonium sulphate to support growth on SDS [13,16–20,35–40], which are often reflected by mesophilic degraders. The use of ammonium sulphate as a nitrogen source is consistent with previous reports [23,43] as this form of nitrogen source is the most easily assimilable form of nitrogen source. Other surfactant degraders like *Citrobacter braakii* required 7.7 g/L ammonium sulphate [23] whereas *Comamonas terrigena* strain N3H showed an optimum growth at 5.4 g/L ammonium nitrate [50].

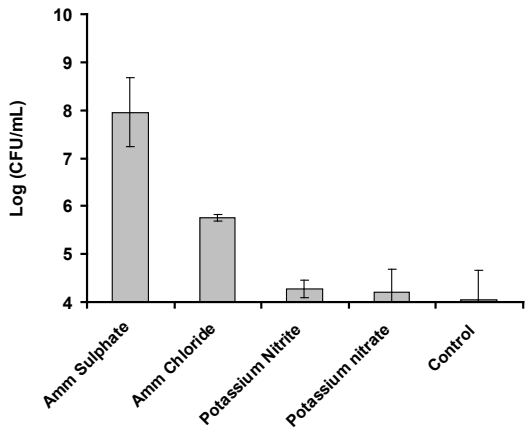

Fig. 3. The influence of different types of nitrogen sources has on the growth of bacterial colonies on 1 g/L SDS. Data is mean (standard error (n=3).

The effects of sodium dodecyl sulphate concentrations on growth

Sodium dodecyl sulphate as being the lone supply of carbon 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 [1,51]. We showed that bacterium was able to utilize SDS as a sole carbon source. We observed the growth rate of bacterium on a series of different concentration of SDS and the highest growth rate was recorded at the concentrations between 0.75-1.75 g/L ($p<0.05$). bacterium exhibited lower growth rate at SDS concentrations higher than 2.0 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 [13,16–20,35–40], which are often reflected by mesophilic degraders.

The ability of bacterium 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). bacterium 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 [46]. 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 [43]. Many SDS degraders reported in the literature are able to grow on simple linear nonaromatic detergent such as SDS while complex detergents including SDBS are either poorly degraded or are strongly inhibited [13,16–20,35–40], due to the membrane and protein-denaturing properties of these strong detergents, which was also observed in this study (data not shown).

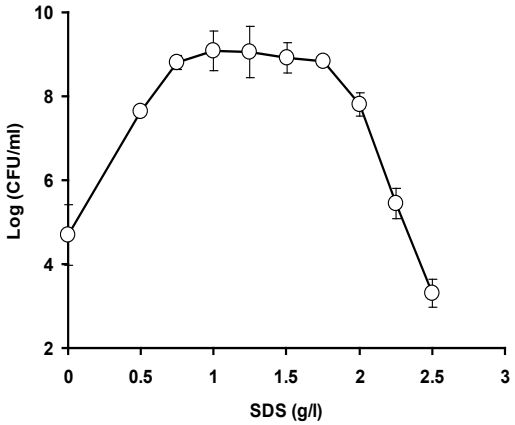

Fig. 4. The influence that different amounts of sodium dodecyl sulfate have on the growth of bacterial colonies. Data is mean (standard error (n=3).

SDS-degradation at different initial concentrations

The capacity of bacteria to digest SDS was investigated further by testing it on a number of SDS concentrations of varying strengths. At a concentration of 2 grams of SDS per liter, we found that bacterial growth was entirely stopped (**Fig. 5**). After four, five, and six days of incubation, respectively, at a concentration of 0.5, 0.75, and 1 g/L of SDS, almost complete

degradation of SDS was observed. However, higher concentrations showed only partial degradation, and after ten days of incubation, no degradation was observed at a concentration of 2.5 g/L SDS.

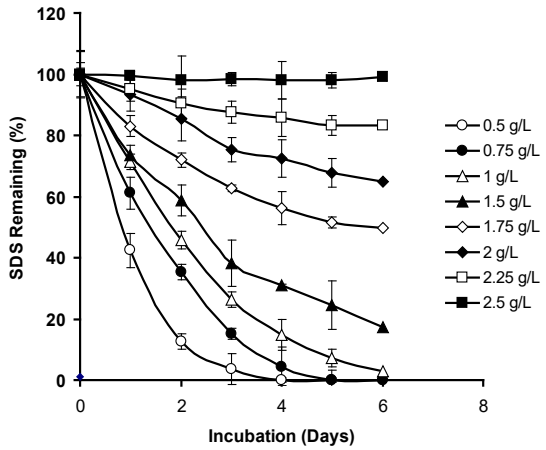

Fig. 5. The influence that different concentration of sodium dodecyl sulfate have on the degradation rate by the bacterium. Data is mean (standard error (n=3).

Growth kinetics studies

A unique equations approach that minimizes sums square of residuals was used to fit the Monod and Haldane growth kinetic models in CurveExpert Professional (Version 1.6). In contrast to the Monod model, which has a low correlation coefficient value of 0.67, the Haldane model matches the curve quite well (0.99) (**Fig. 6**). It was shown that the specific degradation rate q increased with substrate concentration up to a peak value, after which it gradually decreased, betraying substrate inhibition. The calculated q_{max} or maximum degradation rate was 0.917 h^{-1} (95% confidence interval or C.I. from 0.664 to 1.171) while the saturation constant K_s or half velocity constant was 0.178 g/L SDS (95% C.I. from 0.089 to 0.266). The inhibition constant K_i was 0.605 g/L SDS (95% C.I. from 0.358 to 0.941).

Studies of bacterial SDS degradation or growth commonly overlook inhibitory kinetics models [13,16–20,35–40].In the literature, kinetic data on SDS-degradation and -utilization are few. Some substrate-inhibiting models focused on development rather than decay, such as Andrew and Tessier's; Khleifat et al. [45] demonstrated that Andrew's model was the most accurate.(2010) giving μmax, K_s and K_i, values of 0.26 h^{-1}, 0.6 g/L and 1.5 g/L, respectively. The q_{max} value obtained in this work is lower than values reported for a co-culture mixture of several SDS-degrading bacteria such as *Burkholderia* sp., *Acinetobacter calcoaceticus*, *Klebsiella oxytoca* and *Serratia odorifera* that ranges between 0.21 and 0.26 h^{-1} [44,45] although growth and degradation rate cannot be compared. The Haldane model is a robust and three-parameter growth rate inhibition kinetics model and has also similarly been reported as the best model in the SDS-degraders [40] with μ_{max}, K_s and K_i values of 0.13 h^{-1}, 0.707 g/L and 11.303 g/L SDS and *Pseudomonas medocina and Bacillus consortium* [7] with μ_{max}, K_s and K_i values of 1.42× 10^{-6} s^{-1}, 42 mg/L, and 160 mg/L, respectively.

During biodegradation, microorganisms can use surfactants as substrates for energy and nutrients, or they can co-metabolize the surfactants through microbial metabolic processes. Multiple reports have shown that mixed bacterial populations are more effective in biodegradation than single colonies. This group has been singled out because of their capacity to break down complex chemical molecules. Investigating the kinetics of pollutant biodegradation in wastewaters has the potential to enhance the efficacy of process management and contaminant removal in these facilities.

These models estimate how long it will take for a certain concentration of contaminants to be reached, as well as the necessary time to decrease chemical concentration to the designed values, the prediction of chemicals that remain at a certain time, the design of bio-remediation systems ex-situ or in-situ to remove toxic contaminant to a desired concentration, and the estimate of how long it will take to remove toxic contaminant to a desired concentration. It also provides useful data for analyzing and predicting the performance of microorganisms and may be used to estimate the amount of biocatalyst synthesis that is possible at any given moment. Wastewater treatment frequently encounters substrate constraint, and understanding how microorganisms behave under these limiting conditions is crucial for applying bio-kinetic models to system design and optimization. The substrate inhibition kinetics models used in this research provide a crucial first approximation of performance in real-world settings.

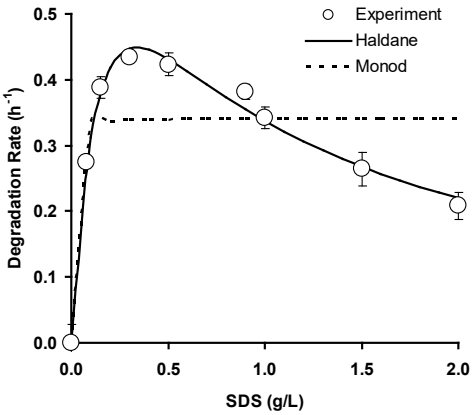

Fig. 6. Growth kinetics of bacterium on SDS. Data represents mean ± SEM (n=3).

Growth of bacterium on heavy metals

To determine the potential ability of bacterium to utilize heavy metals, we tested the growth of bacterium on various heavy metals including zinc (Zn), silver (Ag), nickel (Ni), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and mercury (Hg). We showed that bacterium has a high growth rate on Zn and Ni (**Fig. 7**). Growth on silver and chromium was reduced by 70% while there was 80% growth reduction observed on Cd, Cu and Pb. Growth on Hg was severely inhibited.

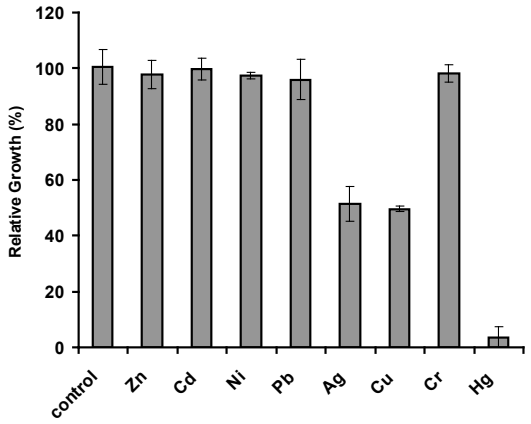

Fig. 7. The effect of various heavy metals on the growth of bacterium. Data is mean ± standard error (n=3).

The ability of microorganisms to grow on heavy metals on easily assimilable substrates has been reported. For instance, *Pseudomonas putida* has been reported to be able to tolerate high concentration of heavy metals such as Cd, Zn and Pb [52,53]. *Paenibacillus* sp. was shown to have high sensitivity against Cu while *Bacillus thuringiensis* has a high sensitivity against Cd and Zn [54]. However, heavy metal tolerant SDS-degrading bacteria or studies on the effect of heavy metals on SDS degradation are limited. Hence, this study offers novel data for comparison on SDS-degrading bacteria isolated in the future.

CONCLUSION

In conclusion, this is the first report of a molybdenum-reducing bacterium having the ability to degrade SDS. The characteristics of the growth on SDS have been explored and show variation in optimal conditions compared to published database. Our attempt to use other detergents as a source of carbon was not successful with the exception of SDBS. The bacterium could completely remove SDS after 10 days at 1 g/L. We also showed that the presence of metal ions such as silver, copper, cadmium, chromium, lead and mercury inhibit the ability of the bacterium to degrade SDS. Growth kinetic studies showed that the growth rate could be modelled using Haldane substrate inhibition kinetics. The characteristics of this bacterium make it suitable for bioremediation of SDS-polluted environment.

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