ORIGINAL ARTICLE

Microbial Remediation: Harnessing Pseudomonas to Tackle Sodium Dodecyl Sulphate Polluted Waters

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ABSTRACT

Background: *Sodium dodecyl sulphate (SDS) is an ionic surfactant that widely use in daily detergent and cleaning products. It one of the abundance water contaminants. This paper focus on SDS bioremediation using Pseudomonas aeruginosa as a sole bacterial species compared with microbial consortium.* ***Methodology****: Four this purpose, two continuous bioreactors were used to degrade 1gl^{-1} SDS as the sole carbon source. Pseudomonas aeruginosa was isolated from peaty wastewater soil and it was identified by VITEK 2 and 16S rRNA. Nevertheless, in bioreactor A, Pseudomonas aeruginosa was used as a pure bacterial culture while in bioreactor B, a peaty soi contains an indigenous microbial community includes Pseudomonas aeruginosa and other synergetic microbial community.* ***Results:*** *The concertation of SDS was declined to zero in bioreactor B compared with 0.18 gl^{-1} in bioreactor A. GC-MS was used to elucidate the SDS degradation products in the effluent of both bioreactors. Decanoic acid and dodecanal were the main SDS degradation products the effluent of bioreactor A while, a complete mineralization was determined in the effluent of bioreactor B. Scan electron microscope (SEM) magnification image highlighted that Pseudomonas aeruginosa was effectively growth and immobilized on the bio-ball surface that used in bioreactor A which contributed in SDS bioretention.* ***Conclusion****: the conducted experiments finding confirmed that microbial community contains Pseudomonas aeruginosa remediated SDS more efficient than Pseudomonas aeruginosa pure culture.*

INTRODUCTION

Water pollution is the contamination of water bodies, rivers, lakes, oceans, and groundwater by the discharge of harmful substances. This can occur through human activities such as industrial and agricultural practices, sewage dumping, oil spills, and littering [1]. Water pollution has serious impacts on aquatic ecosystems, human health, and water availability[2]. Anthropogenic chemicals are one of the main causes of water pollution owing to the adverse effects of hazardous chemicals and waste products that end up in nearby waterways through runoff or accidental spills [3].

Household waste, cleaning products, detergents, and personal care products can contribute to water pollution when flushed down drains and toilets[4]. Municipal sewage and wastewater treatment plants release untreated or partially treated wastewater into nearby waterways, containing harmful chemicals that leak during the treatment process owing to an inappropriate technique[5]. It is essential to monitor and control the release of these chemicals into the environment through sustainable practices that minimize their impact on water resources [2].

Sodium dodecyl sulfate (SDS) is an ionic surfactant widely used in household detergent products [6]. SDS has a negative impact on water. It is toxic to aquatic life, particularly at high concentrations. Exposure to SDS can lead to detrimental effects, reduced oxygen levels, stunted growth, and even death in aquatic biological systems because SDS persists in the environment for long periods as a source of pollution[7]. Moreover, prolonged SDS exposure in water bodies can negatively affect aquatic flora and fauna. SDS is a surfactant agent that reduces water surface tension and changes the behaviour of aquatic organisms[8]. Water contaminated with SDS causes several health hazards, such as skin and eye irritation, gastrointestinal issues, and even neurological damage. It is important to manage SDS-containing products carefully and dispose of them properly to prevent the contamination of water sources[2].

Bacterial species can tackle various environmental contaminations under different conditions [5,9]. Bacteria can degrade SDS through a process called biodegradation. SDS is a carbon source, which means that certain types of bacteria can break it down into simpler components and use it as a food source. *Pseudomonas* belongs to *the Gammaproteobacteria* genus. This species plays a vital role in the degradation of organic waste. It has versatile metabolic capabilities[8]. *Pseudomonas aeruginosa* has been isolated from various environments. This demonstrates the

appropriate motivation to remediate wastewater contaminants [10]. *Pseudomonas aeruginosa* degrades SDS by producing alkylsulfatase, which breaks down SDS [11]. Bacterial species, SDS concentration, and environmental factors such as temperature and pH are the main aspects that control appropriate SDS biodegradation [12,13]. A recent study confirmed the capability of *Pseudomonas* to mineralize organic chemicals as the sole carbon source[14]. These microbes are robust environmental species that can be isolated from the environment[15]. *Pseudomonas* species are substantial in transforming environmentally hazardous chemicals into less toxic materials that can be released safely into the surrounding environment[16]. Bioreactors can be used in practical engineering applications to tackle various environmental contaminants [17,18]. The present research aim to degrade SDS by continuous bioreactor in an efficient green convenient process and monitor the degradation products.

METHODOLOGY

Microbial isolation, cultivation, and characterization

The sample was collected from a from Al-Rustumiya (Iraq) wastewater peaty soil sedimentation tank at a depth of 10–30 cm using an appropriate bag sample and stored at 8◦C for further use. The peaty soil sample was homogenized before use. The prescribed culture medium was used to isolate and SDS degradation bacteria according to PBM, which contained the following ingredients (gl^{-1}): K_2HPO_4 3.5; KH_2PO_4 1.5; NH_4Cl 0.5; NaCl 0.5; Na_2SO_4 0.14; $MgCl_2.6H_2O$ 0.15. Trace elements were added as following $FeCl_3.6H_2O$ 0.0024; $COCl_2.6H_2O$ 0.004; $CuSO_4.5H_2O$ 0.006; $MnCl_2.4H_2O$ 0.003; $ZnSO_4.7H_2O$ 0.031. It was supplemented with 1 gl^{-1} SDS in a 250 ml culture flask and incubated in a shaker at 37 °C and 150 rpm. After bacterial growth was highlighted (two to three days), 1 ml of the culture was transferred to triplicated plates of PBM agar with 1 gl^{-1} SDS as the only carbon source to highlight SDS-degrading bacterial colonies. Colonies were highlighted after incubation for 2 days at 37 °C [7,12]. The acclimatized bacteria with SDS as the sole carbon source were identified using Vitek 2.

Molecular diagnosis of microorganism

The 16S rRNA molecular technique was used to confirm the VITEK 2 outcome. DNA was extracted according to the procedure described below: the target bacteria were cultured for 24 h. and 5 ml was centrifuged at 12000 rpm for 3 min (18). The generated pellet was resuspended in 200 µL of lysis buffer (40 mM Tris-acetate pH 7, 20 mM sodium-acetate, 1 mM EDTA, 1% SDS) to lyse the cells.

The protein was removed by centrifuging the mixture under colling at 12,000 rpm for 10 min. The mixture was added to 66 µl of 5M NaCl solution. The suspension was transferred to a new vial, and a similar amount of chloroform was added. The vial was gently mixed to obtain a white solution. The supernatant was transferred to a new vial and centrifuged at 12, 000 rpm for 3 min.

Vacuum was applied to dry the targeted DNA, which was precipitated by 100% ethanol, washed three times with 70% ethanol, and redissolved in 50 11 1x TE buffer. The polymerase chain reaction used a Verity Thermal Cycler (Applied Biosystems, USA) Each 10 μL PCR mixture contained 1μL (1:10 dilution) DNA (10 –20 ng), 5 μL PCR GoTaq Green master mix 2X (Promega, USA), 0.5 µl of each primer. Nuclease-free water was used to complete the reaction mixture.

The targeted region of *16S rRNA* was amplified using the appropriate *16S rRNA* primers (forward) 5'-GGAGCTTGCTCCTGGATTC-3' and (reverse) 5'-GATGCAGTTCCCAGGTTGAG -3' (19). The reaction began with a 5 min denaturation step at 95°C and 30 s at 95°C for 35 cycles, annealing for 30 s at 59°C, and extension for 30 s at 72°C, with a final extension step for 5 min at 72°C. Electrophoreses were used to check the PCR result (5 μL of the amplification product) on 2% agarose gel in 1X TBE buffer, stained with 0.5 μg/mL ethidium bromide and UV transilluminator was used to visualize the result.

Bioreactor operation conditions

In this study, SDS was degraded using two bioreactors (acrylic column 25×2.5 cm) as described in figure 1. In bioreactor A, peaty soil (20 g) was the bioreactor substrate, while the isolated bacteria was acclimatized on bio-ball and use as a substrate for bioreactor B. A peristaltic pump was used to feed the A and B bioreactors with 300 ml daily flowrate during 30 days. One liter feeding tank was the influent feeding tank, it was filled with synthetic wastewater containing 1 gl^{-1} SDS as sole carbon source. Effluent water samples were collected periodically for monitoring and assessment.

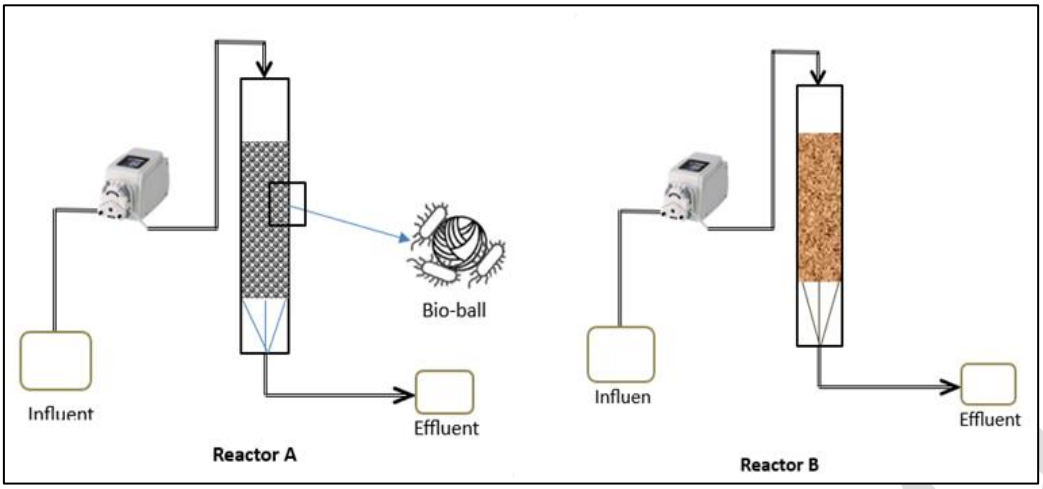

Fig. 1: A schematic diagram for bioreactors A and B.

Gas chromatography mass spectrum (Gc-MS)

GC-MS was used to determine the SDS and their degradation of organic compounds generated during the bioremediation process conducted in both A and B bioreactors. The SDS-degrading spectrum elucidates the role of microorganisms in SDS biodegradation. Gas chromatography-mass spectrometry (7820A, USA) was used to determine the influent SDS and the generated degradation products. The specific column used was Agilent HP-5ms Ultra Inert (30 µm length × 250 µm inner diameter × 0.25 µm film thickness). The operating conditions were as following: 1µl Injection volume, 11.933 psi pressure, inlet temperature of 250 ˚C, AUX heater temperature of 300 ˚C, and helium (99.99%) carrier gas. The oven program was as follows: Ramp1: 65 °C hold to 100 °C. Ramp 2: 60˚C hold to 180 ˚C. Ramp 3: 180˚C to 200˚C. Ramp 4: 200 ˚C hold to 250 ˚C.

Scanning electron microscopy (SEM)

The morphological features and surface characteristics of the samples were obtained using scanning electron microscopy (SEM) with a Hitachi SX-650 scanning electron microscope (Tokyo, Japan). Wide-angle X-ray diffraction (WAXRD) measurements were performed on a D8- Advance XRD diffractometer (Bruker, Germany) at a scan rate of 4° 2θ min−1 from 5 °to 65° with a Cu Kα target.

Spectrophotometric detection

A spectrophotometer was used to determine the periodic SDS concentration during the experiment. The decline in SDS concentration was monitored using the stain-all method, based on the color changes from dark fuchsia to yellowish [19]. A stock stain all solution was prepared by mixing 2 mg of the dye with 2 ml of 50:50 isopropanol and water and stored in a dark container at 5 °C. The solution was used within 30 days. One milliliter of the prepared stock was mixed with a similar amount of formamide and added to 18 ml of deionized water. This solution should be consumed within three days and stored in a dark container. Three milliliters of the effluent samples were mixed with 2.5 ml stain-all solution, and the solution was poured into a spectrophotometer cuvette, and the absorbance was recorded at 510 nm.

RESULTS

The results of PCR amplification for *16S rRNA* demonstrated that a particular band of 556 bp size was present in the isolated strain, supporting the Vitck 2 biochemical test that the bacterium was *Pseudomonas aeruginosa* (Fig. **2**).

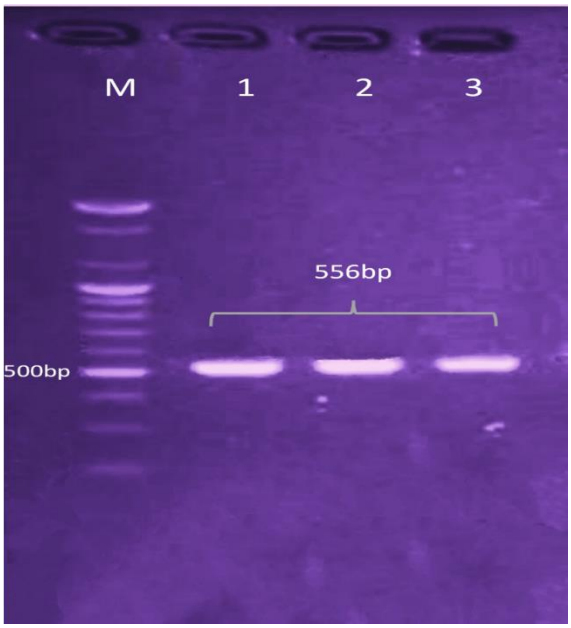

Fig. 2: ***P****seudomonas aeruginosa 16S rRNA* gene PCR products display three replicates of the isolated strain.

The spectrophotometer was set at a wavelength of 510 nm to generate the SDS calibration curve, which was crucial for determining the SDS concentration in the continuous reactor experiment. The SDS concentration was monitored during the continuous reactor experiment, as shown in Fig. **3**. The SDS concentration gradually decreased over time in both reactors. SDS removal was slow in both reactors during the first week. Nevertheless, the concentration of SDS in reactor A, dropped from 1 gl^{-1} to 0.17 gl^{-1} after 30 days, while a completed mineralization was noticed in reactor B.

By inspecting the SDS concentration trendline over 30 d, it was observed that the performance of reactor A, which contained *Pseudomonas aeruginosa* as a pure culture, was slower than that of reactor B, which utilized *Pseudomonas aeruginosa* in addition to the indigenous microbial community in peaty soil. This can probably be attributed to low microbial acclimatization because the only microorganism available was *Pseudomonas aeruginosa*. In contrast, reactor B comprised *Pseudomonas aeruginosa* in addition to the indigenous peaty soil microbial community, which probably increased the acclimatization activity and the contribution of microbial synergism. The influence of microbial synergism could effectively conduct completed mineralization in reactor B.

The GC-MS spectrum of the influent wastewater was analyzed over a retention time of 4–18 min to determine the presence of organic compounds and their degradation outcomes. The time duration before and after this period was ignored for a technical requirement to avoid noise that could influence the accuracy of the reading. According to

Fig, SDS was determined at 12.27 min in the influent wastewater used to feed both bioreactors.

The SDS degradation fragments generated in bioreactor A are shown in Fig. **4**. By examining this figure, it is obvious that the peak of the SDS retreated dramatically compared to the original one in

Fig. The intensity of the SDS peak was detected, which can be noticed by comparing the Y-axis abundance of Figures 4 and 5. In addition, new peaks were generated at 12.77 and 13.18 min, which represented the SDS degradation compound, and the main fragments of this compound were 74 and 91, which were identified as dodecanol and dodecanic acid, respectively (Figure 5). The targeted fragments were dodecanol and dodecanic acid, which are the main SDS degradation products.

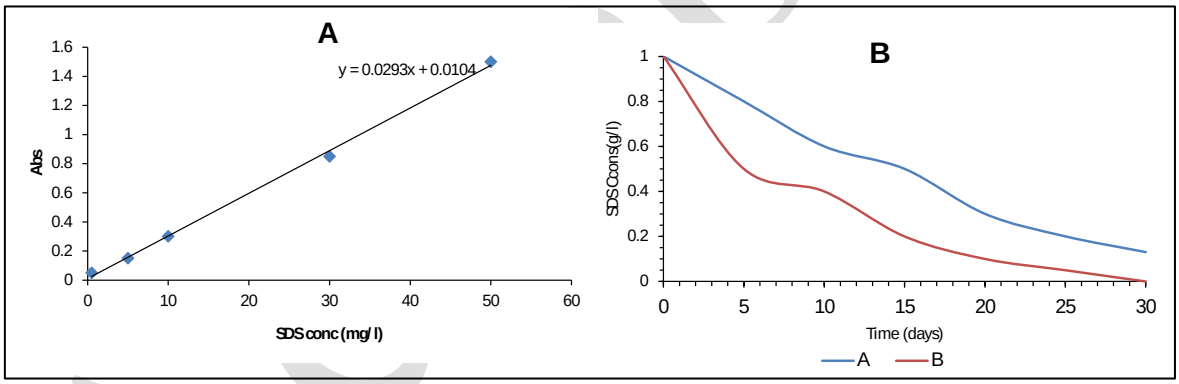

Fig. 3: A spectrophotometer for SDS standard cu was used to determine the SDS concentration. Panel A shows the calibration curve for SDS. While panel B shows the correlation of SDS with time during the column continuous reactors

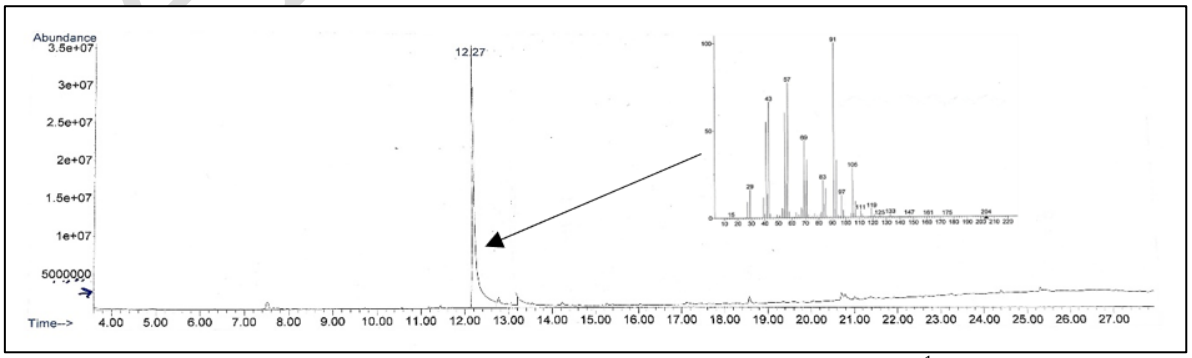

Fig. 4: The GC-MS of the influent contaminated water with at a concentration of 1 g l^{-1} SDS and mass spectrum analysis for SDS gas chromatograph at peak 12.27 min

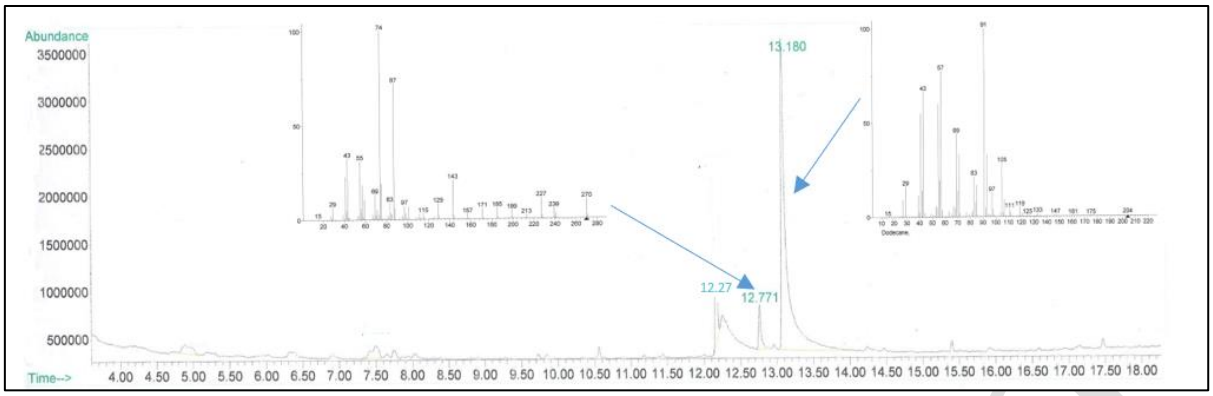

Fig. 4: GC-MS analysis of SDS degradation products by *Pseudomonas aerogenes*.

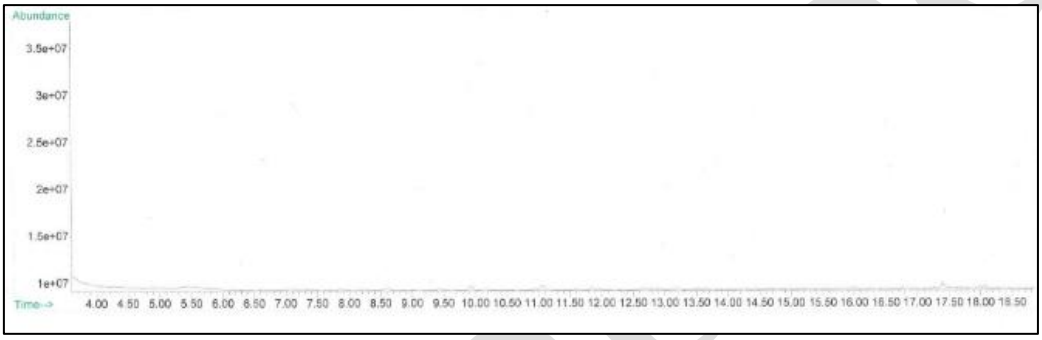

Fig. 5: The gas chromatography of SDS degradation products by bioreactor B.

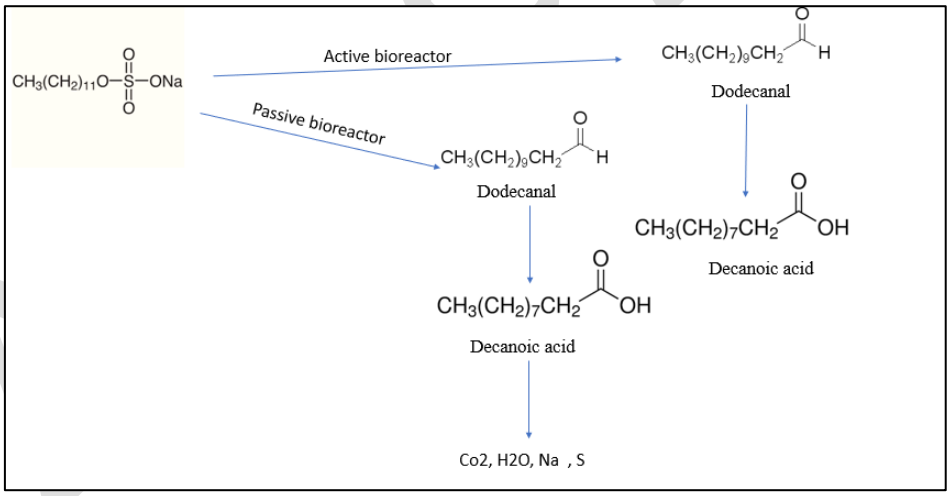

Fig. 6: The SDS degradation products pathway for and bioreactors A and B.

Bio-balls from bioreactor A were selected after the experiment ended. Bio-balls were used to examine the adhesion of bacteria on the outer surface area using a scanning electron microscope. The selected bio-ball was left to dry overnight at room temperature and coated with gold for the best image accuracy. The surface morphology of the bio-ball is shown in Fig. **7**. It can be clearly noticed that *Pseudomonas aeruginosa* was flourished on the surface of the bio-ball. Bacterial colonies are highlighted at 5µm SEM magnification. These adhesive *Pseudomonas aeruginosa* degraded SDS, and the utilized bio-ball increased the treatment surface area, making it an appropriate material for

bacterial biofilms and preventing it from flashing out during the continuous flow rate of the column.

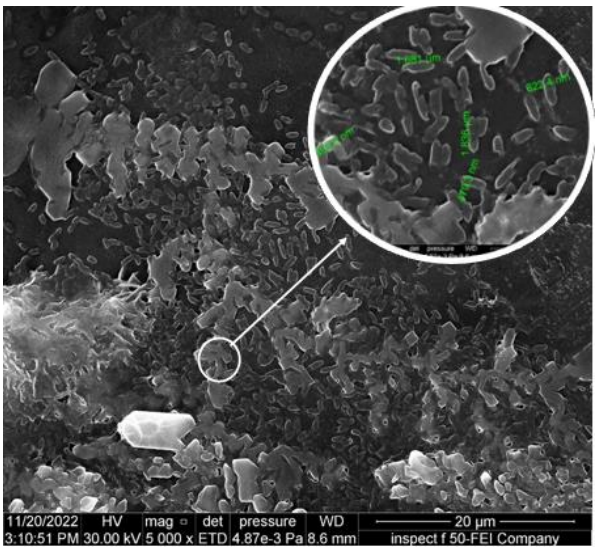

Fig. 7: Scanning electron microscopy image of *Pseudomonas* aeruginosa colonies on bio-balls, which were used as packing material during SDS biomineralization.

DISCUSSION

Pseudomonas species isolated from the environment decomposed approximately 80% of the SDS used in this study. These bacterial species have been described as biotechnological tools for efficient bioremediation approaches[11]. In another study, *Pseudomonas aeruginosa* MTCC 10311, was isolated from contaminated soil and removed approximately 96% of SDS [20].

The current study confirmed that SDS was effectively decomposed by *Pseudomonas aeruginosa* as a sole carbon source, whether it was a pure culture in reactor A or an indigenous species acclimatized with other microbial species when it was present in peaty soil in reactor B. In addition, the presence of this microorganism in the indigenous microbial community accelerated the degradation process and achieved a more efficient outcome. These findings are in agreement with those of previous studies, which also reported SDS degradation as a sole carbon source [11]. Utilizing a mixture of bacteria can be more efficient for SDS bioremediation than using a single microorganism[13]. The SDS colorimetric method using a spectrophotometer was an adequate method to monitor SDS concentration over time [21]. The presence of SDS in a polluted environment with aromatic hydrocarbons supports the bioremediation of aromatic hydrocarbons using bacteria as surfactant agents [22]. Nevertheless, SDS concentration positively influences bacterial viability[6]. The SDS degradation fragment could be a cofactor in the metabolism of the targeted organic hydrocarbons [23]. Bacterial enzyme secretion by *Pseudomonas aeruginosa* is responsible for SDS decomposition[24].

The SDS degradation products for reactor B are shown in Figure 6. In this chromatography, the targeted SDS was completely mineralized, and no side products were highlighted, which was the intrinsic difference compared to reactor A. This finding is in agreement with the spectrophotometer data shown in Fig. **3**, which confirmed that SDS was completely degraded by *Pseudomonas aeruginosa* and the syncretic microbial community in the utilized peaty soil. 1-dodecanal, 2-dodecanol, and 3-dodecanol were highlighted by GC-MS during degradation of SDS by a single pure bacterial culture [11].*Pseudomonas aeruginosa* is a vital bacterial species for hydrocarbon biodegradation; however, the degradation process was reiterated when SDS was added as a surfactant chemical [22]. Nevertheless, the *P. aeruginosa* used in this study was capable of effectively mineralizing SDS, especially when used as an indigenous microorganism associated with other microbial communities.

In the same context, SDS degradation fragment peaks were observed at 8.2 and 14.4 min, and a fragment of 169 corresponded to dodecanal. In addition, the reported information revealed that during SDS bioremediation by *Pseudomonas sp.*, bacterial enzymes such as alkyl sulfatase were responsible for SDS degradation and the generation of dodecanol [23]. Various SDS degradation peaks were highlighted at 12.8 min, 15.8 min, and 16.5 min, and the GC-MS spectrum reported that during SDS bioremediation, dodecanol was oxidized into dodecanoic acid[25]. SDS was degraded by bacteria, and fragment peaks 169, 155, 141, 127, 113, 99, and 85 were represented as C_{11} to C_7 [20]. Dodecanoic acid which distinguished during SDS biodegradation by *pseudomonas sp* was used as a substrate during the metabolic oxidation mechanisms [26]. This might be the reason for not detecting dodecanoic acid in reactor B, as it is used in bacterial metabolism. SDS bioremediation by *Pseudomonas aeruginosa* is probably attributed to the influence of the sulphatase pathway [20]. During SDS detection by GC-MS, dodecene, dodecane, and dodecanol were identified as SDS degradation organic compounds, which are aliphatic products [27]. In another study, it was reported that SDS was noticed at 8.6 min retention time and 1-dodecanol was the main SDS degradation products [28]

According to the GC-MS spectrum, the expected SDS degradation pathway for reactors A and B is illustrated in Figure 8. In reactor A Decanoic acid and dodecanal represented the SDS degradation products. The complete degradation product was determined. This finding is in agreement with Sun et al.[29], Yadav[30], who detected similar degradation products during SDS bioremediation.

In this study, *Pseudomonas aeruginosa* degraded 1 gl^{-1} SDS to decanoic acid and dodecanal as a side product using bioreactor A. The bacteria used were isolated from peaty wastewater soil. Nevertheless, exploiting the peaty soil microbial community, which contains *Pseudomonas aeruginosa* as the main microbial species for SDS degradation, synergistic with indigenous bacterial species, shows that complete mineralization was achieved. Previous research has highlighted that *Pseudomonas* species show various tolerances for SDS degradation under graduated concentrations based on the selected strain and time of incubation. However, the generated degradation compounds cannot be elucidated using spectrophotometric methods[7]. A practical experiment concluded that approximately 36 *Pseudomonas* species could decompose SDS as a sole carbon source under laboratory conditions, which required strict aseptic conditions as a prerequisite for effective outcomes, which imitated the process in plate batch Petri dishes only [11]. *Pseudomonas*, identified by *16S rRNA* as *Pseudomonas laurylsulfatovorans*, has an appropriate attitude to mineralize SDS, but no information was mentioned about the compound degradation products [8]. Additionally, *Pseudomonas fluorescens* can break down SDS, with no evidence reported regarding the related degradation products. *Pseudomonas aeruginosa* has been reported as a convenient bacterium for tackling SDS as a sole bacterial species. All the above-mentioned studies were conducted under laboratory conditions. These conditions make the practical application hard to apply in a convenient approach. Nevertheless, the passive bioreactor used in this study can be applied as a green sustainable substitute for efficient SDS remediation.

CONCLUSIONS

Pseudomonas Aeruginosa was isolated from peaty wastewater and used to degrade SDS using two approaches, passive and active, labelled as bioreactors A and B. Experimental findings revealed that both techniques could degrade SDS; however, bioreactor B was more effective and applicable, as it achieved complete mineralization, compared to 87% in bioreactor A. GC-MS confirmed that complete mineralization was conducted in bioreactor B, and decanoic acid and dodecanal were distinguished in bioreactor A effluent.

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