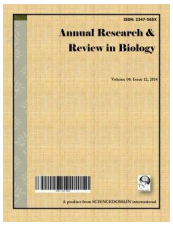

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Biodegradation of Anionic Surfactants (SDS) by Bacteria Isolated from Waste Water in Taif Governate

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Authors' contributions

This work was carried out in collaboration between both authors. Author AGI designed the study, managed literature searches, carried out practical experiments, wrote the protocol and wrote the first draft of the manuscript. Author HEAE performed the statistical analysis and some practical experiments. Both authors read and approved the final manuscript.

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ABSTRACT

Surfactants are synthetic chemicals which are utilized as crude material in cleanser production. Sodium dodecyl sulfate, (SDS) is an anionic surfactant that broadly utilized everywhere throughout the world. Which represent severe hazards effects on the ambient environment. Bacterial strains were isolated from different contaminated sites in Taif Governate (KSA) and screened for SDS degradation. Four bacterial isolates showed high degradation for SDS. The factors that affect the degradation rate of SDS were studied in this work. The selected isolates that can degrade SDS were found to degrade SDS at pH 7-7.5. The optimum temperature was at 30ºC and optimum agitation was at 150 rpm. The degree of SDS degradation was increased when the bacterial isolates

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were combined together. It was found that the four isolates were able to degrade different concentration of SDS until 4%. Different incubation time was studied and it appear that the degradation begin after 24 hrs. but the optimum degradation occur after 15 days. Also different inoculum size was tested. These isolates were physiologically and molecularly identified. These potential strains were biochemically characterized as Gram-negative bacteria. Subsequently, partial sequence of 16S rRNA identified these strains as *Pseudomonas aeruginosa* (H), *Pseudomonas otitidis (A3)*, *Enterobacter cloacae* (A5) and *Klebsiella aerogenes* (A6). This work reveals that the *Pseudomonas aeruginosa* (H), possess greater potential to degrade SDS when compared with other bacterial strains.

Keywords: Anionic surfactant; SDS; biodegradation; isolation; optimization; combination; identification.

1. INTRODUCTION

Anionic surfactants are sets of xenobiotic compounds which contain either sulfonated or ester sulfate groups [1], which are trusted ingredients in a number of professional products such as detergents, cosmetic makeup products, textile industry, agriculture and biotechnology [2,3]. For their large intake worldwide, anionic surfactants contain the potential for huge disposal directly into aquatic and terrestrial surroundings [4]. These chemicals can respond on natural wastewater treatment techniques and cause problems in sewage aeration and treatment facilities because of the high foaming, lower oxygenation potentials and making loss of life of waterborne microorganisms [5]. Large concentrations of surfactants cause skin soreness. The threshold value that can impair aquatic life is 3-12 mg/L. Among the most essential anionic surfactants (by generation volume) are the sodium dodecyl sulphate (SDS), linear alkylbenzene sulfonates (LAS), alcohol ethoxy sulfates (AES), and alkyl sulfates (AS) [6]. Anionic surfactants can attach to bioactive macromolecules, for example, peptides, proteins, and DNA [7]. Official to proteins and peptides may change the collapsing of the polypeptide chain and the surface charge of a particle. This may change biological function [8]. Anionic surfactants such as sodium dodecyl sulphate (SDS) have been use for about 45 years [9]. SDS, in particular, is an essential element of shampoos and foaming agent for toothpaste. Primary criterion for the environmental behavior of surfactants is their biodegradability [10].

SDS has adverse effects on the environment such as making foam on the water surface and avoid the work of oxygen withdrawal of water, make the water smelly and taste, leaving toxic effects on organisms such as humans, water and plants, spread of pathogenic bacteria and help environmental conditions to spread diseases, occurrence of the phenomenon of Eutrophication in terms of consumption of phosphate, and the influence of detergents that rupture membrane of microorganisms leading to the elimination of enzymes [8]. In recent years, the use of bioremediation for the elimination of anionic detergents has been an effective alternative to other different methods, due to its ease and low cost, and the absence of damage to the environment and human [11]. Several authors have announced the danger of SDS and its effects for the survival of aquatic animals, for example, fishes and microorganisms, similar to yeasts and other microscopic organisms [12,13,14]. The target of the present study was to isolate, screen, identify of SDS degrading bacteria from waste water, to obtain the optimum condition for biodegradation, and to determine the degree of biodegradation.

2. MATERIALS AND METHODS

2.1 Isolation of SDS Degrading Bacteria

The water samples were collected from waste disposal sites (Taif Governate, KSA). Water samples transfer to laboratory and will store at 4°C. This water samples were used for the study of anionic detergents degradation capabilities of bacteria. Activated sludge samples obtained from water sewage treatment company in Taif Governate was subjected to 500 mL mineral salt medium, (KH_2PO_4; 3.5 g, $MgCl_2$ $6H_2O$; 0.15 g, dissolved in 1 L of distilled water and the final pH adjusted to 7.1), and containing 1.5 mM sodium dodecyl sulphate ($C_{12}H_{25}OSO_3Na$). The inoculated media were incubated at 30°c with constant Shaking, (150 rpm). After no foams were visible during growth, (due to surfactants utilization), the liquid culture was transferred to solidified, (1% agar) basal salt medium with 1.5 mM SDS in culture plates. Following three

subcultures on the solid media, different bacterial colonies were isolated and identified.

2.2 Screening and Selection of the more Degradable Isolates for SDS Degradation in Liquid Culture

Biodegradation was achieved by methylene blue active substance (MBAS) [15,16]. 50 ml of autoclaved mineral media was taken in sterile conical flasks. 2% detergent was added along with a loop full of inoculum and incubated in rotary shaker at 30°C for 150 rpm. At the end of 3 days, 4 ml of this sample, 4 ml of chloroform and 4 ml methylene blue was mixed well and allowed to settle. The absorbance was measured at 651 nm. Absorbance obtained is a direct indication of the amount of residual surfactant present in the solution.

2.3 Identification of SDS Degrading Bacteria

The identification of the selected bacterial isolates was performed on the basis of macroscopic, microscopic examination and biochemical test according to Bergey's manual of determinative bacteriology [17].

2.4 DNA Extraction and PCR Amplification Conditions

DNA was extracted from the bacterial isolates according to the method described by [18]. Polymerase chain reaction (PCR) reactions were performed under the following conditions: 34cycles of denaturation at 94°C (1 min), annealing at 59°C (1 min) and extension at 72°C (1min). amplification was done using forward primer 5`- AGA GTT TGA TCM TGG CTC AG -3`and reverse primer 5`-TAC GGY TAC CTT GTT ACG ACT T -3`. PCR product was analyzed in 2% agarose gel stained with ethidium bromide. Gels were photographed by Gel Documentation system.

Sequencing steps was performed at Gene Analysis unit, VACSERA. Cycle sequencing was done by using a Bigdye terminator cycle sequencing kit (Applied Biosystems, Foster City). sequencing products were purified by using Centri-sep spin Column and were resolved on an applied Biosystems Model 310 automated genetic analyzer". Phylogenetic and molecular evolutionary analyses of the SDS degrading bacteria based on 16S r-RNA genes were conducted using MEGA version 4 [19].

2.5 Effect of Combination of the Selected Microbial Isolates on SDS Degradation

Examination if the degree of anionic detergent degradation was significantly increased when the isolates were combined together was tested [20]. Into 100 ml mineral salt medium (MSM), SDS was added, followed by the isolates were combined together, 500µl of each isolates (H+A3+A5+A6, H+A3,A3+A6,A5+A6 and A3+A5). The inoculated media were incubated at 30±2ºC for 3 days under shaking at 150 rpm. Degradation was monitored by methylene blue active substance (MBAS).

2.6 Growth Optimization of the Selected Isolates

The selected isolates that can degrade SDS were subjected to various pH values, incubation temperatures and agitations to obtain the best optimization conditions for highly degradation rate.

2.6.1 Effect of pH on the SDS degradation rate

The influence of pH on the growth of the selected isolates was assessed using mineral salt medium (MSM). The pH medium was adjusted at 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 using 1.0 M HCl or 1.0 M NaOH. The prepared medium (100 ml in each flask) was autoclaved and inoculated with one ml (600 nm) of the selected bacterial isolates, and SDS were added, then incubated under shaking (150 rpm) at 30 ±2ºC for 3 days. After incubation period, the activity of isolates for degradation was monitored by methylene blue active substance.

2.6.2 Effect of temperature on the degradation rate

Different incubation temperature degrees were used at 20, 25, 30, and 37ºC for bacterial isolates. Mineral salt liquid medium was prepared and the optimum pH was adjusted as previously mentioned, the medium was autoclaved, inoculated with one ml (600 nm) of the selected isolates and SDS were added and incubated at previously mentioned temperature degree for 3 days under shaking (150 rpm). After incubation period, the activity of isolates for degradation was monitored by the methylene blue active substance.

2.6.3 Effect of agitation on the degradation rate

Different agitation speeds (100, 150 and 200 rpm) were used to determine the best one for high growth rates of the selected isolates.

Mineral salt medium (MSM) was prepared and, the optimum pH and temperature were adjusted as previously mentioned. The medium was autoclaved, inoculated with one ml (600 nm) of the selected isolates and SDS were added and incubated at optimum temperature for 3 days at the different speeds. After incubation period, the activity of isolates for degradation was monitored by the methylene blue active substance.

2.7 Effect of Inoculum Size on SDS Degradation

Effect of different microbial inoculum size on SDS degradation was tested. Into 100 ml mineral salt medium (MSM), SDS was added, followed by (250 µl, 500 µl, 1.0 ml, 1.5 ml, 2.0 ml 2.5 ml, 3 ml, 3.5 ml and 4.0 ml) of each purified microbial culture previously tested for SDS degradation. The medium was incubated at 30±2ºC for 3 days under shaking (150 rpm). The activity of isolates for degradation was monitored by the methylene blue active substance.

2.8 Effect of Incubation Time on SDS Degradation

Into 100 ml mineral salt medium (MSM), SDS was added, followed by one ml (600 nm) of the purified culture previously tested for SDS degradation. The medium was incubated at 30±2ºC for different incubation time (1 day, 3 days, 5 days, 7 days, 10 days and 15 days), under shaking (150 rpm). After the incubation period, the activity of isolates for degradation was monitored by the methylene blue active substance.

2.9 Effect of Different Concentration of SDS on the Degradation Rate

Effect of different conc. of SDS was tested. Into 100 ml mineral salt medium (MSM), one ml (600 nm) of the purified culture previously tested for SDS degradation was added, followed by (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 g/l) of SDS. The medium was incubated at 30±2ºC for 3 days under shaking (150 rpm). The activity of isolates for degradation was monitored by the methylene blue active substance.

3. RESULTS

3.1 Isolation of SDS Degrading Bacteria

The different bacterial colonies (9) were obtained as shown in (Fig. 1).

3.2 Selection of the more Degradable Isolates

The more degradable isolates were selected by methylene blue active substance. There are four isolates (H, A3, A5 and A6) found that were able to degrading high amount of SDS on 30°C. As shown in Figs. 1 and 2.

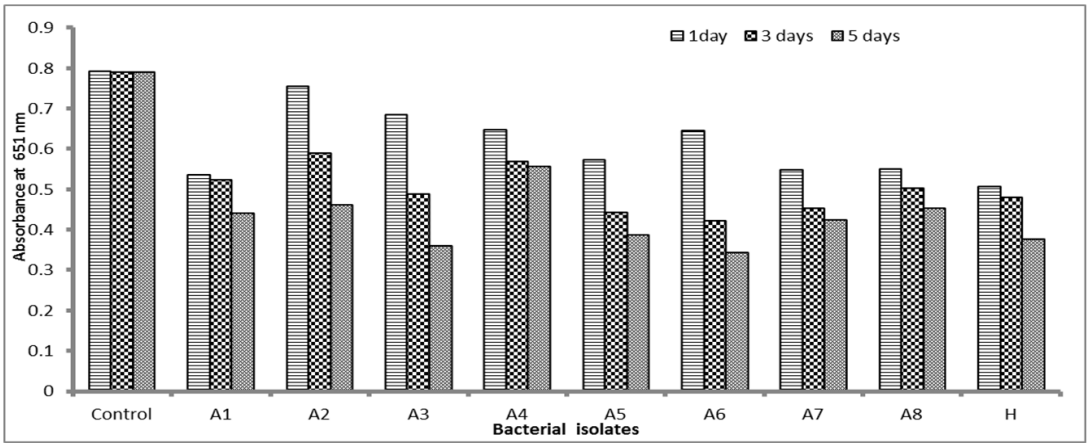


Fig. 1. Absorbance of some bacterial isolates that able to degrade SDS. *Pseudomonas aeruginosa* (H), *Pseudomonas otitidis(A3)*, *Enterobacter cloacae* (A5) and *Klebsiella aerogenes* (A6)

3.3 Isolates Identification

3.3.1 Biochemical tests

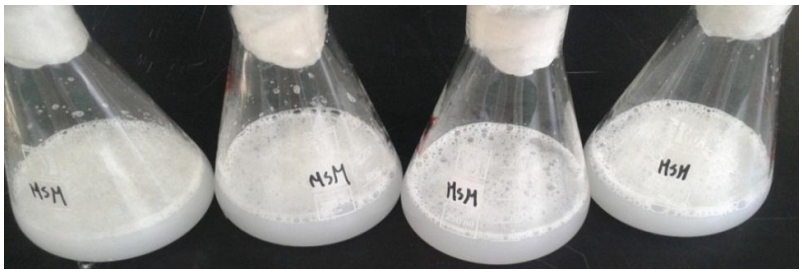
Different biochemical tests were carried out for identification of the four selected bacterial isolates (H, A3, A5 and A6) as described in Table 1, the results revealed that, all the tested isolates were completely different.

The Gram's stain test was carried out for identification of the four selected bacterial isolates (H, A3, A5 and A6) and the results revealed that, all the isolates were gram-negative bacteria.

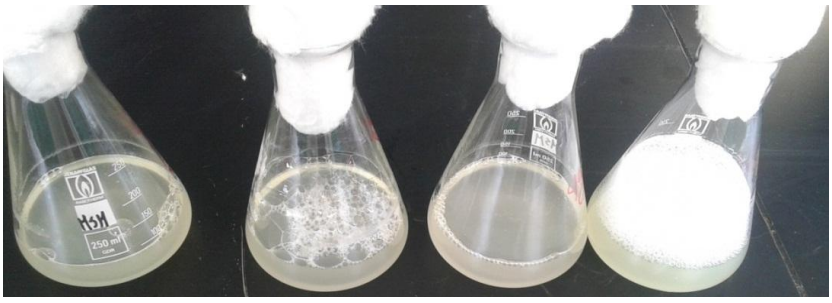
3.3.2 Molecular tools

3.3.2.1 Sequence analysis of 16S r DNA genes

The data presented in Fig. 3, show the PCR amplification results appeared.



A. Before treatment with bacterial isolates



B. After treatment with bacterial isolates

Fig. 2. MSM with SDS before (A) and after (B) treatment with bacterial isolates

Table 1. Biochemical tests of selected bacterial isolates

Test	Isolate H	Isolate A3	Isolate A5	Isolate A6
Gram's stain	-	-	-	-
Shape	Rod	Rod	Rod	Rod
Oxidase	+	+	-	+
Catalase	+	+	+	+
Nitrate Reduction	+	+	+	+
MR	-	+	-	-
VP	-	-	+	+
Gelatin hydrolysis	+	+	-	-
Motility	Motile	Motile	Motile	Non motile
Urease	-	-	-	+
Citrate	+	+	+	+
Indole producation	-	-	-	-
Strain identified	*Pseudomonas aeruginosa*	*Pseudomonas otitidis*	*Enterobacter cloacae*	*Klebsiella aerogenes*

MR/Methyl red test; VP/Voges proskaur test; -/Negative test; +/Positive

3.3.2.2 Alignments and phylogenetic analysis

The selected experimental isolates were identified by partial sequencing the PCR amplified 16Sr RNA genes. The obtained sequences were submitted to the BLAST in order to find a homology with other 16S r RNA sequences. The phylogeny of the bacterial isolates and closely related species was analyze using the multisequence alignment program and the results are presented in phylogenic tree (Figs. 4, 5), showed the similarity between selected isolates and isolates obtained after comparing the sequence of the tested isolates to the submitted sequences in Gen Bank.

The bacterial isolate H was affiliated to *Pseudomonas aeruginosa* where, it revealed 97% similarity to *Pseudomonas aeruginosa* strain ATCC. The bacterial isolate A3 was affiliated to *Pseudomonas otitidis*. Where, it revealed 99% similarity to strain *Pseudomonas otitidis* strain MCC10330. The bacterial isolate A5 was affiliated to *Enterobacter cloacae.* Where, it revealed 99% similarity to *Enterobacter cloacae* strain ATCC 13047. The bacterial isolate A6 was affiliated to the *Klebsiella aerogenes.* Where, it revealed 98% similarity to strain *Klebsiella aerogenes* strain KCTC 2190.

The phylogeny of the bacterial isolates and closely related species was analyze using the multisequence alignment program and the results are presented in phylogenic tree (Figs. 4 and 5).

3.4 Effect of Combination of the Selected Microbial Isolates on SDS Degradation

The mixture of the two bacterial isolates (*H* and *A6*) showed the best ability to degrade SDS more than the individual ones, which appeared as reduction in absorbance at 651nm as shown in Figs. 6 and 7.

3.5 Growth Optimization of the Selected Strains

3.5.1 Effect of pH on the SDS biodegradation rate

In mineral salt medium (MSM), different pHs (4, 5, 6, 7, 8 and 9) were used to determine the effect of pH on the degradation rate for selected bacterial isolates. The results in Fig. 8 showed a relationship between the tested isolates and their degradation rate of SDS at tested pH values. The results in Fig. 8 revealed that the tested isolates showed highest degradation rate of SDS at pH 7-7.5, as shown as a reduction in absorbance at 651 nm. The degradation rate increase when absorbance decrease.

3.5.2 Effect of temperature on the SDS degradation rate

The results in Fig. 9 indicated high degradation rate of the four isolates at temperature 30°C as shown as a reduction in absorbance at 651 nm. The degradation rate increase when absorbance decrease.

3.5.3 Effect of shaking (agitation) on the SDS degradation rate

The data presented in Fig. 10 clarified a high degradation rate of the four tested isolates at 150 rpm, as shown as a reduction in absorbance at 651 nm. The degradation rate increase when absorbance decrease.

3.6 Effect of Inoculum Size on SDS Biodegradation Rate

This experiment demonstrated that SDS degradation was increased with increasing inoculum size (250 µl, 500 µl, 1.0 ml, 1.5 ml, 2.0 ml, 2.5 ml, 3 ml, 3.5 ml and 4.0 ml) of the culture for the selected isolates as shown as a reduction in absorbance at 651 nm in (Fig. 11). However, the reduction appeared by *Pseudomonas aeruginosa* (H) was higher than other selected strains. The degradation rate increase when absorbance decrease.

3.7 Effect of Incubation Time on SDS Biodegradation Rate

Data presented in Fig. 12 showed that the degradation rate of SDS was increased with increasing the incubation time of the four tested isolates. SDS degradation increased as the incubation period increased (1 day, 3 days, 5 days, 7 days ,10 days and 15 days) as shown as a reduction in absorbance at 651 nm.

3.8 Effect of Using Different Concentration of SDS on the Degradation Rate of the Selected Bacterial Isolates

Data presented in Fig. 13 showed that the bacterial isolates were able to degrade

different concentration of SDS (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 g/l), and the isolate H was the most degradable isolate in SDS degradation, as shown as a reduction in absorbance at 651 nm.

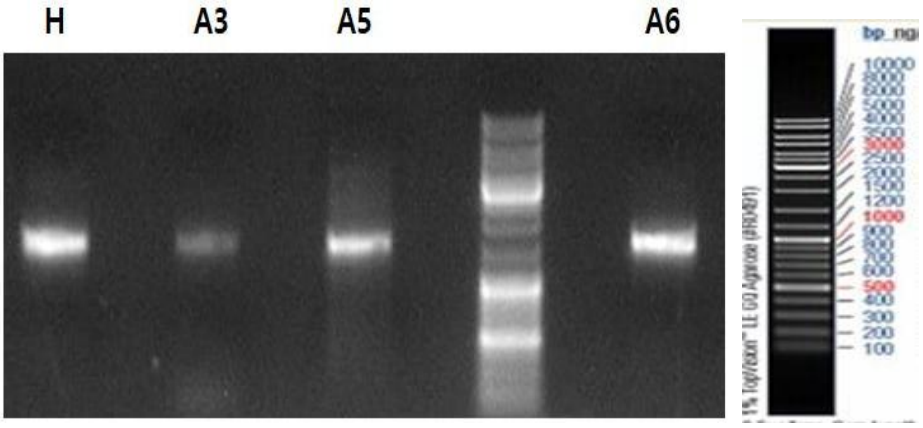


Fig. 3. PCR amplification of the 16Sr DNA gene from the bacterial isolates
Lane4 from left : molecular wt. marker (10000bp)

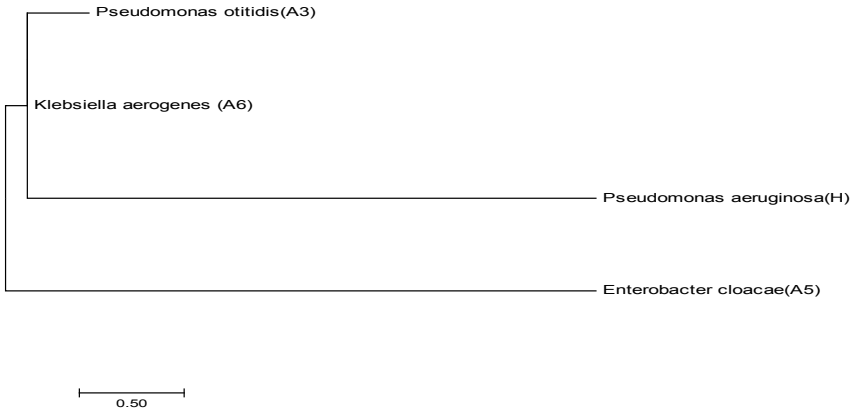


Fig. 4. Molecular phylogenetic analysis between selected isolates by maximum likelihood method

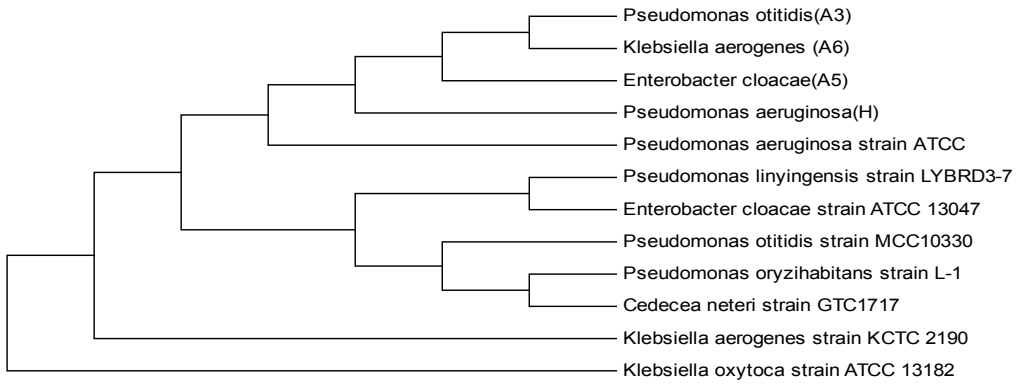


Fig. 5. Molecular phylogenetic analysis showed the similarity between selected isolates and isolates obtained after comparing the sequence of the tested isolates to the submitted sequences in Gen Bank by maximum likelihood method

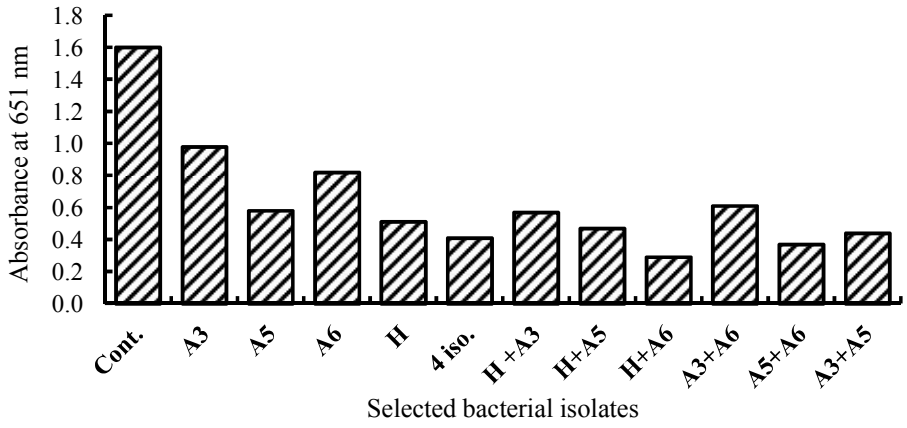


Fig 6. Effect of combination of the selected bacterial isolates on SDS degradation appeared as reduction in absorbance. *Pseudomonas aeruginosa* (H), *Pseudomonas otitidis(A3)*, *Enterobacter cloacae* (A5) and *Klebsiella aerogenes* (A6)

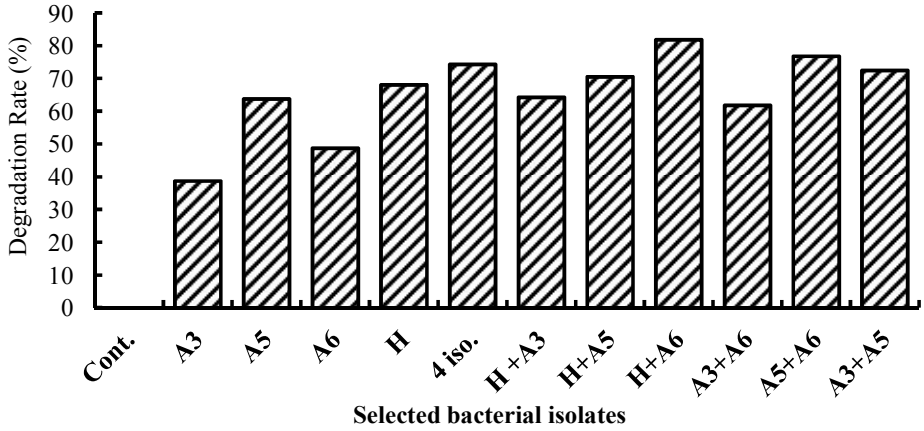


Fig. 7. Effect of combination of the selected bacterial isolates on the degradation rate of SDS. *Pseudomonas aeruginosa* (H), *Pseudomonas otitidis (A3)*, *Enterobacter cloacae* (A5) and *Klebsiella aerogenes* (A6)

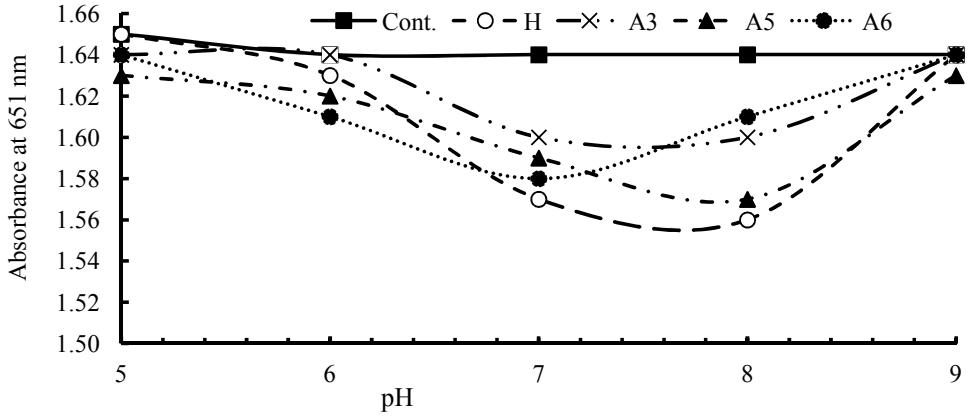


Fig. 8. Effect of pH values on SDS biodegradation rate by selected bacterial isolates. *Pseudomonas aeruginosa* (H), *Pseudomonas otitidis(A3)*, *Enterobacter cloacae* (A5) and *Klebsiella aerogenes* (A6)

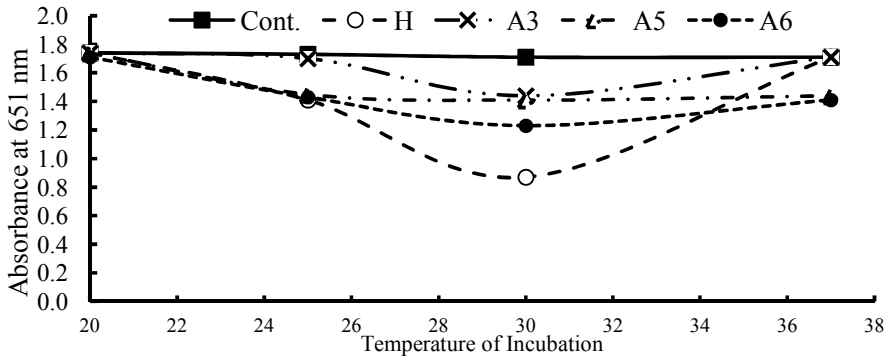


Fig. 9. Effect of different incubation temperature on SDS biodegradation rate by selected bacterial isolates. *Pseudomonas aeruginosa* (H), *Pseudomonas otitidis(A3)*, *Enterobacter cloacae* (A5) and *Klebsiella aerogenes* (A6)

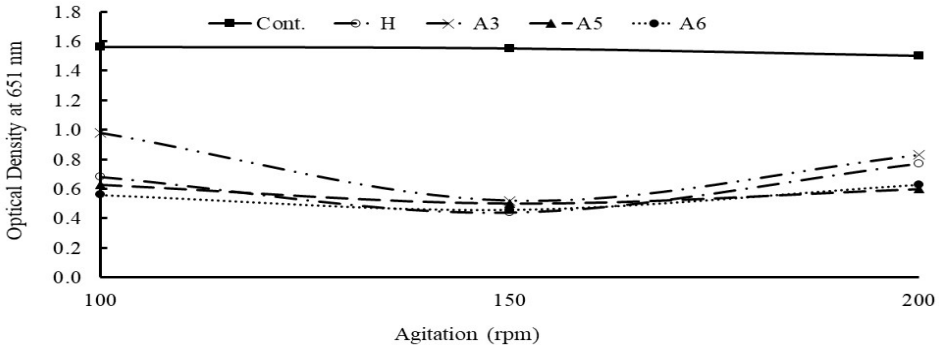


Fig. 10. Effect of agitation on SDS degradation rate by selected bacterial isolates. *Pseudomonas aeruginosa* (H), *Pseudomonas otitidis(A3)*, *Enterobacter cloacae* (A5) and *Klebsiella aerogenes* (A6)

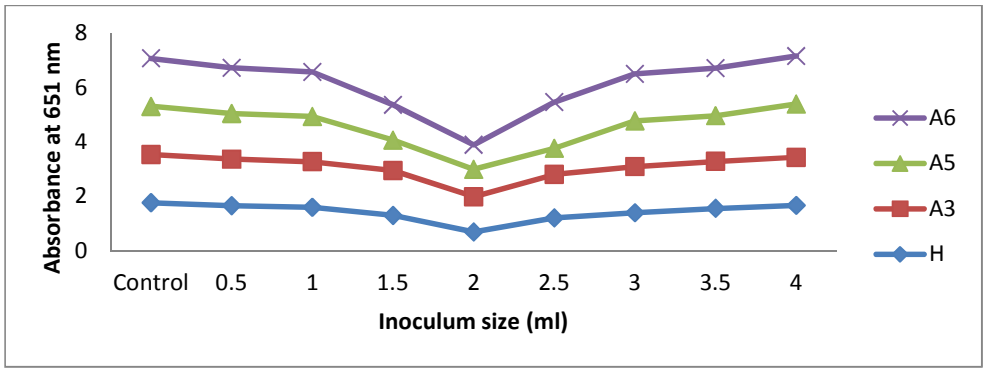


Fig. 11. Effect of the inoculum size on SDS biodegradation rate by absorbance at 651nm. *Pseudomonas aeruginosa* (H), *Pseudomonas otitidis(A3)*, *Enterobacter cloacae* (A5) and *Klebsiella aerogenes* (A6)

4. DISCUSSION

The ability of a combination of microbial strains on SDS degradation resulted in a high degree of SDS degradation. A reasonable explanation of this phenomenon is that, they complement each other in their biodegradation action on SDS surfactants. Thus, each strain alone has failed to produce the same level of surfactants degradation obtained by combined with each other. Furthermore, it was possible to improve the synthesis of two different alkylsufatase

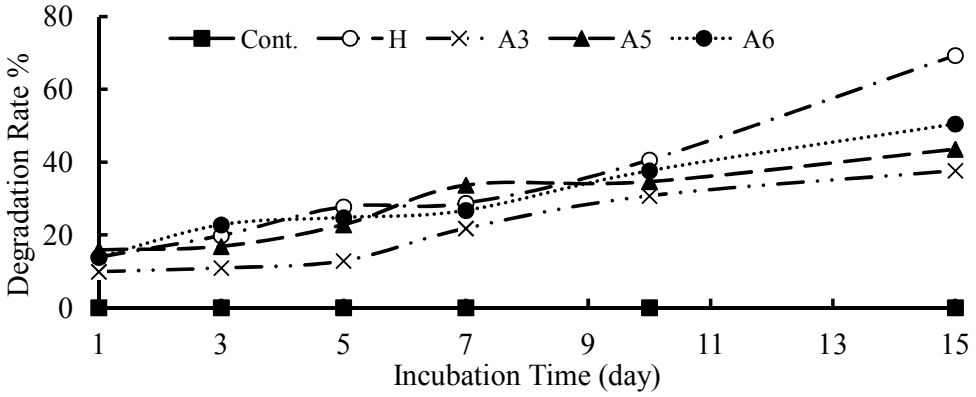


Fig. 12. Effect different incubation time on SDS biodegradation rate by absorbance at 651nm. *Pseudomonas aeruginosa* (H), *Pseudomonas otitidis(A3)*, *Enterobacter cloacae* (A5) and *Klebsiella aerogenes* (A6)

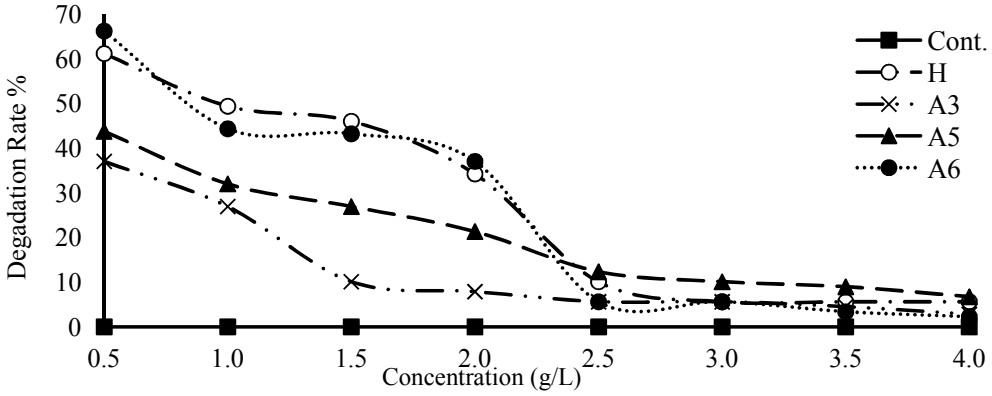


Fig. 13. Effect different conc. of SDS on the degradation rate of the selected bacterial isolates by absorbance at 651nm. *Pseudomonas aeruginosa* (H), *Pseudomonas otitidis(A3)*, *Enterobacter cloacae* (A5) and *Klebsiella aerogenes* (A6)

enzymes that catalyze various mechanisms of sulfate ester bond breakage by growing mixed culture.

These results were agreement with [21], reported the coexistence of constitutive and inducible enzymes with overlapping specificities during growth of different *Pseudomonas* species on 2-butyloctyl sulfate and SDS.

Also a reasonable explanation of this phenomenon is that the strongest strain can produces a biosurfactant and a surface-active compound [22]. Biosurfactants produced by the bacteria are secondary metabolites they seemingly provide nutrient for the growth of the organisms thus enhancing growth of the organism and thereby degradation. The obtained results were agreement with [22] who indicated high degree of polyethylene degradation by a combination of the strains *Sphingomonas* and *Pseudomonas.* Also similar results proved by [23], who indicated that degradation rate of anionic surfactants was increased by *Bacillus subtilis* and *Bacillus cereus* together.

Biological degradation of surfactant is generally affected by various variables. The environmental conditions (e.g. nutrient supply, temperature, pH, shaking) are known to impact the degradation rate [24]. In the present study effect of temperature, pH and shaking on the degradation of SDS were studied.

Best degradation occur at temperature 30^{o}C for selected bacterial isolates, similar study with *Citrobacter braakii*, *Delftia acidovorans* strain

SPB1, *Pseudomonas* strain C12B, *Acinetobacter calcoaceticus* and *Pantoea agglomerans* all required 30°C for ideal SDS degradation [25,26, 27,28], while *Comamonas terrigena* strain N3H showed optimum growth at 28°C [28]. [29] announced that the degradation of SDS by *Pseudomonas* sp. occur at lower temperature.

An essential factor for the degradation activity of bacteria is the pH [30,31,32]. Results obtained in this study showed the highest degradation rate of SDS was at pH 7-7.5 for the tested strains. These results was agreement with [26], who revealed that the optimal growth on SDS is occurred by many SDS-degrading bacteria such as *Citrobacter braakii* at pH 7.0, *Comamonas terrigena* strain N3H at pH 7.4 [*33*] and pH 7.5 to 8.0 for *Pseudomonas* strain C12B [25]. In another report, development on SDS by a novel consortium of *Acinetobacter calcoaceticus* and *Pantoea agglomerans* required pH 8.5 for effective degradation [26]. These results could be taken as pointer for good adjustment to this factor and thusly advantage in utilizing those isolates for bioremediation.

In the present study, shaking effect revealed high growth rate at 150 rpm for the tested strains. Similar results were obtained using different agitation speeds for assessment the development of *Pseudomonas* sp. [34]. Past examinations with *Acinetobacter calcoaceticus* and *Pantoea agglomerans* demonstrated that agitation at 250 rpm improved the SDS and LAS biodegradation [27].

SDS degradation increased with the increase of the inoculum size of the tested cultures. The increase stopped when volume of the cultures was too more and this results was agreement with [35], who reported that poly-ethylene degradation increased with the increase of the inoculum size of the mixed culture (*Sphingomonas* and *Pseudomonas)*. The increase stopped when volume of the cultures was more than 2 mL.

SDS degradation increased significantly as the incubation period increased (1, 3, 5, 10 and 15 days). These results were agreement with [3], who announced that *Pseudomonas beteli* could degrade 94% SDS levels after 120 hours whereas Schlcheck et al. 2003 revealed that 90% of surfactant usage by *Citrobacter sp* within 35 hours of growth.

The effect of SDS as a carbon source to growth of selected isolates was studied using SDS concentrations (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 g /l. The isolates showed an increase in cellular growth as SDS concentrations was increased culminating to an optimum SDS concentration of between 1.0 and 2.0 g/l. The bacterial growth decreased dramatically from 2.0 to 4.0 g/l. SDS as the sole source of carbon is needed in large quantities as carbon is the basic structural unit of all organic compounds. However, at certain concentrations, SDS can be poisonous and dangerous to microorganisms. This is because, in microorganisms, SDS adsorption produces a depolarization of cell membrane and consequently diminishes the assimilation of supplement and modifies the release of substance from cell metabolism. The bacteria may also be killed by the possible detergent effects which strip the lipopolysaccharide outer layer of Gram negative bacteria when the SDS concentration is high [27,36]. Finally, the viability of microorganisms will decrease [37]. The result from this work shows that these isolates required 2.0 g/l of SDS for optimum growth. Previous study showed that *Citrobacter braakii* demonstrated optimum growth at surfactant concentration of 1.0 g /l [26]. Bacterial growth on higher SDS concentration at 4 g/l has been reported [26]. To date, the most tolerant SDS-degrading bacterium is *Pseudomonas* strain CL12B that could grow optimally on 0.025 M SDS or 7.2 g l-1 [25]. In all cases, drastically diminishes at higher concentrations of SDS.

Gram negative bacteria similar to the ones obtained in the present study have been reported to utilize surfactants. [38], reported the isolation of *Staphylococcus scuiri* and *Bacillus cereus* capable of degrading or utilizing surfactants as their carbon source.

The investigation of 16S rRNA partial sequence alignment identified H as *Pseudomonas aeruginosa*, A3 as *Pseudomonas otitidis*., A5 as *Enterobacter cloacae* and A6 as *Klebsiella aerogenes*. The phylogenetic tree demonstrated the linkage of the four isolates; this was constructed using other isolates from GenBank.

5. CONCLUSION

Synthetic surfactants discharged into the water and prevent aeration due to its high foaming and Low oxygenation capacity. Microbes are shown to be an efficient degrader of anionic surfactants. *Pseudomonas aeruginosa* showed a high degradation rate of SDS. The optimum

temperature for *Pseudomonas aeruginosa* was found to be 30°C and pH was found to be7-7.5. Biodegradation efficiency for the removal of synthetic surfactants was increase at 150 rpm, increase with increase inoculum size and incubation time but the degradation rate decrease with increase SDS concentration.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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