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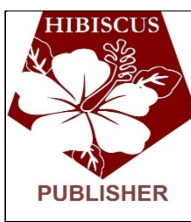

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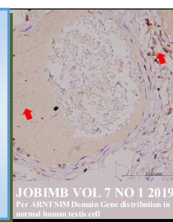

Growth Characterization of *Bacillus amyloliquefaciens* strain KIK-12 on SDS

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ABSTRACT

The pollution caused by sodium dodecyl sulfate (SDS) arise mainly from its utilization as detergent in industrial washing, which results in the high effluent level of this contaminant. SDS as anionic surfactant is ubiquitously toxic to the aquatic ecosystem. In this study, the potentials of a previously isolated molybdenum-reducing *Bacillus amyloliquefaciens* strain KIK-12 to degrade and utilize SDS as sole source of carbon was investigated. The bacterium grew optimally at pH between 6.0 and 7.0, temperature between 30 and 45 °C in 1 g/L SDS as the sole source of carbon, with ammonium sulphate (1 % w/v) as the best nitrogen source. The growth characteristics of strain KIK-12 on various concentrations of SDS (as a carbon source) reveals optimum growth 500 mg/L but was able to tolerate and grow at 1500 mg/L. However, concentrations higher than this results in growth termination. Heavy metals such as mercury, silver and copper significantly inhibit growth of strain KIK-12 on SDS. The ability of this bacterium to tolerate and detoxify multiple toxicants makes it suitable for their bioremediation.

INTRODUCTION

Anionic surfactants are favourite additives and preservatives due to their cost effectiveness for greater industrial purposes [1]. The most common anionic surfactant is sodium dodecyl sulphate (SDS), which is mostly preferred because of its excellent detergency in neutral solutions at low temperature. The sulphate or sulfonated ester groups of anionic surfactants dissociate in aqueous solution generating negatively charged ions originating from xenobiotic substances that are traditionally used in numerous manufacturing processes [2]. The hydrophobic and hydrophilic parts in SDS makes it easier to interact with both polar and non-polar substructures in macromolecules. Consequently, the hydrophilic and hydrophobic groups can cause them to occur around the interfaces in between oil and water or air and water and in addition decreasing the surface tension of the system.

Interestingly, anionic surfactants can bring about positive effects in numerous biological systems and scientific operations by lessening the energy of interaction and the solvation energy of substances [3]. Surfactants are generally categorized into non-ionic, anionic, cationic or amphoteric depending on their overall charge in aqueous solutions. SDS composing of the linear alkyl benzene sulfonates (LASs) is among the major anionic surfactants used in surface cleaners and household detergents that have alkyl chains from C_{10} to C_{14}. In Malaysia for example, SDS is specifically used as an anionic surfactant in commercial products used for cosmetics and personal hygiene [4–9].

Perhaps, large quantities of surfactants are utilized in commercial cleaning agents, which appear in numerous marine ecosystems as a result of inefficient wastewater treatment processes to remove them, coupled with the recalcitrant properties in terms of biodegradability of some of the active surface substances [10–14]. Upon discharged into the water bodies, detergents possess damaging consequences to marine life [15,16]. Concentrations as low as 0.0025 mg/L for sodium dodecyl sulphate (SDS) and sodium dodecyl benzene sulfonate (SDBS) has been shown to be toxic to the aquatic organism, *Daphnia magna* [17,18]. SDS was reported to disrupt cellular membrane integrity by altering with the ionic gradients and membrane potential, which result in bacterial membrane leakage which eventually leads to death. Similarly, SDS also binds to surface protein and enzymes leading to their denaturation [19].

Researches have indicated potential of bacteria to biodegrade SDS, thereby managing its release into the environment [1,9,20,21]. Though, the search for remediating agents for surfactants is ongoing, but bioremediation has currently been considered as eco-friendly than the physicochemical methods. A number of microorganisms with alkylsulfatase enzyme can assimilate SDS and use it for growth [19]. To date, several SDS-degrading bacteria from the genus *Pantoea, Acinetobacter, Pseudomonas, Acinetobacter* and *Klebsiella* have been isolated [7,19,22–28]. However, the current need for bacterium with high tolerance to remediate multiple toxicants due to the ever-increasing number of pollutants is highly sought. In this study, the ability of a previously isolated heavy metal-reducing bacterium to degrade and grow on SDS is reported.

MATERIALS AND METHODS

Maintenance and growth of *Bacillus amyloliquefaciens* strain KIK-12

Previously isolated Mo-reducing bacterium [29] was screened for its ability to grow and degrade SDS on basal salt (BS) medium containing $(NH_4)_2SO_4$ (7.7 g/L), $MgSO_4$ (0.01 g/L), KNO_3 (0.5 g /L), KH_2PO_4 (1.36 g/L), Na_2HPO_4 (1.39 g /L) and $CaCl_2$ (0.01 g/L). The following trace elements were also suplemented into the medium: $ZnSO_4.7H_2O$ (0.01 g l^{-1}), $MnCl_2.4H_2O$ (0.01 g/L), H_3BO_4 (0.01 g/L), $CoCl_2.6H_2O$ (0.01 g/L), $FeSO_4.2H_2O$ (0.01 g/L), $CuCl_2.2H_2O$ (0.01 g/L) and $Na_2MoO_4.2H_2O$ (0.01 g/L) [30]. To conduct the growth characterization, filter-sterilized SDS was added into the medium as a sole source of carbon at the final concentration of 1.0 g/L.

Methylene Blue Active Substances (MBAS) Assay

Methylene blue assay (MBAS) was used to quantify the SDS degradation by this bacterium [31]. Into 5 mL SDS calibration samples and standards, 100 µL of methylene blue reagent with slightly acidic pH of 5 to 6 was added, followed by addition of 200 µL sodium tetraborate solution of pH 10.5. Then chloroform (1 mL) was added and final mixture thoroughly mixed in a separating funnel and allowed to separate by leaving it to stand for 5 min. The blue layer of chloroform was removed and measured spectrophotometrically at 650 nm using a glass cuvette.

Statistical Analysis

One-way analysis of variance was performed using a statistical software INSTAT GraphPad version 3.0.

RESULTS AND DSICUSSION)

The potentials of a bacterium to tolerate and degrade or transform multiple contaminants is intensively sought as most polluted sites are reported to contain mixture of both organic and inorganic pollutants including heavy metals [7,8,32–36]. This work report on SDS-degrading potential of a previously isolated molybdenum-reducing *Bacillus amyloliquefaciens* strain KIK-12. This is the second report on bacterium with this capacity as [21] previously reported similar multi-detoxifier. The characterization work, particularly the effect of various environmental factors such as temperature, pH and heavy metals as well as optimization studies via one-factor-at a time is needed before this bacterium can be used in field bioremediation works. The data obtained will be very useful either in formulating further optimization works using methods such as response surface methods (RSM) and artificial neural network (ANN) or for direct usage in bioremediation works.

Optimization of pH

The effect of pH on SDS degradation was conducted using phosphate buffer spanning the pH range of 5.8 to 7.5, which is within the pKa range for phosphate (**Fig. 1**). pH strongly affects bacterial growth, so also its degradation capacity, which makes it necessary to optimize the pH condition that supports growth and hence the bioremediation potentials. The optimum pH facilitating SDS degradation in this bacterium was 7.0. Most bacteria function well at neutral pH to a slightly alkaline pH range. However, rarely soils in Nigeria are neutral with most being acidic, which require soil additives to ensure neutrality is achieved so that bioremediation of xenobiotics in soils can be carried out efficiently [37–43].

SDS degradation decreases at higher pH with more than 50% growth inhibition occurring at the highest pH tested. Previous studies reveal different optimum pH for different bacteria, though are still within the pH range of 5 to 9. For example, *Citrobacter braakii* [44] and *Enterobacter* sp. strain Neni-13 [30] show optimum pH of 7.0, *Delftia acidovorans* shows optimum pH of 7.2 [28], whereas, *Pantoae agglomerans* require optimum pH of 8.5 [45]. In a most recent finding, *Bacillus cereus* WAW2 and *Staphylococcus aureus* WAW1 grow optimally at pH 7.5 [9].

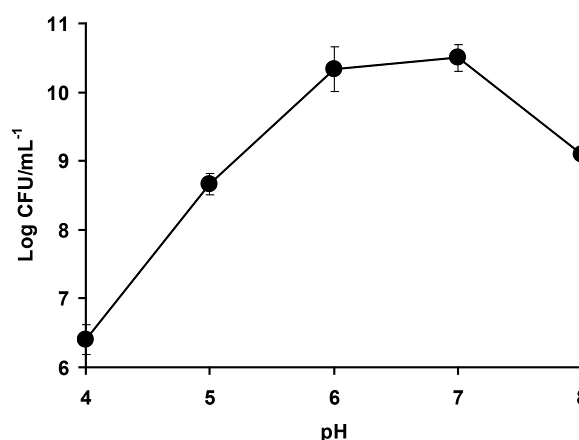

Fig. 1. The effect of pH on SDS degradation by strain KIK-12 at 1 g/L SDS and 1% (w/v) ammonium sulphate. Data represent mean ± SEM of triplicates.

Optimization of temperature

The effect of temperature on growth and SDS (1 g/L) degradation was studied from the temperature range of 20 °C to 45 °C (**Fig. 2**). Temperature is among the factors that influence microbial degradation of xenobiotics, with relatively slow metabolic activity at lower temperature, while higher temperature above the optimum inhibits degradation [46].). In this bacterium, growth was found to be optimal between 30 and 37 °C (Fig. 2) corresponding to the optimum temperature range in previously isolated bacteria, such as *Acinetobacter calcoaceticus* and *Pantoe agglomerans* [45]. *Citrobactor braakii* [44] and *Delftia acidovorans* [28] had optimum temperature at 30 °C, *Staphylococcus aureus* WAW1 and *Bacillus cereus* WAW2 [9] however, were optimum at 35 °C, while in *Pseudomonas* sp. SDS degradation was optimum at 28 °C [47] and 37 °C for *Enterobacter* sp. Strain Neni-13 [30]. Antarctic bacterium was the lowest so far with optimum growth at 10 °C [48].

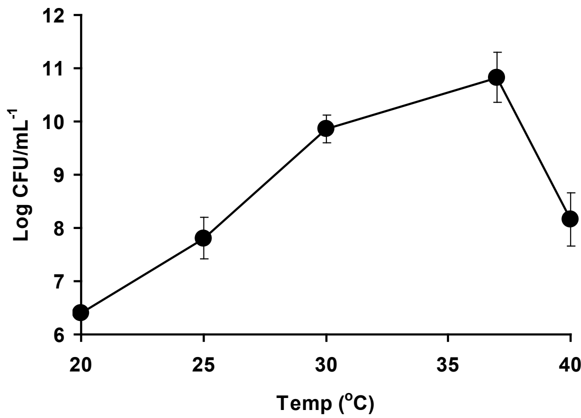

Fig. 2. The effect of temperature on SDS degradation by strain KIK-12 at 1 g/L SDS and 1% (w/v) ammonium sulphate. Data represent mean ± SEM of triplicates.

Optimization of nitrogen sources
The effect of various nitrogen sources on growth and SDS degradation by strain KIK-12 was conducted since SDS cannot be utilized as nitrogen source, which needs to be supplemented into the growth medium [44]. Ammonium sulphate was the best nitrogen source supporting growth in this bacterium (**Fig. 3**), which is in agreement with previous on *Enterobacter* sp. strain Neni-13 [30], *Citrobacter braakii* [44], *Comamonas terrigena* [49] and *Pseudomonas* sp. strain DRY15 [48]. However, in *Staphylococcus aureus* WAW1 and *Bacillus cereus* WAW2, 1 g/L ammonium chloride was used as nitrogen source [9]. Cheaper nitrogen source would be used when conducting bioremediation in the field to offset the price of ammonium sulphate [50].Therefore, there is need to find the best nitrogen source that support growth for effective bioremediation for surfactant, since source of nitrogen is an absolute requirement for microbial growth and degradation.

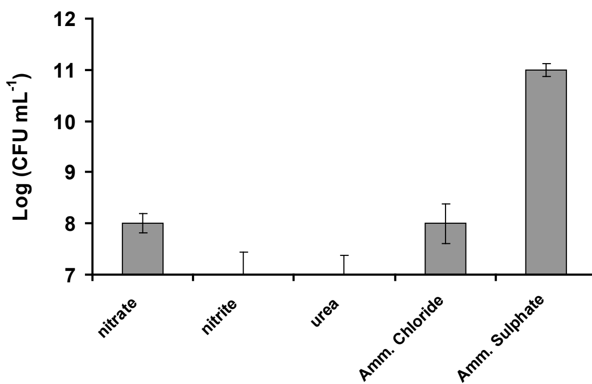

Fig. 3. The effect of 1% (w/v) for various nitrogen sources on growth of strain KIK-12 on 1 g/L SDS. Data represent mean ± SEM of triplicates.

The effect of SDS concentration as a carbon source
The ability of this bacterium to grow and metabolize SDS was tested at maximum concentration of 2000 mg/L. Growth was found to be optimal at 500 mg/L of SDS, however, concentration of 1000 mg/L SDS significantly decreased growth, while total inhibition of growth was observed at 2000 mg/L (**Fig. 4**). Microbial tolerance to SDS varied amongst the previously isolated bacteria. For instance, a bacterial consortium of *Pantoea agglomerans* and *Acinetobacter calcoaceticus* was able to degrade higher concentration of SDS up to 4000 mg/L [45]. In a recent study, [9] reported the degradation of 3615 mg/L and 5055 mg/L SDS by *Staphylococcus aureus* WAW1 and *Bacillus cereus* WAW2 respectively, indicating incomplete degradation was observed at very high concentration of SDS, especially at concentrations higher than the CMC. This is further exemplified in the work on *Klebsiella oxytoca strain* DRY14, which degrades 80% of 2000 mg/L of SDS within 4 days of incubation [48]. *Enterobacter* sp. strain Neni-13 [30] grew best at 1200 mg/L SDS but was able to tolerate as high as 2000 mg/L SDS. *Pseudomonas aeruginosa* MTCC 10311 tolerates 1500 mg/L of SDS [15,16].

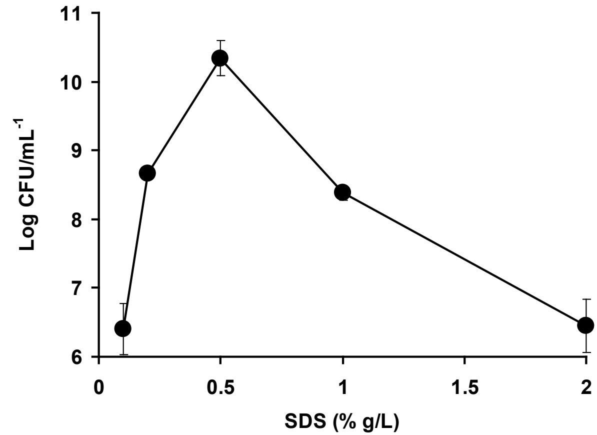

Fig. 4. The effect of various SDS concentrations on the growth of strain KIK-12 in a medium containing 1% (w/v) ammonium sulphate. Data represent mean ± SEM of triplicates.

The effect of heavy metals on growth on SDS
To date, almost all previous works on growth characterization on SDS do not study the effect of heavy metals, which is very necessary as many pollution sites contained both organic and inorganic pollutants including heavy metals [51]. The result reveals that 1 mg/L mercury, silver and copper strongly inhibits growth on SDS (**Fig. 5**). This indicates that some metals detoxification additives or treatments need to be added to ensure remediation of SDS is not affected. There appears to be a dearth of information regarding the inhibition of growth on SDS in the presence of heavy metals. A previous study on *Enterobacter* sp. strain Neni-13 showed that the bacterium was strongly inhibited by mercury, silver and copper at 1 mg/L while growing on 1000 mg/L SDS [30]. The presence of heavy metal meant that degradation will be strongly inhibited and ways to overcome this need to be studied.

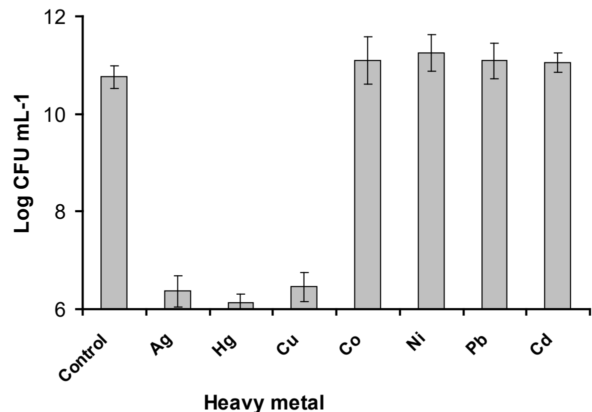

Fig. 5. The effect of heavy metals on the growth of strain KIK-12 on 0.5 g/L SDS. Data represent mean ± SEM of triplicates.

Growth and degradation of SDS at 500 mg/L

Bacillus amyloliquefaciens strain KIK-12 was able to grow and completely degrade 500 mg/L SDS following 7 days incubation, while abiotic control shows negligible degradation (**Fig. 6**). There has been variation amongst different species of bacteria for both tolerable concentration and the duration taken to completely degrade SDS as reported by many researchers. For instance, a consortium of *Pantoea agglomerans* and *Acinetobacter calcoaceticus* were able to degrade as high as 4000 mg/L SDS in approximately 5 days [45].

Recently, *Staphylococcus aureus* WAW1 degraded 3615 mg/L SDS at a rate of 15.06 mg/L/h with percentage SDS degradation of 36.8%, while *Bacillus cereus* WAW2 degraded 5055 mg/L of the initial SDS in the setup at a degradation rate of 21.07 mg/L/h, which resulted in a total SDS degradation of 51.4% following 10 days of incubation [9]. Similarly, *Klebsiella oxytoca* strain DRY14 was reported to degrades 80% of 2000 mg/L SDS within 4 days of incubation [48]. *Enterobacter* sp. strain Neni-13 grow well and completely degrade 500 mg/L SDS after 7 days of incubation. One of the most efficient SDS-degrading bacterium isolated is a mutated strain of *Pseudomonas aeruginosa* MTCC 10311 that degrades 1500 mg/L of SDS within two days of incubation [15,16].

At concentrations lower than the CMC, a much complete degradation and at a faster rate are observed. For instance, *Pseudomonas aeruginosa* sp. degrades 100% of 1000 mg/L of SDS within 2 days of incubations [26]. The SDS-degrading bacteria *Pseudomonas betelli* and *Acinetobacter johnsonii* degrades 500 mg/L SDS within 5 days of incubation [24]. Incidentally, the critical micelle concentration (CMC) for SDS is from 1700 to 2300 mg/L, and many detergents exhibit intense inhibition to bacterial growth at the CMC values [52]. At high concentrations, SDS disrupts cellular membrane integrity. This leads to disturbances to the ion gradients resulting in the leakage of bacterial cytosolic contents [27]. Another mechanism of SDS toxicity is through surface protein and enzymes denaturation [15].

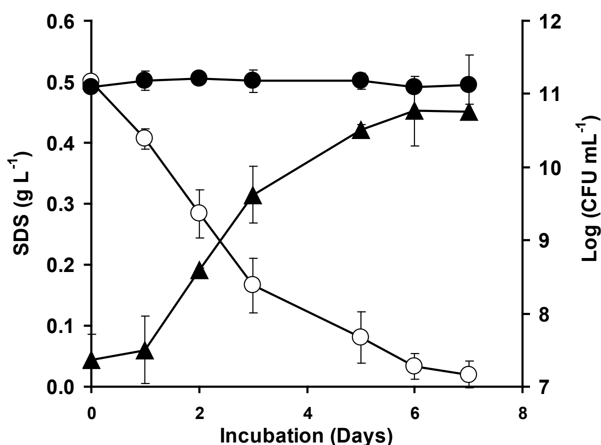

Fig. 6. Sodium dodecyl sulphate degradation (○), abiotic control (●) and cellular growth (▲) of strain KIK-12 after growth optimizations. Data is mean± standard error of triplicates.

CONCLUSION

A previously isolated molybdenum-reducing bacterium has been identified with potentials to degrade SDS. The bacterium grows best at neutral pH and temperature of 37 °C when 0.5 g/L SDS was used as sole source of carbon, the degradation was near complete in 7 days of incubation. Growth on various concentrations of SDS as a carbon source shows that the bacterium can tolerate SDS concentrations as high as 1500 mg/L while concentrations higher than this caused the cessation of growth, which coincidentally is near the limit of the CMC for SDS. The heavy metals mercury, silver and copper inhibit growth on SDS. The ability of this bacterium to detoxify SDS is an important tool for the remediation of sites containing detergent, but the inhibitory effect of other heavy metals needs to be addressed in the future.

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