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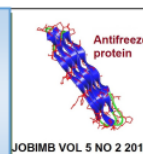
Submission date: 14-Oct-2022 11:42AM (UTC+0800)

Submission ID: 1924933496

File name: Characterization_of.pdf (267.95K)

Word count: 4275

Character count: 22133



Characterization of the Growth on SDS by *Enterobacter* sp. strain Neni-13

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HISTORY

Received: 27th August 2017
Received in revised form: 15th Nov 2017
Accepted: 28th of Dec 2017

KEYWORDS

biodegradation
Enterobacter sp.
characterization
Sodium Dodecyl Sulfate
SDS-degrading

ABSTRACT

Sodium dodecyl sulphate (SDS) is an anionic surfactant which is mainly utilized in washing detergents and industrial products and solutions. Its toxicity and contamination of the surroundings environment are ubiquitous. In this study, the SDS-degradation capacity of a previously isolated molybdenum-reducing bacterium; *Enterobacter* sp. Strain Neni-13 is reported. At 1 g/L of SDS as the sole carbon source, the bacterium grew maximally at pH 7.0, the temperature range from 20 to 45 °C support maximal growth and require ammonium sulphate as the best nitrogen source at the optimal concentration of 1 % w/v. Growth on various concentrations of SDS as a carbon source shows that the bacterium can grow maximally in between 800 and 1200 mg/L and was able to grow at the maximum concentration of 1500 mg/L while concentrations higher than this caused the cessation of growth. The heavy metals mercury, silver and copper inhibit growth on SDS. The ability of this bacterium to detoxify dual toxicants, which include the degradation of high concentration of SDS is highly sought.

INTRODUCTION

SDS, being an anionic surfactant is preferred primarily because of excellent detergency at low temperatures in neutral solutions. They generate negatively charged ions in aqueous solution, originated in sulphate or sulphonate groups. Anionic surfactants have either ester sulphate or sulfonated groups coming from xenobiotic substances are traditionally used in numerous manufacturing purposes [1]. SDS forms an essential number of organic substances in detergents because they have got greater solubility of organic and inorganic substances. SDS and other detergents have been found in numerous water bodies in Malaysia [2]. The hydrophilic and hydrophobic parts of SDS makes it easier to interact with both non-polar and polar substructures in macromolecules. Consequently, anionic surfactants can bring about positive aspects in numerous heterogeneous levels in numerous biological systems and scientific operations by lessening the energy of interaction and the solvation energy of substances [3]. anionic surfactants have hydrophilic and hydrophobic parts of which 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. Surfactants is usually categorized into non-ionic, anionic, cationic or amphoteric depending on the charge they have in aqueous solutions. Anionic surfactants are preferred

additives and preservatives due to their monetary cost and greater purposes in the industrial sector [4]. The most common anionic surfactants group of is sodium dodecyl sulphate (SDS). The applications of SDS in Malaysia specifically are most popular as an anionic surfactant in commercial products used for cosmetics and personal hygiene [5–10]. SDS is one of the most abundant anionic surfactants used in surface cleaners and household detergents and compose of the Linear alkylbenzene sulfonates (LASs) group which have alkyl chains from C₁₀ to C₁₄.

Large quantities of surfactants are utilized in commercial cleaning agents particularly from anionic surfactants. Anionic surfactants are essential in term of marketing for pretty much 50 % of the share of the market of surfactant manufacturing. They appear in numerous marine ecosystems a result of the less than efficient of wastewater treatment plants to remove them as well as the recalcitrant properties in terms of biodegradability of some of the active surface substances [11–15]. Upon discharged into the aquatic environment, they can harm the environment. Detergents possess damaging consequences to marine life [16,17]. Concentrations as low as 0.0025 mg/L for the surfactants sodium dodecylbenzene sulfonate (SDBS) and sodium dodecyl sulfate (SDS) has been demonstrated to show the toxic effect to the aquatic organism *Daphnia magna* [18,19].

The toxicity of SDS is the disruption of cellular membrane integrity that leads to the disturbances of the ion gradients. This results in the leakage of the content of bacteria leading to death. SDS also binds to surface protein and enzymes leading to denaturation. This is another mechanism of SDS toxicity [20].

Researchers have stated that biodegradation course of action by employing bacteria can manage the amount of SDS released to the environment [4,10,21,22]. The search for remediation agents for surfactants is ongoing with bioremediation being evaluated extensively as it is more economical and safe in the long run compared to physicochemical methods. Several microorganisms can assimilate SDS and use it for growth through the action of the enzyme alkylsulfatase [20]. Numerous SDS-degrading bacteria include bacteria from the genus such as *Pantoea*, *Acinetobacter*, *Pseudomonas*, *Acinetobacter* and *Klebsiella* [8,20,23–29]. However, there is a current need for a bacterium that is able to remediate multiple toxicants due to the ever-increasing amount of numerous pollutants being found in one particular pollution site. In this study, a heavy metal-reducing bacterium is reported to be able to degrade SDS, and the characterization of growth on SDS by this bacterium is reported.

MATERIALS AND METHODS

Maintenance and growth of *Enterobacter sp. strain Neni-13*

The bacterium was previously isolated as a Mo-reducing bacterium with SDS-degradation capacity [30]. The basal salts (BS) medium contained the following: KH_2PO_4 , (1.36 g l⁻¹), Na_2HPO_4 , (1.39 g l⁻¹), KNO_3 , (0.5 g l⁻¹), MgSO_4 (0.01 g l⁻¹), CaCl_2 (0.01 g l⁻¹) and $(\text{NH}_4)_2\text{SO}_4$ (7.7 g l⁻¹). The medium also contained the following trace elements: $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.01 g l⁻¹), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.01 g l⁻¹), H_3BO_3 (0.01 g l⁻¹), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (0.01 g l⁻¹), $\text{FeSO}_4 \cdot 2\text{H}_2\text{O}$ (0.01 g l⁻¹), $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.01 g l⁻¹) and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (0.01 g l⁻¹). Filter-sterilized sodium dodecyl sulphate was added into the medium as a carbon source at the final concentration of 1.0 g l⁻¹. The characterization works for the bacterium was carried out in the above medium using SDS as the sole carbon source.

Methylene Blue Active Substances (MBAS) Assay

Quantification of SDS degradation was carried out using the Methylene Blue assay (MBAS) [31]. Briefly, 5 mL of the SDS calibration samples or standards were added with 100 μL of methylene blue reagent that is slightly acidic (pH of 5 to 6). The mixing was carried out in a separating funnel. This is followed by adding about 200 μL of pH 10.5 of a solution of sodium tetraborate. The final mixture was vigorously mixed. Then about mL of chloroform was added the mixture was mixed vigorously again for 1 min. Finally, the mixture was allowed to separate and settle down by leaving it to stand for 5 min. The blue layer of chloroform layer was taken out and read at 650 nm using a glass cuvette.

RESULTS AND DISCUSSION

The ability of bacterium to degrade or transform more than one pollutant is intensively sought as more and more polluted sites are reported to contain the combination of organic and inorganic pollutants including heavy metals [8,9,32–36]. This study report on the SDS-degrading capacity of a previously isolated molybdenum reducing bacterium. This is the second bacterium with such capacity as previously, another similar multi detoxifier has been reported [22]. Before this bacterium can be used in field works as a bioremediation agent, its characterization especially the effect of various environmental

factors such as pH, temperature and heavy metals as well as optimization studies via one-factor-at a time is needed. The data obtained will be very useful either for 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

Optimization of pH provides growth important data since pH strongly affects bacterial growth and also important bioremediation works. The effect of pH on SDS degradation was studied using phosphate buffer spanning the pH range from 5.8 to 7.5, which is within the pKa range for phosphate (Fig. 1). From this study, the optimum pH for degradation of SDS was at pH 7.0. Most bacteria functions well at neutral pH neutral to a slightly alkaline pH range. However, soils are rarely neutral with soils in Malaysia are acidic in properties, and require soil additives to ensure neutrality or near neutrality is achieved so that bioremediation of xenobiotics in soils can be carried out efficiently [37–43]. The degradation decreased at higher pH with more than 50% inhibition of growth occurring at the highest pH tested. Previous studies have shown that different pHs are optimum for different bacteria, but are still within the pH range from 5 to 9, for instance, *Citrobacter braakii* shows an optimal pH at 7.0 [44], *Delftia acidovorans* shows pH 7.2 as the optimum [29] and *Pantoea agglomerans* require pH 8.5 for optimal pH [1]. In the most recent data, *Staphylococcus aureus* WAW1 and *Bacillus cereus* WAW2 are optimally grown at pH 7.5 [10]

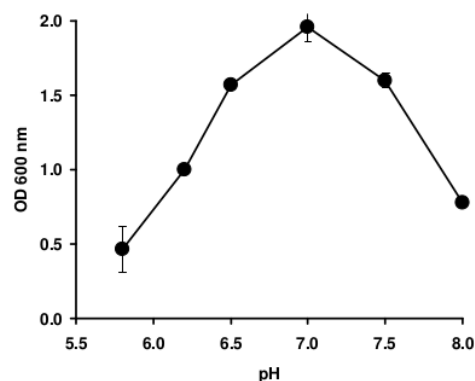


Fig. 1. The effect of pH on the degradation of SDS by *Enterobacter* at the SDS concentration of 1 g/L. The ammonium sulphate concentration was 1% (w/v). Data represent mean \pm SEM, n=3.

Optimization of temperature

The effect of temperatures on growth on 1 g/L of SDS was studied from the temperature range of 20 °C to 45 °C (Fig. 2). Temperature is one of the factors that influence biodegradation with low temperature affecting the metabolic rate while higher temperatures will inhibit degradation [45]. The results show maximal growth occurring in between 30 and 35 °C (Fig. 2). The result obtained in this study showed within the range of optimum temperature such as *Acinetobacter calcoaceticus* and *Pantoea agglomerans*, where both are optimum between 30 to 37°C [1], 30°C for *Citrobacter braakii* [44] and *Delftia acidovorans* [29], 35 °C for *Staphylococcus aureus* WAW1 and *Bacillus cereus* WAW2 [10], 28°C for the degradation of SDS by *Pseudomonas* sp. [46] and the lowest so far is 10 °C by an Antarctic bacterium [47].

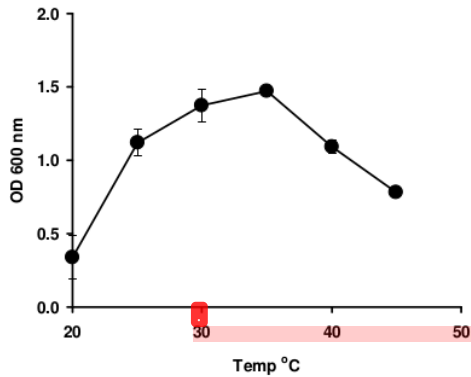


Fig. 2. The effect of temperature on the degradation of SDS by *Enterobacter* at the SDS concentration of 1g/L. The ammonium sulphate concentration was 1% (w/v). Data represent mean ± SEM, n=3.

Optimization of nitrogen sources

As SDS cannot be a nitrogen source, this needs to be added into the growth medium [44]. The study showed the effect of different nitrogen sources on the degradation of SDS. The results show that the best nitrogen source was ammonium sulphate (Fig. 3) at the concentration of 1% (w/v) (Fig. 4). But from previous studies, the best nitrogen source is ammonium sulphate and include the reports on bacteria such as *Citrobacter braakii* [44], *Comamonas terrigena* [48] and *Pseudomonas* sp. strain DRY15 [47] while *Staphylococcus aureus* WAW1 and *Bacillus cereus* WAW2 both are grown on 1 g/L of ammonium chloride as the nitrogen source [10]. Finding the optimum nitrogen source would help in bioremediation strategies for surfactant contamination as most soils are deficient in nitrogen source. In the field, cheaper nitrogen source will be used to offset the price of ammonium sulphate [49].

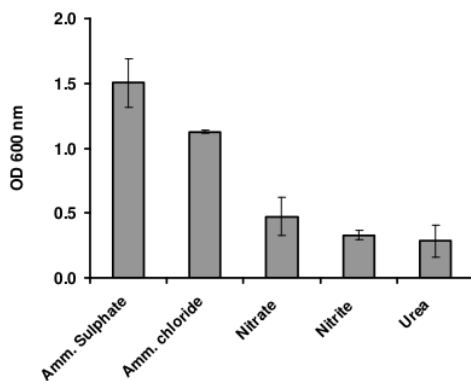


Fig. 3. The effect of 1% (w/v) of different nitrogen sources on growth on 1 g/L of SDS by *Enterobacter* sp. strain Neni-13. Data represent mean ± SEM, n=3.

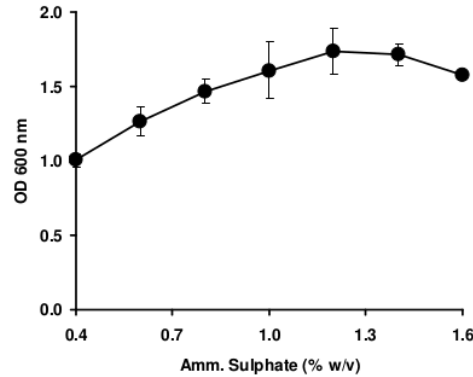


Fig. 4. The effect of various concentrations of ammonium sulphate on the growth of 1 g/L of SDS by *Enterobacter* sp. strain Neni-13. Data represent mean ± SEM, n=3.

The effect of SDS concentration as a carbon source

The bacterium grew optimally in between 800 and 1200 mg/L of SDS with no significant differences in the values as analysed by ANOVA (Fig. 5). Concentrations higher than 1500 mg/L inhibits growth. There exists a variety of range both for concentration and number of days for complete degradation reported for bacterial growth on SDS. For instance, a bacterial consortium of *Pantoea agglomerans* and *Acinetobacter calcoaceticus* is able to degrade a very high concentration of SDS at 4000 mg/L in approximately 5 days [1]. In another recent study, *Staphylococcus aureus* WAW1 degraded 3615.37 mg/L of SDS at a rate of 15.06 mg/L/h, which resulted in a total degradation of 36.8% of SDS, while *Bacillus cereus* WAW2 degraded 5055.70 ppm of the initial SDS in the setup at a degradation rate of 21.07 mg/L/h, which resulted in a total degradation of 51.4% of SDS after 10 days of incubation [10] indicating incomplete degradation is observed at very high concentration of SDS, especially at concentrations higher than the CMC for SDS. This is further exemplified in the work on *Klebsiella oxytoca* strain DRY14, which degraded 80% of 2000 mg/L of SDS within 4 days of incubation [47]. 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 [16,17].

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 [27]. The SDS-degrading bacteria *Pseudomonas betelli* and *Acinetobacter johnsonii* degrades 500 mg/L SDS within 5 days of incubation [25]. 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 [50]. At high concentrations, SDS disrupts cellular membrane integrity. This leads to disturbances to the ion gradients resulting in the leakage of bacterial cytosolic contents [28]. Another mechanism of SDS toxicity is through surface protein and enzymes denaturation [16].

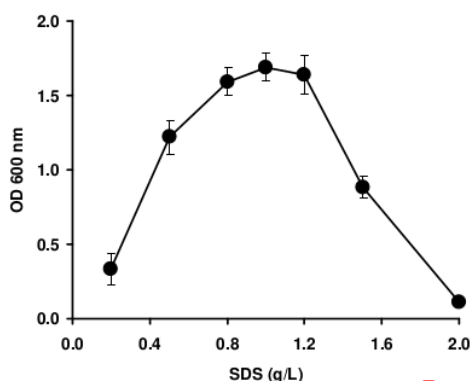


Fig. 5. The effect of various concentrations of SDS on the growth of *Enterobacter* sp. strain Neni-13. The ammonium sulphate concentration was 1% (w/v). Data represent mean \pm SEM, n=3.

The effect of heavy metals on growth on SDS

Almost all studies done on growth on SDS do not study the effect of heavy metals, and this should be studied since many polluted sites contained not only organic but inorganic pollutants including heavy metals [51]. The results indicate that the heavy metals mercury, silver and copper, all at 1 p.p.m strongly inhibits growth on SDS (Fig. 6). This indicates that some form of heavy metals detoxification additives or treatments need to be added to ensure remediation of SDS is not affected.

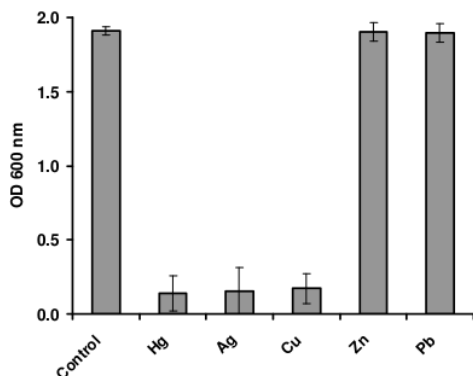


Fig. 6. The effect of heavy metals on the growth of *Enterobacter* sp. strain Neni-13 on 1 g/L of SDS. The ammonium sulphate concentration was 1% (w/v). Data represent mean \pm SEM, n=3.

CONCLUSION

SDS-degrading capacity by a bacterium previously isolated as a molybdenum-reducing bacterium is reported. At 1 g/L of SDS as the sole carbon source, the bacterium grew maximally at neutral pH and exhibited a broad range of temperature for maximal growth. 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 dual toxicants, which include the degradation of high concentration of SDS is an important tool for the remediation of sites containing detergent and molybdenum, but the inhibitory effect of other heavy metals needs to be addressed in the future.

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