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Optimal conditions for enhancing sodium dodecyl sulfate biodegradation by *Pseudomonas aeruginosa* KGS

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ABSTRACT

The anionic surfactant sodium dodecyl sulfate (SDS) was degraded by novel strain of *Pseudomonas aeruginosa* KGS under accession No. JQ328193, which was isolated from car wash wastewater. The purpose of this research was to study different optimization conditions required for enhancing the biodegradation of sodium dodecyl sulfate *P. aeruginosa* KGS. Influence of different Physicochemical factors such as nitrogen and carbon sources, pH, temperature, inoculation percent and different concentrations of SDS on the biodegradation of SDS were investigated by measuring the degradation rate of SDS using methylene blue active substance (MBAS) method. The optimum conditions determined for the this selected bacterium strain for degradation of SDS were 1.5mM SDS, inoculation percent 7%, pH 7.5, temperature 37°C, ammonium nitrate (nitrogen source) when basal salt medium was supplemented with glucose as a co substrate. This bacterium is able to degrade about 98% of the SDS after 24h of incubation under optimized conditions of biodegradation. The results presented in this research indicate that *Pseudomonas aeruginosa* is a suitable candidate for SDS biodegradation.

Key Words: biodegradation, optimization, *pseudomonas aeruginosa*, sodium dodecyl sulfate.

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Introduction

In recent years, surfactants have been widely used in industries and daily life for their surface or interfacial functions (1, 2). In order to make their applications safe, it is essential to remove these agents rapidly from the environment to avoid secondary pollution (3).

Synthetic surfactants are components of household and industrial detergents (4). Surfactants are organic chemicals that accumulate at gas-liquid or solid-liquid interfaces (5). After utilization, large quantities of surfactants and their derivatives are discharged into aquatic and or terrestrial environments (6). These compounds can cause problems in sewage aeration and treatment facilities due to their high foaming capabilities, lower oxygenation potentials and consequently, cause the death of waterborne organisms (7). Sodium dodecyl sulfate (SDS; $C_{12}H_{25}OSO_3Na$) is an essential component of shampoos and car wash soap, and a foaming agent in toothpastes (8). It consists of a hydrocarbon chain (C_{12}) to which a sulfate group is attached (8, 9).

Due to the widespread use of SDS, its bioremediation by suitable microorganism has gained much importance (10). Systems involving surfactant, oil and water are being studied due to their high oil recovery potential. Soil washing studies showed greater oil removal with SDS than other surfactants employed. Domestic and industrial wastewaters are major pollution sources of receiving water bodies. These wastewaters included a significant amount of surfactants. Wastewater treatment plants can experience operational difficulties due to the excess of foam generated by these substances (11). The biodegradability of alkylsulfate surfactants in wastewater treatment, marine and estuarine environments is well established (12). It seems therefore likely that bacteria may be able to mobilize organically bound sulfur for growth. There is evidence that bacterial sulfatase could play a role in sulfur scavenging. In most cases, degradation of alkylsulfate esters was found to be initiated by alkylsulfatase enzymes that catalyze hydrolytic cleavage of the ester bond resulting in liberation of inorganic sulfate. The resulting parent alcohol is further degraded and converted to CO_2 and H_2O by a β-oxidation process or incorporated into cellular lipids (13, 14). In this study, an SDS-degrading bacterium was isolated from a car wash wastewater in Tehran. The effects of physicochemical factors on SDS biodegradation using the novel strain of *Pseudomonas aeruginosa* were studied.

Material and Methods

Microorganism and culture medium

The novel strain of *Psudomonase aeruginosa* (*P. aeruginosa* KGS) used was isolated from wastewater of a car wash location in Tehran. The basal salt medium used for growth was composed of KH_2PO_4 3.5 g/l, K_2HPO_4 1.5 g/l, NH_4Cl 0.5 g/l, NaCl 0.5 g/l, Na_2SO_4 0.14 g/l, $MgCl_2$.6H2O 0.15 g/l. SDS concentration in the medium, pH, inoculation volume, temperature, and shaking speed were variables tested as described below. Growth was for 24 hours (8, 15, 16).

Methylene blue active substance (MBAS) assay

Concentrations of SDS were determined using the MBAS method. 0.01µl of sample was added to a 100 ml funnel containing 9.9 ml deionized H_2O, 2.5 ml methylene blue solution (0.5%) and 1 ml of chloroform. The funnel was shaken vigorously until the aqueous and

organic (chloroform) phases were created, after which the organic phase was drawn off into a second funnel. Then 5.0 ml of wash solution (Phosphate buffer) was added and the mixture was shaken vigorously for 20 seconds. The organic phase was drawn off into a 10 ml volumetric flask. The absorbance was read at 652 nm against blank chloroform in a quartz or glass cuvette (17, 18).

Medium optimization for maximal SDS biodegradation by *Pseudomonas aeruginosa* strain KGS

A culture of *P. aeruginosa* KGS was prepared in basal medium. SDS was added at concentrations ranging from 0.5 to 15.5 mM. The effects of incubation temperature in the range of 30-42˚C, pH in the range of 5.5 to 8.5, aeration by shaking in the range of 100, 150, 200 rpm, and inoculation percent in the range of 3% to 9% were also evaluated. An investigation into the effects of various carbon sources including glucose, sucrose, lactose and fructose, and various nitrogen sources including ammonium chloride, ammonium nitrate, tryptone, yeast extract and casein on SDS biodegradation by *P. aeruginosa* KGS was carried out under optimized conditions for SDS biodegradation (19).

Data were analyzed using one-way ANOVA and Tukey's HSD test at 5% significance level.

3. Results

Medium optimization for SDS-degrading by *Pseudomona aeruginosa* strain KGS

The effect of SDS concentrations on growth

The effect of SDS as a carbon source for the growth of KGS isolate was studied using SDS concentrations of up to 15 mM. The isolate exhibited an increase in cellular growth as the SDS concentrations was raised culminating to an optimum SDS concentration of 1.5 mM (Fig.1). The bacterial growth decreased dramatically in the presence of 1.5 mM to 15.5mM SDS.

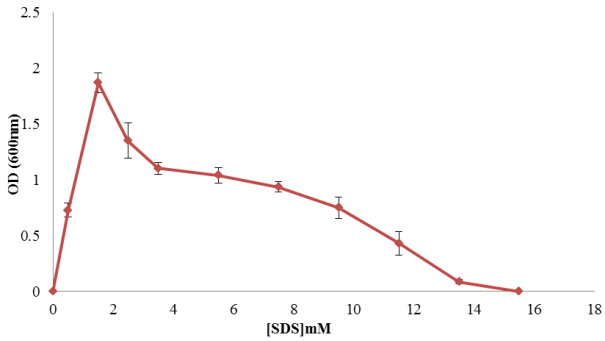

Figure 1. The effect of SDS concentrations on the growth of strain KGS.

3.1.2. Effect of carbon sources on SDS biodegradation

Various carbon sources including glucose, sucrose, lactose and fructose were added separately at fixed concentrations of 1.5 mM to the minimal medium before the biodegradation capability of the surfactant was examined. Figure 2 shows that almost all tested carbon sources were able to enhance the biodegradation of SDS after 24 hours of incubation. Slight advantage for this degradation ability was observed in the presence of glucose as a carbon source ($p<0.05$). A positive control bacterial culture growing under similar conditions but lacking additional supplementation of carbon source was also examined. The addition of the carbon source caused a slowdown in the degradation process of SDS as indicated by incubation period (24h) required to achieve elevated levels of degradation. Furthermore, lactose failed to promote a significant degradation of this surfactant within the above incubation interval.

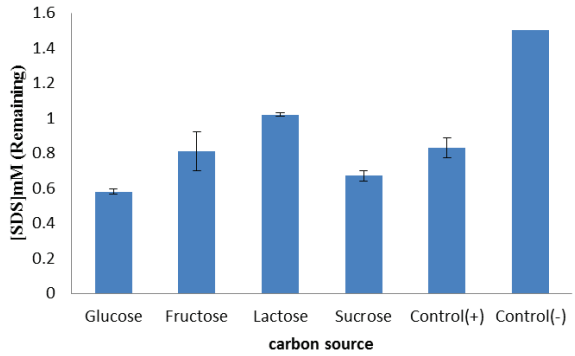

Figure 2. Effect of carbon sources on SDS biodegradation.

The effect of nitrogen sources on SDS biodegradation

The addition of various nitrogen sources to the basic medium increased SDS biodegradation (Fig.3). Ammonium nitrate, in particular, produced maximum SDS biodegradation Biodegradation was poorly stimulated by casein ($p<0.05$).

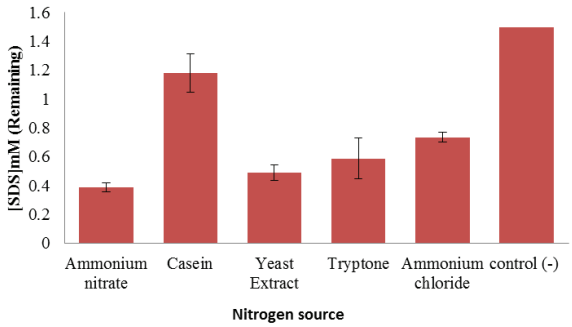

Figure 3. The effect of nitrogen sources on SDS biodegradation.

A control culture containing the SDS but without bacterial inoculation of bacterial was used throughout the biodegradation experiments. Such control permitted the estimation of possible SDS chemical degradation that might take place in the culture during prolonged incubation period. Under these conditions, no chemical degradation was observed.

Effect of shaking rates on SDS biodegradation

To test the effect aeration on SDS biodegradation, three shaking rates were tested (Fig.4). Effective SDS biodegradation was achieved at all three speeds tested (100, 150, and 200 rpm), but the medium shaking rate (150 rpm) led to maximum increase in degradation of the surfactant ($p<0.05$).

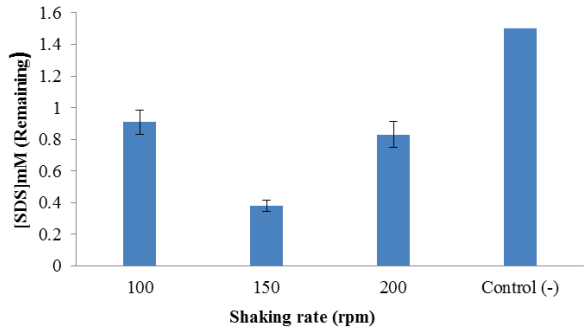

Figure 4. Effect of agitation rates on SDS biodegradation.

Effect of inoculation percent on SDS biodegradation

The effect of inoculation percent (v/v) in the range of 3% to 9% in basal salt medium was studied at 30°C, pH 7.1 and 150 rpm within 24 hours of incubation time. Strain KGS was able to degrade high concentrations of SDS in basal salt medium containing 1.5mM SDS at 7% inoculation percent within 24 hours of incubation time (Fig.5).

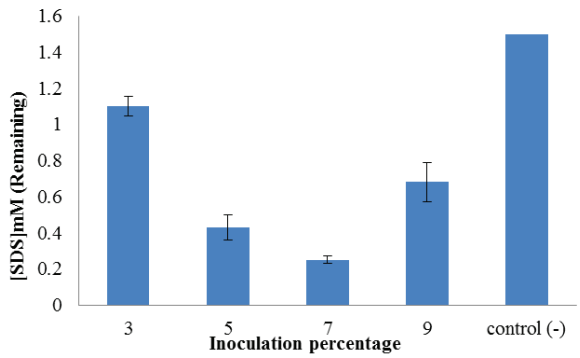

Figure 5. Effect of inoculation percent on SDS biodegradation.

The effect of pH on SDS biodegradation

A range of pH 5.5-8.5 was used to examined (Fig.6). Maximum degradation of SDS by strain KGS was observed at pH 7.5. An intermediate level of degradation of SDS was observed at pH 8.5 and pH 6.5, while the lowest degree of degradationwas achieved at pH 5.5 ($p<0.05$).

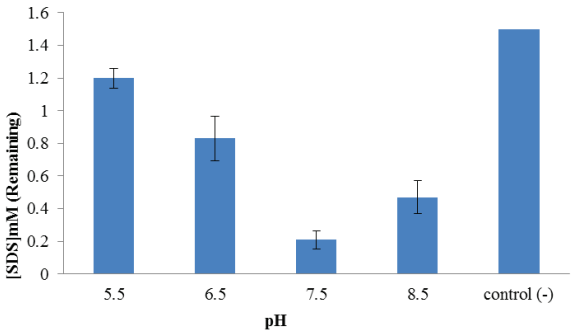

Figure 6. Effect of pH on SDS biodegradation.

The effect of incubation temperature for SDS biodegradation

Optimum incubation temperature for SDS biodegradation was 37˚C, while the lowest extent of degradation was observed at 42 ˚C ($p<0.05$) (Fig. 7).

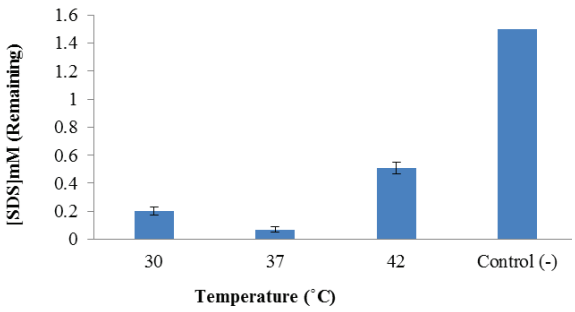

Figure 7. Effect of incubation temperature on SDS biodegradation.

3.2. SDS degradation study

Subsequent to the optimization of conditions required for SDS degradation, the bacterial isolate *P. aeruginosa* KGS was shown to degrade almost 98% of 1.5 mM SDS after 24 h of incubation.

Discussion

Due to their amphiphilic properties alkylsulfates such as sodium dodecyl sulfate, they are common components of detergents and are consequently discharged into wastewater after use of the detergents. It is realized that rapid removal of surfactants from the environment will make their application safer and more widespread. Using microorganisms to degrade surfactants is one promising methodfor their elimination (20). In the present study, we have made an attempt to isolate SDS degrading bacteria from car wash wastewaters in Tehran, Iran. It was known that the car wash soaps used at the locations included SDS.

Past works have shown that anionic surfactants biodegradation is exclusively conducted by bacteria (8). Chaturvedi and Kumar (21) isolated SDS degrading bacteria from two surfactant contaminated ponds by enrichment technique in minimal medium. The kinetics of degradation of SDS by these isolates was studied by monitoring disappearance of SDS with time and also by measuring the growth of the isolate. In the present study on SDS degradation, SDS was measured by the MBAS method. Optimization of biodegradation SDS by *p. aeruginosa* KGS were performed. Previous studies by Hosseini (8) and Sigoillot and Nguyen (22) indicated that the bacteria are actually utilizing SDS as their sole carbon source.

In this study concentrations was raised culminating to an optimum SDS concentration of 1.5 mM. The bacterial growth decreased dramatically in the presence of SDS

concentrations 1.5 mM to 15.5mM. This is because, in microorganisms, SDS is able to increase the cellular permeability, which might interfere with the bacterial cell membrane integrity and also alter the hydrophobicity of the cell membrane (23, 24, 25).

Bacteria that can degrade SDS at the higher concentrations of 14 mM and 3.47 mM have been reported (18). The most tolerant SDS-degrading bacterium thus far reported is *Pseudomonas* strain C12B that can grow in medium containing SDS at a concentration of 25 mM (26). In this study the degradation was achieved when the minimal medium was supplemented with defined carbon or nitrogen sources. In this process, the first step sugars such as glucose, sucrose, and fructose were used as the inducing carbon sources of biodegradation and SDS was metabolized into less available organic molecules by *P. aeruginosa* KGS. In the second step, the further degradation of the organic molecules caused regrowth of this bacterium.

High level of degradation rate of SDS by strain KGS demonstrated the strong degradability potential of this strain. But it seems that the lactose has a negative effect on enzyme activity of SDS degrading, as indicated by reduced rate of SDS biodegradation in the presence of this sugar. Furthermore, *Pseudomonas aeruginosa* grown in minimal medium supplemented with a carbon source such as glucose or a nitrogen source such as ammonium nitrate was able to degrade high concentrations of SDS. It is likely that some enzymes present in the metabolic pathways of anionic surfactants are induced by high levels of carbon and nitrogen nutrients (19, 27).

Past studies have reported elevated SDS biodegradation in the presence of glucose or sucrose by a consortium of the mixed facultative anaerobes, *Acinetobacter calcoaceticus* and *Pantoea agglomerans* (19). In other work Roig and coworkers (28) showed that *Comamonas terrigena* grows optimally with ammonium nitrate as the nitrogen source while ammonium sulfate was reported to be the optimum nitrogen source for SDS degradation by *Citrobacter braakii* (29). A more complex nitrogen source in the shape of nutrient broth has been used to increase SDS biodegradation using a bacterial consortium. However, the use of this complex nitrogen source did impose problems involving SDS-degrading enzyme induction (19). Identification of the optimum nitrogen source for biodegradation could help in designing effective biodegradation strategies for surfactant contaminants such as SDS (18).

Wang and Bartha (30) reported these nutritional conditions are needed for the full biodegradation of petroleum hydrocarbons. High and medium rates of aeration (250 rpm and 150 rpm) have been shown to be substantially more effective on SDS degradation by a novel consortium of *Acinetobacter calcoaceticus* and *Pantoea agglomerans* (18,19).

In present work, the selection of optimum incubation conditions like temperature and pH managed to improve the degradation efficiency of SDS. The preference of neutrality in terms of optimal growth on SDS is shared by many other SDS-degrading bacteria such as *Citrobacter braakii* at pH 7 (29), *Comamonas terrigena* strain N3H at pH 7.4 (25) and pH 7.5 to 8 for Pseudomonas strain C12B (28).

In another report, growth on SDS by consortium of *Acinetobacter calcoaceticus* and *Pantoea agglomerans* required pH 8.5 for effective degradation (19). Marchesi and coworkers (31) reported a lower temperature for the degradation of SDS by *Pseudomonas*

sp. at 25˚C. Further differences in the SDS degradation were detected when the effects of pH, aeration and temperature of incubation on the biodegradation process were studied. In particular the degradation data at 42˚C incubation temperature suggest that alkylsulfatase enzyme in the pathway of SDS degradation is labile at this temperature.

Another property of the isolate *P. aeruginosa* KGS is its ability to degrade high biomass of the SDS under optimized conditions of biodegradation (98%) after only 24 hours. A much higher degradation of SDS (7 mM SDS) has been reported using a consortium *Acinetobacter calcoaceticus* and *Pantoea agglomerans* with complete degradation occurring after 5 days incubation (19). Shukor and coworkers (18) have isolated a strain of *Klebsiella oxytoca*, which was able to degrade 1g/l SDS in 3 days incubation time. But the rates of SDS degradation by these isolates are very low as compared to our isolate. Hosseini and coworkers (8) have isolated two bacterial strains from activated sludge that were identified as *Pseudomonas betelli* and *Acinetobacter johnsoni*, These strains degraded 93.6% and 84.6% of SDS (522 mg/L) within 5 days respectively.

The available data suggest that the use of SDS in various industry and household products is increasing at an alarming rate. The consequences arising from its overuse and subsequent disposal in waterways are of serious concern especially for health of humans. Thus the results of this research show that growth of simple bacteria such as *Pseudomonas aeruginosa* in household and industrial wastewaters can be an effective way to achieve complete destruction of anionic surfactants.

In conclusion, we have isolated an SDS-degrading bacterium from an SDS-polluted car wash wastewater sample from Tehran and evaluated its biodegrading potentials. Optimization studies of the isolate were performed using various physicochemical parameters to enhance degradation rates in order to apply the optimized parameters in the field. The isolate exhibited mixed results in terms of degradation optimizations using SDS as the carbon source compared to other published work. Another characteristic property of this bacterium is the ability to degrade high biomass of the SDS surfactant. Hence the results obtained in our study show maximum degradation (98%) by *P.aeruginosa* KGS in basal salt medium containing 1.5mM SDS at inoculation percent 7%, pH 7.5, temperature 37˚C and 150 rpm after 24h of incubation.

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