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EXPLOITING THE POTENTIAL OF ISOLATED SDS DEGRADING BACTERIA FOR DEGRADATION OF HOUSEHOLD DETERGENTS.

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ABSTRACT Sodium dodecyl sulfate (SDS) is one of the main surfactant components in detergents and cosmetics, used in high amounts as a detergent in products such as shampoos, car wash soap and toothpaste. Therefore, its bioremediation by suitable microorganisms is important. Alkylsulfatase is an enzyme that hydrolyses sulfate -ester bonds to give inorganic sulfate and alcohol. The purpose of this study was to isolate SDS-degrading bacteria from soil samples and study bacterial alkylsulfatase enzyme activity. The isolation of SDS (sodium dodecyl sulfate) degrading bacteria were carried out from the soil and water samples collected from detergent contaminated sites near college area. Out of the 12 bacteria isolated, six were found to be tolerating high levels of SDS. The amount of SDS degradation and alkyl sulphatase enzyme activity was detected using Methylene blue Active Substance Assay (MBAS) (MBSA) method. These isolates were found to degrade other domestic detergents as well. The results obtained indicated that these isolates can be used for degradation of detergent contaminated areas in terrestrial and aquatic habitat.

KEYWORDS : Sodium dodecyl sulfate, SDS degrading, Alkylsulfatase, Methylene blue Active Substance Assay (MBSA)

INTRODUCTION

In India, man-made ponds have been exploited as an alternative supply of drinking water. However, washer men and locals use these ponds to wash clothes and bathe (Prakash et al., 2009). Many ponds surround temples and are used for bathing by people who visit the temples for worship, as well as for the disposal of temple garbage (Sharma et al., 2009). In certain ponds, sewage water and waste water from local cottage enterprises are released. Tyagi et al. (2006) conducted a comprehensive examination of a few ponds across the city, revealing that the majority of the ponds are eutrophic.

Sodium Dodecyl Sulfate (SDS) otherwise referred to as Sodium Lauryl Sulphate is the most widely used anionic detergent in household products, such as toothpastes, shampoos, shaving foams, bubble baths, cosmetics and detergents (Dhouib et al., 2003). In the industries, however, it is used as leather softening agent, wool cleaning agent, penetrant, flocculating agent, de-inking agent in the paper industry; and it is the major components of fire-fighting devices, engine degreasers, floor cleaners, and car wash soaps. The occurrence of SDS in the environment stems mainly from its presence in domestic and industrial effluents as well as its release directly from some applications (Fendinger et al., 1994). Several authors have reported the toxicity of SDS and its effects on the survival of aquatic animals such as fishes, microbes, like yeasts and bacteria (Sandbacka et al., 2000). It has also been reported to be toxic to mammals, like mice and humans though to a lesser extent. The excessive use of detergents domestically and industrially is becoming a serious problem due to the fact that they have detrimental effects on aquatic organisms via the discharge of surfactant-laden wastewater into water bodies and channels (Chukwu, 2001).

Liwarska-Bizukojc et al. (2005) reported that surfactants are ubiquitous and in many untreated effluents, certain classes of surfactants can be present in sufficient concentrations to constitute toxicity problems to aquatic organisms because most of the massive amounts of surfactants used industrially and domestically end up in wastewater flows. Petterson et al. (2000) reported that anionic surfactants have toxic effects on various aquatic organisms even at concentrations as low as 0.0025 mg/L thus necessitating the removal of these compounds before they build up to a considerable high concentration in the environment especially water bodies. Cserhati et al. (2002) reported that SDS and other surfactants are considered to be biodegradable by aerobic processes; however, the mass loadings of these compounds into water bodies suggest that, even at these natural removal rates, appreciable amounts of surfactants are released into receiving waters to the extent that a variety of surfactants has been identified in both surface and drinking water (Isobe et al., 2004). This has necessitated the need for a system capable of degrading surfactants discharged into water system as a means of augmenting the natural biodegradation of these compounds.

Surfactants in wastewater have been treated in a variety of ways, including the employment of microbes capable of degrading them (Zeng et al., 2007). The first study describing bacteria's capacity to breakdown SDS was published by Payne and Feisal in 1963. This present study, we have investigated the ability of bacteria isolated from the soil and water samples collected from detergent contaminated sites near college area to utilize SDS as a sole source of carbon and degrade SDS in a batch culture system spiked with SDS as the carbon source. In recent years, surfactants have widely been used in industries and daily life for their interfacial functional capabilities. The rapid removal of these compounds from the environment to avoid secondary pollution will make its application safer and more widespread (Mulligan et al., 2001).

Isolation Of Sds Degrading Bacteria From Soil And Water Samples

Soil and water sample were collected from the detergent contaminated sites in and around housing areas in Kalyan. Enrichment of the soil and water samples was carried out using 50ml of sterile minimal mineral salt medium [Na2HPO4 (1.6g/L), KH2PO4 (1.0g/L), NH4Cl (0.5g/L), K2SO4 (0.06g/L), CaCl2 (0.025g/L), Trace element solution (2.0 ml/L - FeSO4.7H2O; MnCl2.4H2O; ZnSO4.7H2O), SDS (10g/L), pH = 7.2 adjusted]. The flasks were incubated at 28±2°C for 3 days. The culture was streaked on Sterile Nutrient agar plates and further incubated at 28±2°C for 24 to 48 hrs. The bacterial isolates obtained were further cultured on sterile Nutrient agar slants and used for the further experiments. The cultures were maintained at refrigerator conditions during the project work. The cultures were preserved in 30% glycerol stocks at 10 ° C. Further Gram staining and screening the bacterial isolates for SDS degrading abilities was carried out.

Screening for SDS degradation ability:

The screening of the isolates was selected by Methylene blue Active Substance Assay (MBAS) (Chitikela and Dentel 1995). 100 microliters of bacterial culture were added to 100ml separating funnel containing 9.9ml distilled water, followed by addition of 2.5ml of methylene blue solution and 1ml of chloroform. The funnel was shaken vigorously for 15 seconds, and the combination was allowed to separate, followed by the removal of the chloroform layer into test tube. The extraction was repeated 3 times using 1ml of chloroform each time. Prior to adding 5.0ml wash solution, all chloroform extracts were combined and shaken vigorously for 15 seconds. The chloroform layer was drawn off into test tube. The wash solution was extracted twice with 1ml of chloroform. All extracts were combined and diluted

to 10ml mark with chloroform. The absorbance was read at 652nm against blank chloroform in a cuvette using a colorimeter (CL 157).

Optimization of SDS degradation

The most trivial step of SDS remediation is optimizing the growth of the bacterial strain in SDS, which involves multiple enrichment steps in minimal mineral salt medium. Previous study revealed that pH and temperature played a critical role in detergent degradation (Abboud et al. 2007) and thus optimization of SDS degradation by isolated bacterial cultures was carried out around these factors. The bacterial suspension was taken having optical density of 1.0 at 620 nm in saline solution. SDS degradation was optimized at different concentrations of pH (6.0, 7.0, and 8.0) and temperatures (25 ± 2 and 35 ± 2 °C) in minimal mineral salt medium. The isolates were inoculated at different operational conditions in 100 ml of SDS medium in 500 ml Erlenmeyer flasks for 24 hours. Cell-free supernatant at 24 hours was used for SDS degradation analysis by MBAS assay.

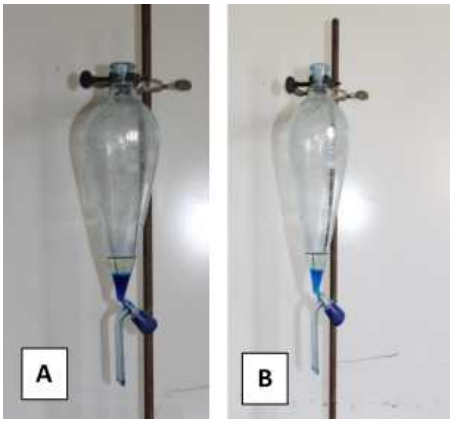


Figure 1: (A) at 1% SDS concentration (B) After 24 hours of degradation

Isolation Of Alkylsulphatase Enzyme

For enzyme isolation purpose bacteria were grown on well aerated conical flasks in mineral salt medium supplemented with 0.1 % SDS. The cultures were incubated overnight at C. After 28±2°C sufficient growth cells were harvested by centrifugation at 10,000 g for 10 min at 4°C. The cell pellet was washed twice with phosphate buffered saline pH 7.4 at 4°C. The cells were resuspended in a small volume of phosphate buffered saline and further incubated at 37°C water bath for 1 h. 1.0 mM PMSF was added to the tube after ½ hr. interval as it has a short half- life. The cells were ruptured by sonication (10 pulses, 10 times at 1 min interval). The homogenate was centrifuged at 20,000 g for 10 min at 4°C. The supernatant was transferred to vials and used for enzyme alkylsulphatase assay.

Estimation of alkylsulphatase enzyme activity

Alkylsulphatase assay was done using modified MBAS assay method (Ellis et al., 2002). About 0.5 ml of the cell extract was reacted with equal amount of 0.05 mg of SDS/ ml of phosphate buffer saline. Immediate reaction get starts, after 1 hour of incubation at room temperature, the residual amount of SDS in the reaction mixture was assessed by MBAS assay at different intervals. The decrease in the SDS with the time in the reaction mixture was used to determine the enzyme action (Ellis et al. 2002).

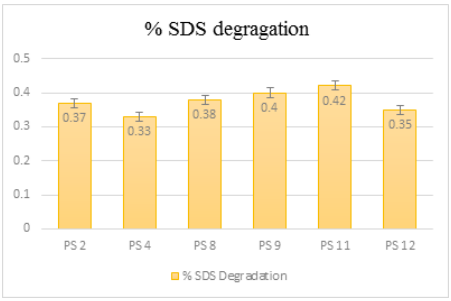
RESULTS AND DISCUSSION

A total of twelve bacterial (PS 1 to PS 12) isolated were obtained from the soil and water samples. Out of twelve bacterial cultures, six bacterial cultures (PS 2, PS 4, PS 8, PS 9, PS 11, PS 12) showed SDS degradation activity. Gram nature and the colony characteristics for all the isolates were studied.

Table 1: Colony Characters of the Isolated bacterial isolates

Isolates	Size	Shape	Colour	Margin	Elevation	Opacity	Consistency	Gram nature
PS1-2	1mm	Circular	Off white	Entire	Convex	Opaque	Smooth	Gram negative rods
PS2-4	1mm	circular	Off white	Entire	Raised	Opaque	Mucoid	Gram negative coccobacilli
PL1-8	2mm	Circular	Off white	Entire	Raised	Translucent	Smooth	Gram positive cocci
PL1-9	1mm	Circular	Off white	Entire	Convex	Translucent	Mucoid	Gram positive rods
PL2-11	2mm	Circular	White	Entire	Flat	Translucent	Mucoid	Gram negative coccobacilli
PL2-12	1mm	Circular	Off white	Entire	Raised	Translucent	Smooth	Gram positive cocci

All six organisms were then grown in minimal mineral salt media supplemented with 1% of SDS for 24 hours. The growth was monitored by visually observing turbidity against the control tube. The culture PS2-4, PL1-8, PL1-9 and PL2-12 showing better turbidity. After 24 hours the quantification of SDS left over is carried out by Methylene blue active substance assay method and it was measured by colorimeter at 652nm.



Graph 1: Degradation of SDS by bacterial isolates at 28°C, pH=7.2 for 24 hours.

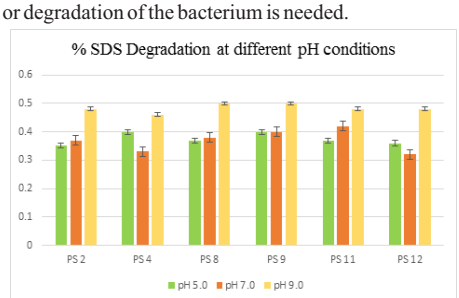
The acquired data suggested that the bacterial isolates were capable to grow at 1% SDS concentration. The isolates PS1-2, PS2-4, PL1-8 and PL2-12 shows higher degradation than PL1-9 and PL2-11. Thus, organisms represented good organisms that can tolerate and degrade SDS to greater extent. Hence this organism was found to be good organisms for further studies i.e. optimization at different pH concentration and temperature.

Othman et al (2019), explore S. marcescens strain DRY6's capacity to breakdown SDS. S. marcescens strain DRY6 was obtained from dirt near the State Museum in Taiping, Perak, Malaysia, and previously demonstrated the capacity to reduce molybdenum to molybdenum blue (Yunus et al., 2009). They demonstrated that S. marcescens strain DRY6 was capable of degrading SDS. SDS-degrading bacteria identified in the literature include Acinetobacter calcoaceticus and Pantoea agglomerans (Abboud et al., 2007), Pseudomonas betelli and Acinetobacter johnsoni (Hosseini et al., 2007), Klebsiella oxytoca (Shukor et al., 2009), Burkholderia sp., and Serratia odorifera (Khleifat, 2006, Khleifat et al., 2010).

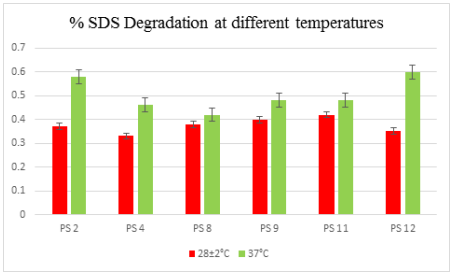
Bacterial degradation of SDS at different temperatures and pH conditions

The bacterial cultures were inoculated into minimal mineral salt medium (pH 7.0), were incubated at room temperature i.e. 28°C and in incubator i.e. 37°C. The bacterial isolates grown at room temperature shows maximum degradation than the isolates which are incubated at 37°C. By this report, it was suggested that the optimum temperature for SDS degradation was a room temperature. All the six isolates were grown in minimal mineral salt medium at room temperature that is 28°C shows better degradation than at 37°C.

All the six isolates were seen to carry out the degradation more efficiently at an alkaline pH of 9.0. The study of pH optimal is important for two reasons. The first is for mass production of the bacterium in bioaugmentation exercise and the second is to assess whether pH adjustment of soil in polluted sites to match optimal growth or degradation of the bacterium is needed.



Graph 2: Bacterial degradation of SDS at different pH conditions

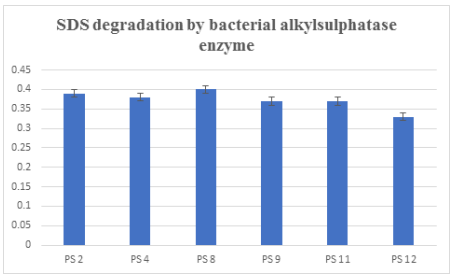


Graph 3: Bacterial degradation of SDS at different temperatures conditions

The temperature range that affects SDS breakdown by bacteria ranges between polar and tropical extremes. Examples include the SDS-degrading bacterium Pseudomonas Strain C12B (Payne and Feisal, 1963), which degrades SDS best at 30 °C. Marchesi et al. (2006) found that the optimal temperature for mesophilic Pseudomonas sp. is 25 °C. Roig et al. (1998) discovered that Comamonas terrigena strain N3H grew best at 28 °C, but Citrobacter braakii and Delftia acidovorans strain SPB1 grew best at 30 °C (Schulz et al., 2000; Dhouib et al.,2003). In contrast, psychrotolerant SDS-degrading bacteria may degrade at far lower temperatures (less than 10 °C) (Margesin and Schinner, 1998). Several additional SDS-degrading bacteria have shown a predilection for neutral to slightly alkaline pH. Klebsiella oxytoca strain DRY14 grows optimally at pH 7.25 (Shukor et al., 2009). Delftia acidovorans strain SPB1 grew best on SDS at a pH of 7.2 (Schul et al., 2000). Citrobacter braakii required pH 7.0 (Dhouib et al., 2003), while Comamonas terrigena strain N3H needed pH 7.4 (Roig et al., 1998). At pH 9.5, the development of S. marcescens strain DRY6 dropped considerably, most likely due to the high alkaline circumstances. Bacteria's capacity to adjust cytoplasmic pH allows them to endure a certain pH range. However, excessively acidic and alkaline circumstances impact the state of ionisation of enzyme active sites, causing changes in the electrical configuration of the active site and eventually inhibiting substrate binding. This results in a loss of activity (Booth, 1985).

Enzymatic degradation of SDS – alkylsulphatase assay

The crude cell extract of enzyme shows degradation in 1 hour of incubation at room temperature. This was almost similar to degradation of isolates carried out in 24 hours of incubation at room temperature. The cell enzyme extract showed better activity, in the crude extract form, than directly incubated isolates.



Graph 4: Alkylsulphatase enzyme activity after 24 hours of incubation

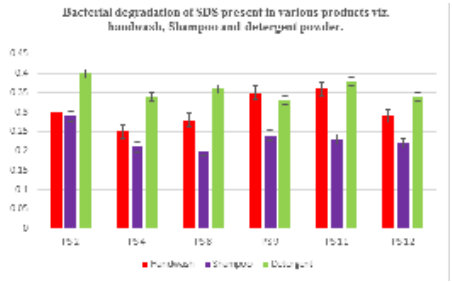
Quantification of SDS degradation using detergent products (containing SDS) and detergent powder (without SDS) by MBAS assay.

Bacterial degradation of SDS was done using a laboratory SDS and it showed that the six isolates are tolerating to the SDS as well as they have ability to degrade SDS with the help of alkysulphatase enzyme.

Whether the bacterial isolates are capable to degrade the detergent products which containing SDS and detergent powder which having other detergents than SDS; this is carried out using liquid hand wash, shampoo and detergent powder.

Degradation Of Liquid Hand Wash

The bacterial isolates were grown in the minimal mineral salt medium for 24 hours of incubation at 28°C and supplemented with 1% of liquid hand wash solution contains SDS as an ingredient. After 24 hours of incubation, the growth occurred in the form of turbidity which reported that, the isolates were capable to grow in medium of liquid hand wash.



Graph 5: Bacterial degradation of SDS present in various products viz. handwash, Shampoo and detergent powder.

Degradation of liquid hand wash

The bacterial isolates were grown in the minimal mineral salt medium for 24 hours of incubation at 28°C and supplemented with 1% of liquid hand wash solution contains SDS as an ingredient. After 24 hours of incubation, the growth occurred in the form of turbidity which reported that, the isolates were capable to grow in medium of liquid hand wash. Therefore, the degradation activity was carried out using MBAS method. The sterile uninoculated medium showed higher reading than the inoculated medium; by this results it was concluded that the isolated bacteria are degrading the liquid hand wash. PS1-2, PS2-4, PL1-8, PL2-12 shows maximum of degradation while PL1-9, PL2-11 showed less degradation by comparing with uninoculated TEST medium.

Degradation Of Shampoo

The bacterial isolates were inoculated with 1% shampoo containing SDS; the assay showed that all the bacterial cultures viz. PS2-4, PL1-8, PL1-9, PL2-11 and PL2-12 were degrading maximum of SDS except PS1-2 showed less degradation as compared to uninoculated TEST medium. The isolates were inoculated in medium supplemented with 1% of detergent powder; after 24 hours of incubation at 28°C showed less degradation. Therefore, it was reported that the bacteria which were grew in medium supplemented with SDS, by the enrichment got only those bacteria which was specifically degrade only SDS and no other detergents. The bacterial isolates could tolerate the other detergents which resulted with the formation of turbidity in medium after inoculation, but they didn't have ability to degrade it.

The high prevalence of SDS in the household and industry has increased the disposal of this anionic detergent into the ecosystem. The influence and relevance of surfactants in man's life is too immense, that totally avoiding them from our day-to-day life seems to be impossible and unpractical. Since anionic surfactants are essential as cleaning and processing chemicals, they are produced in large quantities and, are mostly discharged, after use, in large quantities either to wastewater treatment plants or, in coastal regions, directly to estuaries, seas and oceans. Thus become a great hazard to the environment.

Margesin and Schinner (1998) claimed that their microbe consortia can digest 0.5 to 1 g L-1 SDS in 4 days at 10°C. The tropical isolate Klebsiella oxytoca strain DRY14, isolated from a detergent-polluted location, shows no lag phase during the degradation of 2 g L-1 SDS, showing that the genes for detergent degradation are immediately produced upon contact with a detergent like SDS (Shukor et al., 2009). Better surfactant usage and disposal management is becoming increasingly important, both in the industrial and home sectors. Strict controls should be implemented to ensure successful surfactant treatment prior to disposal. In this scenario, surfactant remediation, particularly bioremediation, is extremely important prior to disposal in the environment.

CONCLUSION

The isolation of SDS degrading bacteria was carried out from soil and water samples contaminated with SDS using enrichment culture technique. Total twelve bacteria were isolated; the occurrence Gram positive bacteria was greater than the Gram negative bacteria. Out of all cultures, PS1-2, PS2-4, PL1-8, PL1-9, PL2-11 and PL2-12 were screened for SDS tolerance using Methylene blue active substance assay method. The PS2-4 observed to be potent culture for study in detail. The Gram staining method and colony characteristics was carried out for identification of Gram nature and morphology of these

culture respectively.

The alkylsulphatase enzyme was extracted from the bacterial cells, and the enzyme activity was determined to comparative study of SDS degradation by bacterial cell and by extracted enzyme with the help of Methylene blue active substance assay method. The enzyme shows maximum activity within one hour of incubation and the isolates from soil and water sample retained their alkylsulphatase enzyme activity in the presence of 1% of SDS in the minimal mineral salt medium. Thus, it can be concluded that there is biochemical link between alkylsulphatase enzyme and SDS degradation.

Proper environmental conditions are fundamentally important for microbial growth and survival, and its relevance in biodegradation in nature cannot be overstated. If environmental conditions such as pH, temperature are not adequate, microbial growth and survival will be adversely affected. Consequently, Biodegradation may not occur at optimal rates. The rate of SDS degradation was found to vary with different temperatures, pH. Therefore, it was concluded that the optimum temperature and pH for growth in SBS media was found to be 30°C and 7 respectively.

The analysis of SDS degradation by all six organisms was carried out by using a different detergent products such as liquid handwash, and shampoo (having a SDS as an ingredient) and detergent powder (having detergent other than SDS) with the help of Methylene blue active substance assay and it shows the degradation of liquid handwash and shampoo but isolates not showed the degradation of detergent powder; by this report it was concluded that the bacterial isolates can be specifically used for the SDS degradation only.

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