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# Qualitative and Quantitative Analysis of Sodium Dodecyl Sulphate (SDS) Degradation by Pseudomonas Species

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**Abstract:**
Sodium dodecyl sulphate (SDS), an anionic surfactant widely used in cleaning and cosmetic products, poses significant environmental risks when untreated effluents are discharged into terrestrial and aquatic ecosystems. In this study, a potential bacterial isolate identified as Pseudomonas sp. FD-2aii, which could grow in the presence of SDS, was successfully isolated from industrial wastewater. Bacteria were used in the SDS degradation study based on their ability to utilise SDS as the sole carbon source. Isolate FD-2aii showed growth after incubating at 30 °C with shaking at 150 rpm for 24 h. The residual SDS concentration and percentage of SDS degradation in the bacterial cultures were quantified using a methylene blue active substances (MBAS) assay. This study focused on the reduction in SDS concentration over a ten-day period and provided a clear indicator of the degradation efficacy of isolate FD-2aii.

**Keywords:** Sodium dodecyl sulphate, anionic surfactant, biodegradation, effluent, Pseudomonas sp.

## Introduction

Sodium dodecyl sulphate (SDS), commonly known as sodium lauryl sulphate, is an anionic surfactant with a 12-carbon alkyl tail connected to a negatively charged sulphate group. SDS is the main component of various products used in cleaning and cosmetics, such as dishwashing liquids, hand washes, and laundry detergents(Belhaj et al., 2019). SDS poses environmental risks, particularly affecting the survival of aquatic life forms such as fish, yeasts, and bacteria(Martínez-Jerónimo & Guillermo, 2007). The SDS structure consists of a linear alkyl chain that is susceptible to biodegradation, whereas surfactants with longer or branched alkyl chains exhibit slower biodegradation rates(Knapp & Bromley-Challoner, 2003). The bioremediation of contaminated wastewater containing SDS using microbes is an economical, eco-friendly, and efficient approach. SDS-degrading bacteria are found in various environments including soil, water, and industrial wastewater treatment plants. Bacteria can use SDS as a carbon source and eliminate it from their environment. According to Yadav et al (2021), successfully isolated SDS-degrading bacteria were dominated by Pseudomonas sp. This research focused on a detailed analysis of the SDS-degrading capability of selected bacteria isolated from two new sources, a palm oil mill and textile effluent, using the methylene blue active substances (MBAS) assay.

## Materials and Methods

### Sample Collection

In this study, SDS-degrading bacteria were isolated from industrial wastewater. Samples were collected from a palm oil mill and textile effluents in Johor, Malaysia. The sample temperatures (35–45 ˚C) and pH (4–10) were recorded.

### Bacterial Culture Preparation

The growth medium utilized in this study was a basal salt medium (BSM) composed of 3.5 g/L $KH_2PO_4$, 1.5 g/L $K_2HPO_4$, 0.5 g/L $NH_4Cl$, 0.5 g/L NaCl, 0.14 g/L $Na_2SO_4$, and 0.15 g/L $MgCl_2.6H_2O$. The pH of the medium was adjusted to 7.1 with 1 M NaOH. After autoclaving for 15 min at 121 ℃ and 101.3 kPa, 0.1% SDS as the sole carbon source was added to the medium (Furmanczyk et al., 2017). The chemicals used in this study were of analytical grade. Subsequently, 10% (v/v) of the inoculum was transferred to 22.5 mL of BSM containing SDS at 0.1% SDS. The culture was then incubated for 10 days at 30 ºC with shaking at 150 rpm. The optical density (OD) at 600 nm was used to monitor growth.

### Methylene Blue Substance Assay (MBAS)

The residual SDS concentration in the culture was determined by using a modified version of the MBAS assay described by Ellis et al. 2002. First, 0.1 mL of methylene blue solution was added to 0.4 mL of 0.825 mM phosphate buffer (pH 7.2) and 1 mL of sample in a centrifuge tube. The mixture was vortexed five times for 3 seconds each. Next, 4 mL of chloroform was added, vortexed vigorously for 5 s, and left to stand at 4 ℃ for 5 min. The suspension was then centrifuged at 2000 rpm for 4 min. A dropper was used to extract the chloroform layer. Absorbance of the chloroform layer was measured at 655 nm.

## Results and Discussion

### Quantification of residual SDS in bacterial cultures

Bacterial culture samples were collected over 10 days to monitor SDS degradation using the MBAS assay. Methylene blue, a cationic compound, reacted with anionic-charged SDS to form a complex; chloroform was used to extract the methylene blue-SDS complex. As shown in Figure 1, chloroform was the lower layer and the methylene blue solution was the upper layer of the mixture. The results in Figure 1 show that the intensity of the blue colour of the chloroform layer was changed in the culture. The intensity of the blue colour in the chloroform layer decreased over time, implying that SDS degradation occurred. The uninoculated medium was used as a control, and the intensity of the blue colour remained unchanged. This assay showed that the intensity of the blue colour was directly proportional to the residual SDS concentration in the culture.

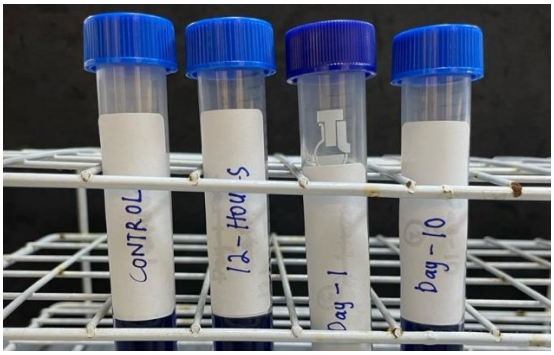


**Figure 1: Results of the MBAS assay following a ten-day incubation period**

In addition, changes in the intensity of the blue colour were quantified by analysing the absorbance of the chloroform layer at 655 nm. Figure 2 shows the lower intensity of the blue colour in the chloroform layer, indicating a higher percentage of SDS degradation.

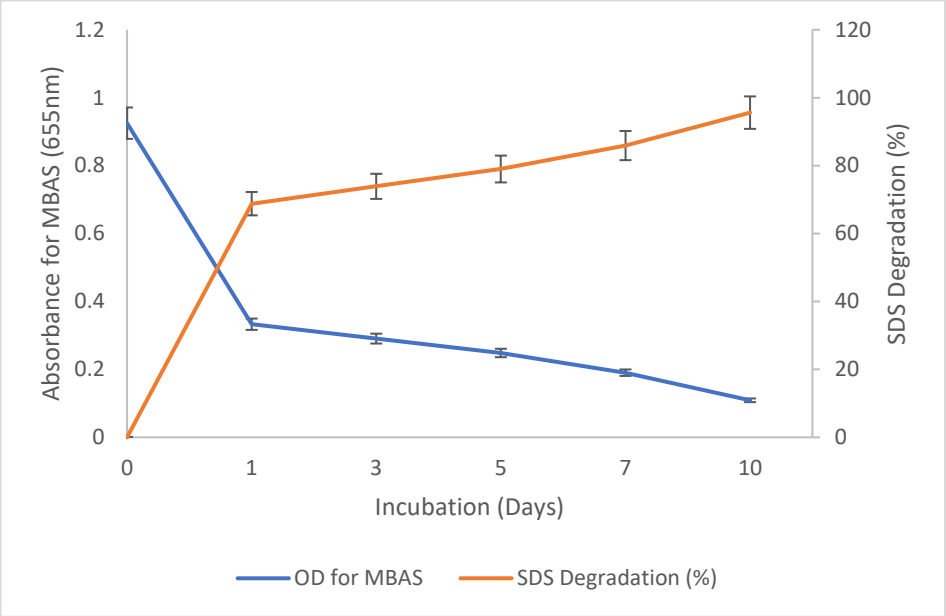


**Figure 2: Intensity of blue colour of the chloroform layer in relation to SDS degradation**

**Degradation of SDS in relation to bacterial growth**

In this study, the ability of isolate FD-2aii to degrade SDS was investigated. Figure 3 shows the relationship between bacterial growth and residual SDS concentration in the cultures. Bacterial growth did not exhibit a typical growth profile, as it showed rapid growth in one day. The growth of isolate FD-2aii was at the end of the exponential phase on the first day, when the maximum absorbance reached 1.421. Several reports have shown that Pseudomonas sp. can grow in the presence of SDS and reach the maximum absorbance in less than 24 h(Chaturvedi & Kumar, 2011; Furmanczyk et al., 2017).

Moreover, as bacterial growth progressed, there was a significant reduction in SDS concentration within the cultures. This suggests that isolate FD-2aii consistently utilised SDS as the sole carbon source for metabolic activity and cellular division. Isolate FD-2aii demonstrated the ability to degrade over 60% of the initial SDS concentration of 0.1% after incubation for 24 h. Compared to the findings of Chaturvedi & Kumar (2011), Furmanczyk et al. (2017), and Hosseini et al. (2007), the maximum absorbance observed in this study correlated with the complete degradation of SDS, surpassing the 90% degradation rate. However, our study found that both bacterial growth and SDS degradation rate showed a sharp increase during the first 24 h and entered a gradual phase, as indicated by a decline in absorbance, concurrent with reduced SDS concentration in the cultures. These results suggest that isolate FD-2aii required a short acclimatisation period and metabolised SDS as a carbon source for growth. However, after one day of incubation, the available carbon in the cultures may have become insufficient for further growth. After ten days of incubation, further degradation was observed, as isolate FD-2aii achieved more than 95% degradation rate.

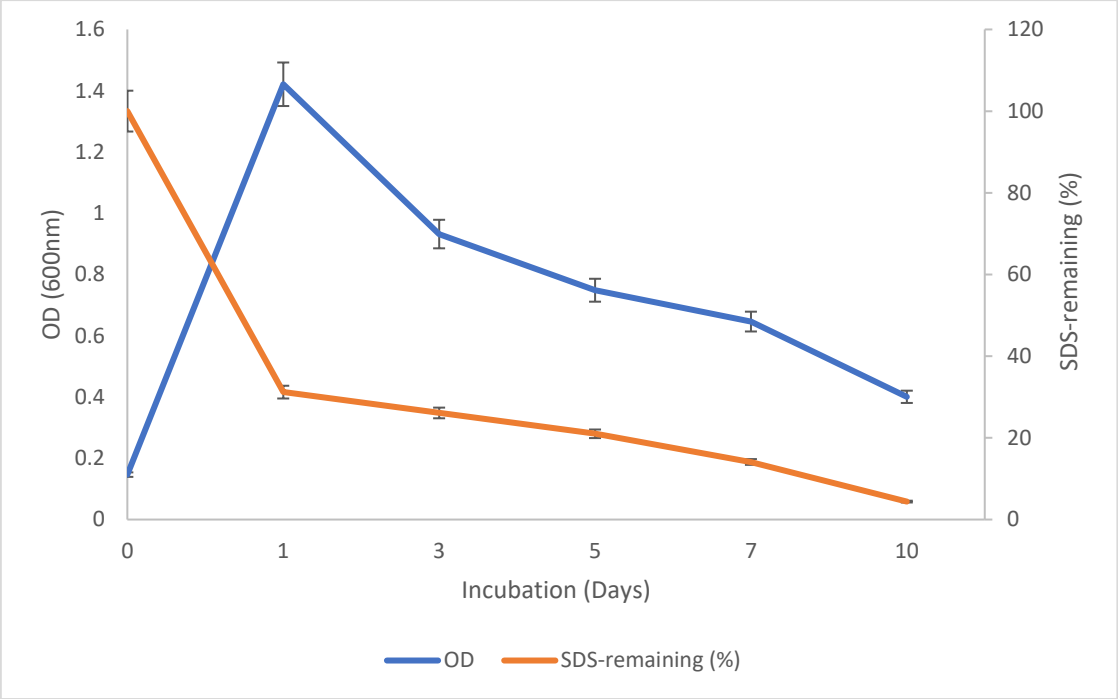


**Figure 3: Correlation of growth (optical density) of isolate FD-2aii in BSM supplemented with SDS (0.1%) and SDS degradation**

## Conclusion

In conclusion, the SDS degradation capability of isolate FD-2aii was qualitatively and quantitatively analysed using the MBAS assay. This assay provides a clear visualisation and an indicator of SDS reduction in bacterial cultures. Isolate FD-2aii was found to degrade the initially added SDS by more than 60% in less than 24 h. Current research for the determination of products using LCMS is in progress.

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