

Bioremediation of surfactants by bacteria isolated from spume used to scavenge surfactants along with dyes in washing textile effluents.

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Abstract: Surfactants are organic chemicals that reduce surface tension in liquids. Surfactants are broadly used industrially and in households, the effluent let out in river water is toxic and there is anticipation of alarming consequences to aquatic organisms. To counter-balance its appalling repercussions, microbial degradation offers a competent approach. For the purpose bacteria were isolated from spumes as they are potent surfactant degraders. Samples were collected from Arabian Sea & Indian Ocean during tides and were analyzed. Samples were enriched in Zobell Marine Broth and were then screened for surfactant degradation using basal medium consisting SDS & Nirma as sole carbon source. Eight bacterial strains were isolated (S1, S2, S3, S4, S5, N1, N2, N3), all tested strains were gram positive, motile except S4, aerobic, rod and cocci, oxidase positive except S4, and requires sodium for growth except strain N1. Best degradation was observed at room temperature, in alkaline conditions after 72hours incubation, maximum degradation efficiency was obtained with strain S1 & S3 -14% and N1-17% using MBAS assay. Better degradation was observed with consortium 16% for SDS degraders and 19% for Nirma degraders. Varying substrate concentrations were used, Nirma degradation was observed at 0.5% and 1% concentration and SDS degradation was observed only with 1% concentration. Finally textile washing effluent was treated and 100% surfactant degradation was observed using consortia, also the dye contained in effluent was degraded which was confirmed by azo reductase activity. The bioremediation of surfactants along with dyes was also seconded by FTIR (Fourier-transform infrared spectroscopy).

Index Terms: Spume, Surfactants, SDS, Nirma, Bioremediation, Effluent, Marine, Dye.

I. INTRODUCTION

This study aims to avert causalities caused by surfactants present in washing effluent, as they are toxic, increase solubility of pollutants & disturbs the equilibrium of aquatic system. The problem can be dealt effectively by biodegradation for which bacteria are isolated from spumes aligning to the explanation that, spume is sea foam or ocean foam formed due to agitation of organic matter present in sea and under turbulence air bubbles are trapped forming a stable foam. This foam serves as a habitat and food source to many marine microbes and thus these microbes can be potent surfactant degraders. Also the harmful dye particles are simultaneously degraded. Surfactants degraded for this study were SDS (Sodium dodecyl sulfate) and NIRMA detergent. The two were chosen as maximum surfactant in waterways hail from laundry industries and most of the industries use higher order detergents that consist of SDS as its prime surfactant. Secondly, most of the poor people cannot afford good quality detergents so they go for affordable ones like NIRMA and again due to lack of resources they do not have water supplies in their houses and wash their utensils and clothes on the river banks. Eventually in the same river water they bathe their domesticates and use water for other activities and there is anticipation of alarming consequences to all living organisms.

II. MEDIA

1. Sterile Zobell Marine Broth (Modified) (Hi-Media Composition)
2. Sterile Basal Agar Medium (Modified) (Jean-Clawde sigoillot *et al.*)
3. Sterile Potassium Requirement Test Broth (Jean-Clawde sigoillot *et al.*)
4. Sterile Sodium Requirement Test Broth (Jean-Clawde sigoillot *et al.*)

III. RESEARCH METHODOLOGY

3.1 Sampling

Foam samples were collected into sterile containers from three sampling sites Rameswaram (Indian Ocean), Tithal Beach & Dandi Beach (Arabian Sea) and were analyzed for pH, temperature, TSS, TDS, Acidity & Alkalinity. Sampling was done during, late onset or early offset of high tides considering the tide charts.

3.2 Enrichment

1ml of samples were enriched in 9ml Sterile Zobell Marine Broth and incubated for 72 hrs. at room temperature and turbidity was checked by measuring absorbance after every 24 hrs. time interval by colorimetric technique at 660nm.

3.3 Screening and Isolation

Enriched samples were screened for surfactant degrading bacteria by providing SDS and NIRMA as sole source of carbon in Sterile Basal Agar Medium by spread plate technique and incubated at room temp. (Jean-Clawde sigoillot *et al.*) Surfactant degrading bacteria developed on Sterile Basal Agar Plates after three days of incubation and were then isolated on the same medium. The colonies were studied for Gram's stain, Motility, Oxidase Test and Sodium /Potassium requirement test.

3.4 Degradation at varying substrate concentrations

Degradation of SDS and NIRMA was tested at various concentrations by providing 0.5%, 1% and 1.5% of NIRMA and SDS in Sterile Basal Agar Broth, incubated at room temperature for three days and turbidity was measured after incubation period at 620nm.

3.5 MBAS Assay

Degradation efficiency of individual strains was tested using MBAS Assay with modified Jack Bell Lab. Protocol. Centrifuge 2ml of sample after adding 0.1mg/ml methylene blue reagent and 2ml alcohol. Absorbance of supernatant to be taken at 660nm. Same experiment was performed using consortia of SDS Degraders and NIRMA Degraders separately. Percentage efficiency was calculated by the following formula.

$$\% \text{ EFFICIENCY} = \frac{\text{CONTROL Absorbance} - \text{SAMPLE Absorbance}}{\text{CONTROL Absorbance}} \times 100$$

As surfactant was degraded absorbance gradually decreased.

3.6 Scavenging surfactants and dyes from textile washing effluent

Textile washing effluent was collected from the washing machine outflow and treated using consortia of SDS degraders and NIRMA degraders separately by adding 10ml inoculate per 200ml of effluent. Surfactant before degradation and after degradation was measured using COD colorimetric technique (APHA guidelines).

Table 1: COD testing

| Tubes | Sample / d/w | Digestion Solution | Sulfuric Acid Reagent | Total Final Volume |
|--------|--------------|--------------------|-----------------------|--------------------|
| Sample | 2.5 ml | 1.5 ml | 3.5 ml | 7.5 ml |
| Sample | 5 ml | 3 ml | 7 ml | 15 ml |
| Sample | 10 ml | 6 ml | 14 ml | 30 ml |
| Blank | 2.5 ml | 1.5 ml | 3.5 ml | 7.5 ml |
| Blank | 5 ml | 3 ml | 7 ml | 15 ml |
| Blank | 10 ml | 6 ml | 14 ml | 30 ml |

After which cod vials were kept in COD digester for 2 hours and then cooled. Absorbance was measured 420nm. The difference between absorbance of a given digested sample and the digested blank is measure of the sample COD.

Simultaneously dye degradation was also obtained that was confirmed using Azo Reductase activity.

Samples were centrifuged at full speed for 15 minutes and azo reductase enzyme was collected in supernatant whereas cells settled down. Azo reductase activity was performed by the following procedure.

Table 2: Azo Reductase activity

| Tubes | Buffer | Reactive black Azo Dye | Enzyme Solution | NADH |
|------------|--------|------------------------|-----------------|------|
| SDS Test | 2 ml | 1 ml | 1 ml | 1 ml |
| NIRMA Test | 2 ml | 1 ml | 1 ml | 1 ml |
| Blank | 2 ml | 1 ml | 1 ml | 1 ml |

After which absorbance was taken at 420nm with an interval of 2 minutes till complete reduction was observed. (Roopa K. B. & Reena Desai).

One unit of enzyme activity was defined as the amount of enzyme required to reduce 1 μ mol of dye per min. under the assay conditions.

3.5 FTIR (Fourier-transform infrared spectroscopy)

To understand the degradation of surfactants along with dye, Fourier-transform infrared spectroscopy was performed for which controls along with degraded samples were analyzed. Infrared absorption spectra were recorded on Thermo Nicolet, AVATAR 330 FTIR system with a spectral resolution and wave number accuracy of 4 and 0.01cm⁻¹, respectively. Total 6 samples were analyzed SDS uninoculated Control (SU), SDS inoculated Sample (SI), NIRMA uninoculated Control (NU), NIRMA inoculated Sample (NI), Azo Dye Control (Azo-D) and Inoculated Effluent dye Sample (IE).

IV. RESULTS AND DISCUSSION

4.1 Screening and Isolation

Total eight surfactant degraders have been screened and isolated, of which five are SDS degraders (S1, S2, S3, S4, S5) and three are NIRMA degraders (N1, N2, N3). The isolates were morphologically characterized by the Gram's Reaction, Motility, Colony Characteristics, Oxidase Test, Sodium and Potassium Requirement Test. All strains obtained were Gram Positive, aerobic rod and cocci, motile except strain S4, oxidase positive except for S4, and requires sodium and potassium both for growth except for strain N1(Requires only sodium) and strain N2 (Requires only potassium). Based on tests strains S1, S2, S5, N2 & N3 are assumed to be *Planococcus halophilus*; strain S4 *Lactobacillus halotolerance* and strains S3 & N1 *Bacillus subtilis*.

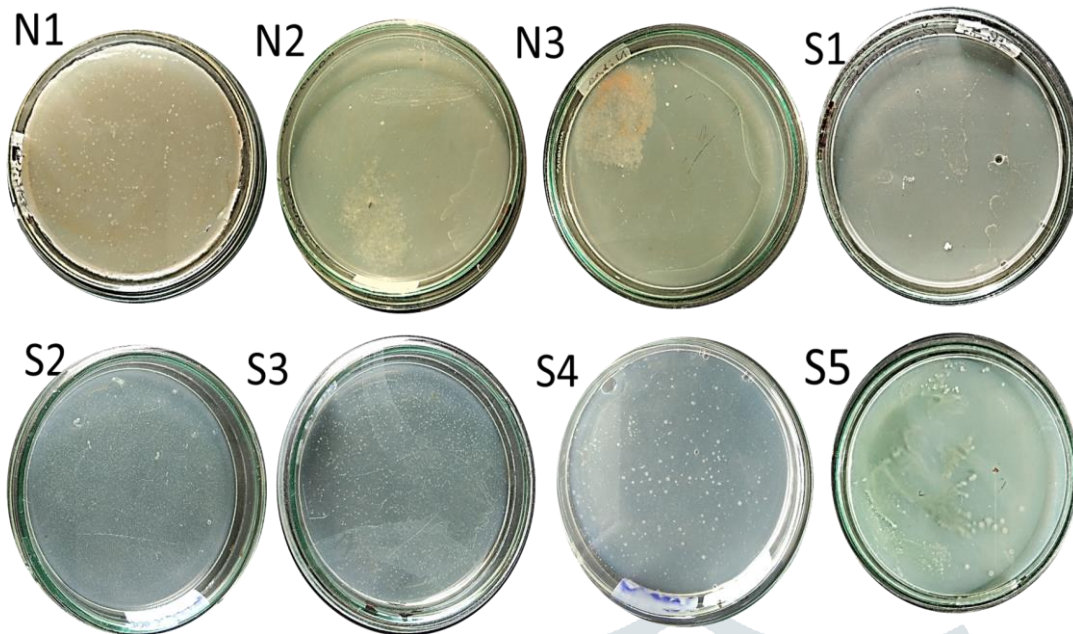


Figure 1: Screening of surfactant degraders

4.2 Results of growth at varying substrate concentrations.

NIRMA was degraded at 0.5% and 1% concentrations while SDS was degraded only at 1% concentration.

4.3 Results of MBAS Assay.

As days passed absorbance decreased as surfactant concentrations decreased due to degradation. Maximum SDS degradation efficiency was obtained with strain S1 and S3 and maximum NIRMA degradation was obtained with strain N1; better degradation was obtained using consortium of NIRMA degraders and SDS degraders.

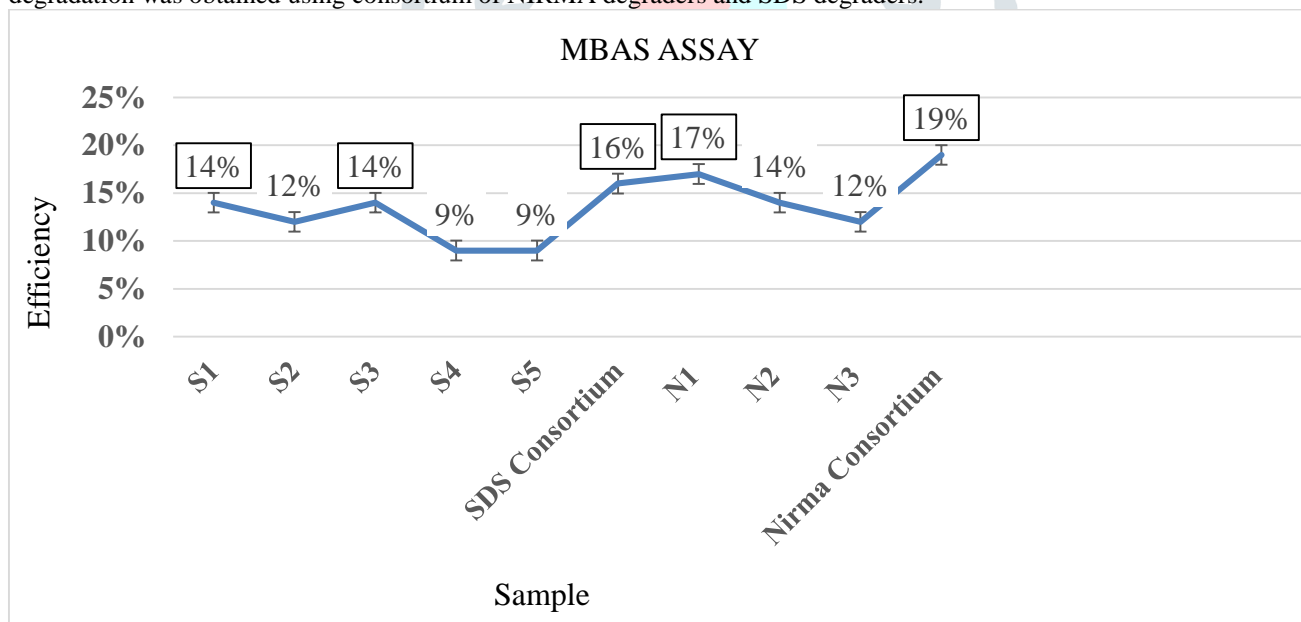


Figure 2: Efficiency testing by MBAS Assay

4.4 Results of treatment of textile washing effluent



Figure 3: Textile washing effluent before and after treatment

100% degradation was obtained after 15 days of treatment.

TABLE 3: COD BEFORE TREATMENT

| | |
|---------|----------|
| VOL 2.5 | 200 MG/L |
| VOL 5 | 184 MG/L |
| VOL 10 | 160 MG/L |

TABLE 4: COD AFTER TREATMENT

| VOLUME | AFTER 72 HRS. | | AFTER 15 DAYS | |
|--------|---------------|----------|---------------|--------|
| | SDS | NIRMA | SDS | NIRMA |
| 2.5 | 200 MG/L | 188 MG/L | 0 MG/L | 0 MG/L |
| 5 | 76 MG/L | 76 MG/L | 0 MG/L | 0 MG/L |
| 10 | 36 MG/L | 31 MG/L | 0 MG/L | 0 MG/L |

As time interval increases azo reductase activity decreases as enzyme is being used up. Total dye reduction was obtained after 8 Mins.

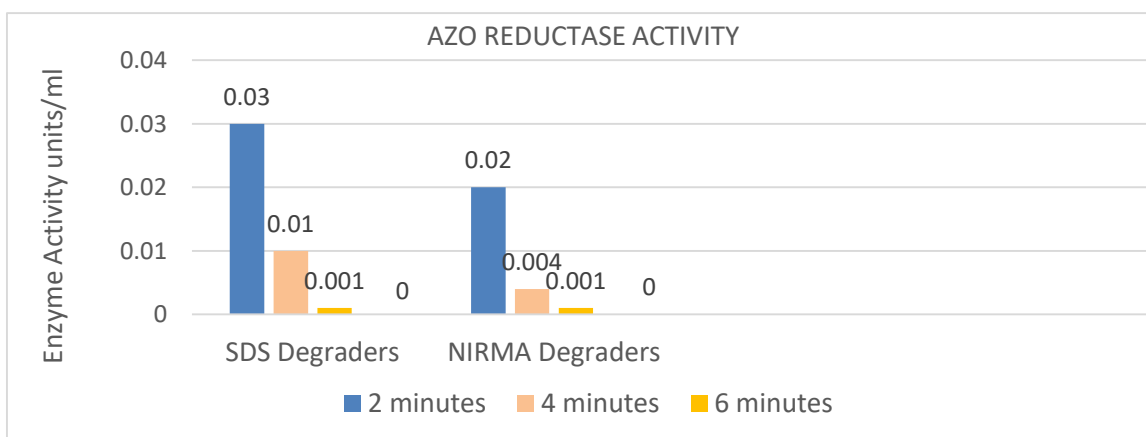
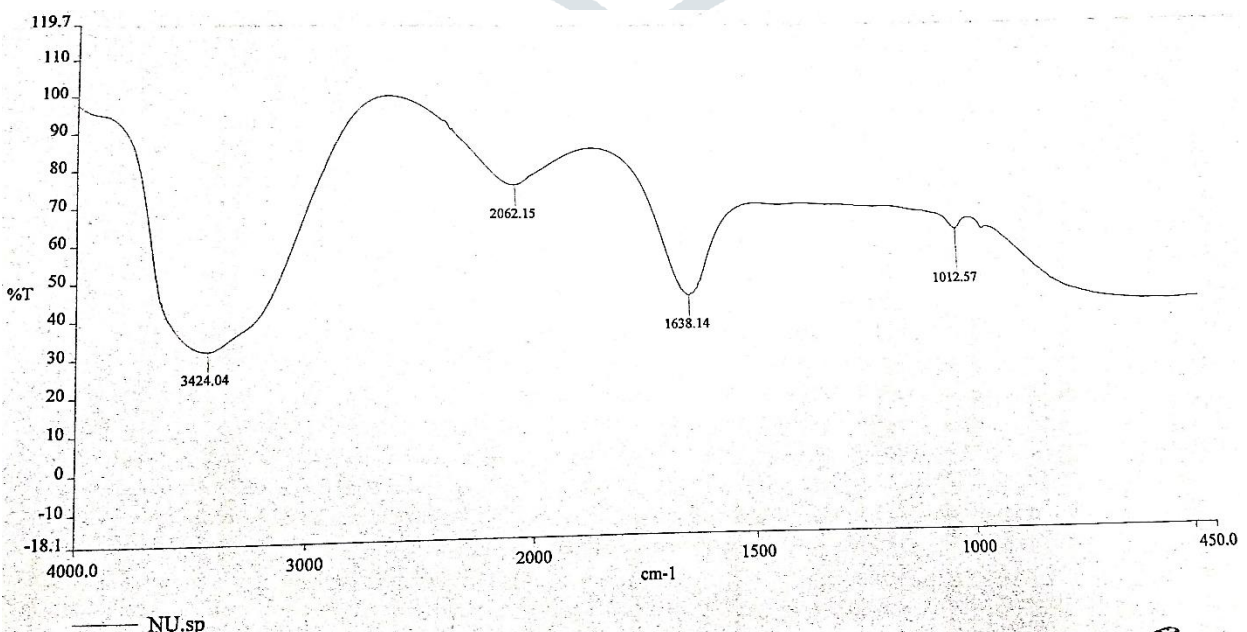
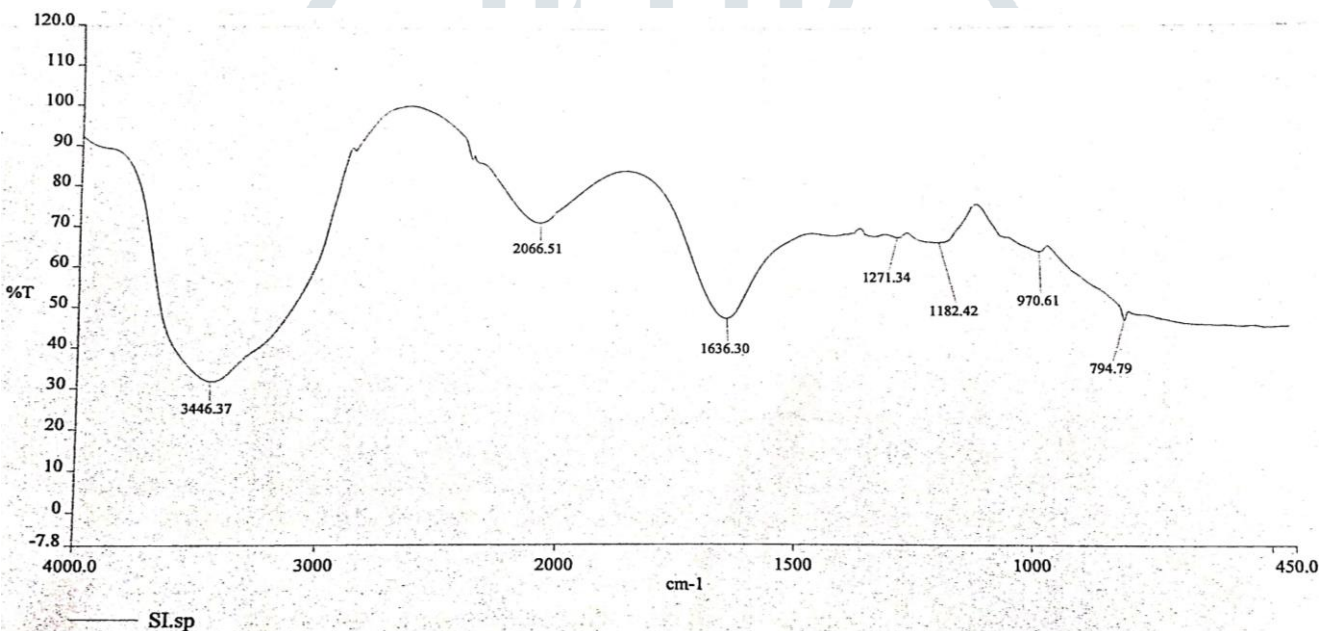
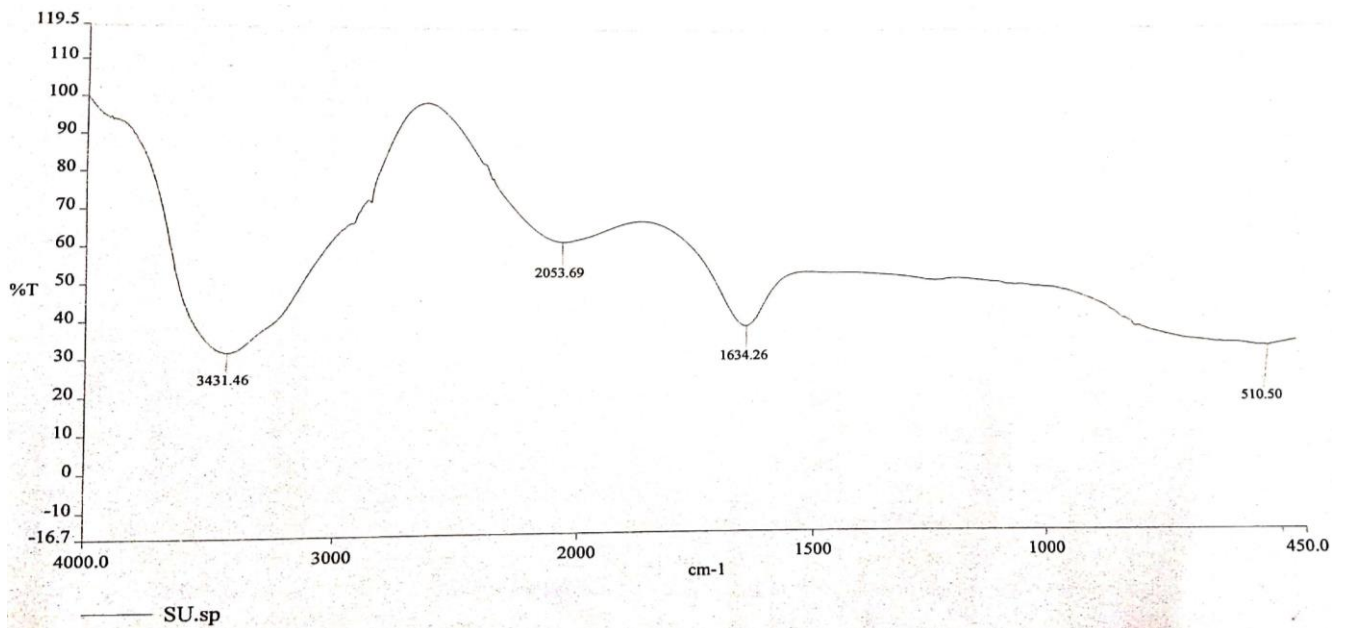
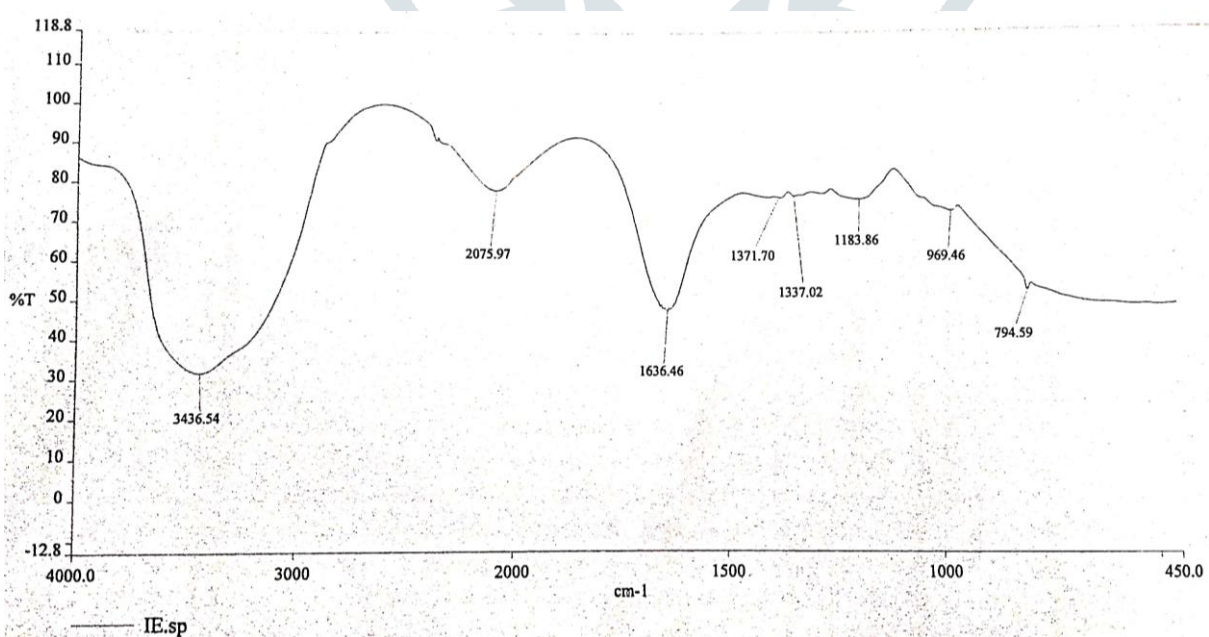
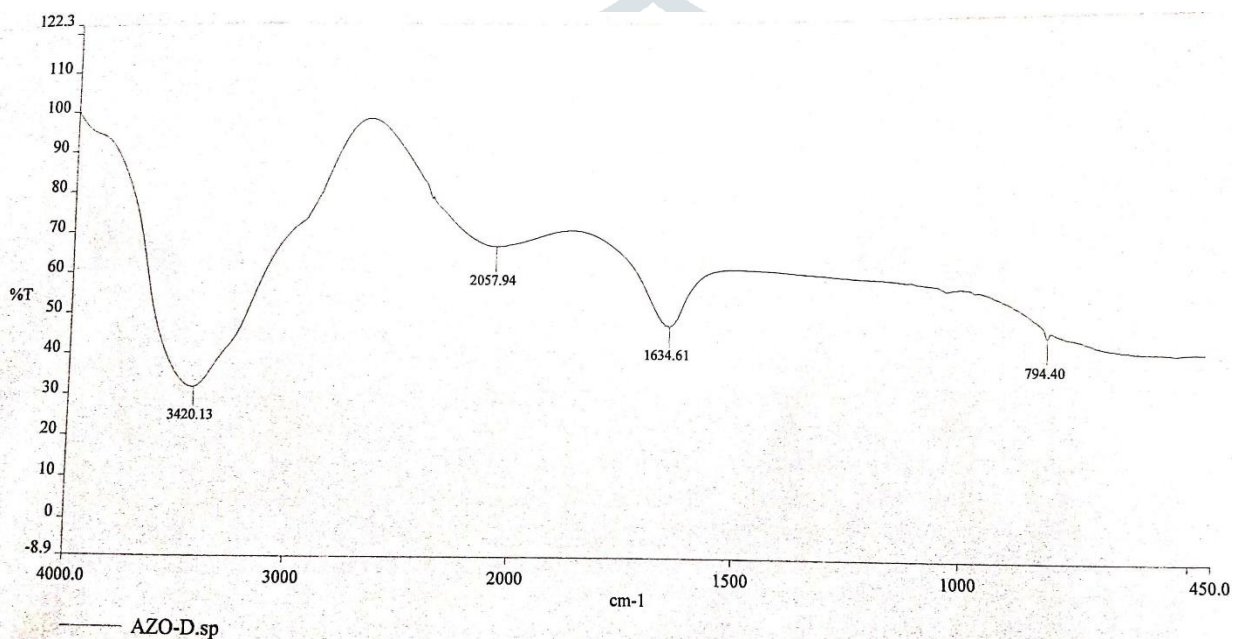
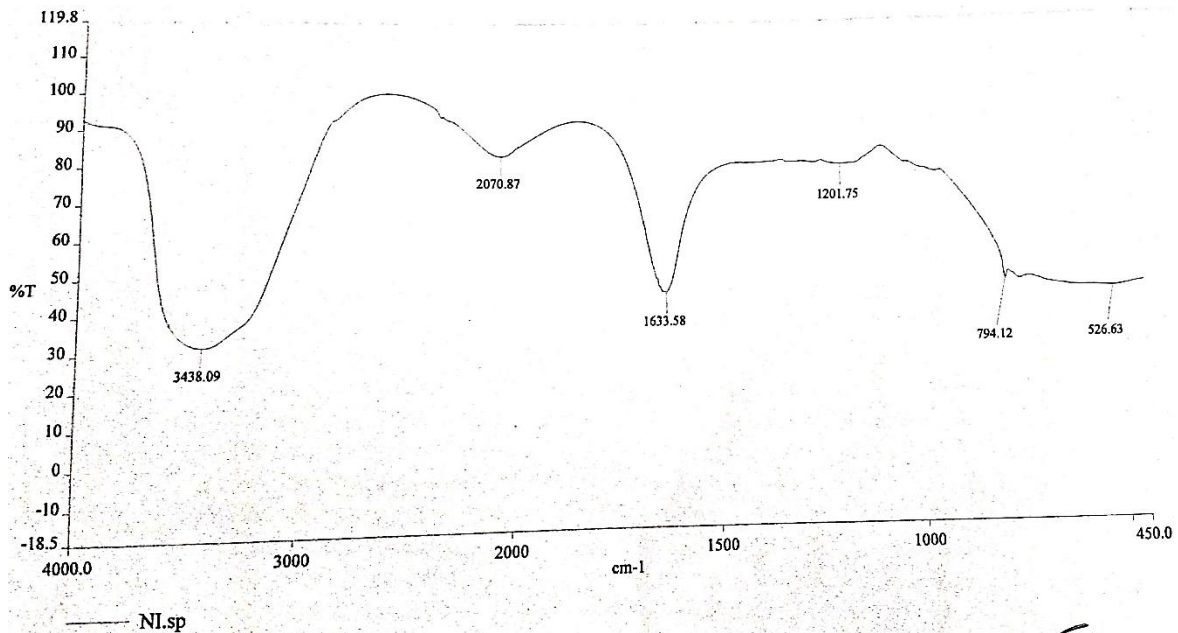


Figure 4: Azo reductase activity

4.5 Results of FTIR (Fourier-transform infrared spectroscopy)





- The control sample of SDS shows a single absorption peak in the low frequency region indicating presence of halocompound while SI-SDS sample shows 4 absorption peaks in the fingerprinting regions indicating presence of ether, carboxylic acid, alcohol, fluoro compound, aromatic rings and 1,2,3 trisubstitutes that may have been obtained as a product of breakdown of SDS thus confirming breakdown.

- The low frequency region of control NIRMA shows a single peak indicating presence of alcohol, ether and fluoro compound while 3 peaks are obtained in NI-NIRMA sample indicating presence of aromatic ring, halo compounds and 1,2,3 trisubstitutes in addition to alcohol, ether and fluoro compound as a part of the breakdown process.
- The low frequency region of Azo-D control shows a single peak indicating presence of aromatic ring and halo compound while IE-DYE sample shows five peaks indicating presence of primary amine, aliphatic nitro, aromatic nitro, fluoro compound, alcohol, ether and 1,2,3 trisubstitutes in addition to aromatic ring and halo compound and absence of azide group obtained as a result of breakdown process.

Thus the differences in the absorption peaks of IR Spectra of controls and samples give an indication of the difference of compound class present before and after breakdown thus seconding the degradation process as a result of which toxic compound are converted to non-toxic ones which are safe to be released into the environment.

CONCLUSION

In the present study eight bacterial strains degrading surfactants have been isolated, of which five are SDS degraders and three are NIRMA degraders. All strains obtained were Gram Positive, aerobic rod and cocci, motile except strain S4, oxidase positive except for S4, and requires sodium and potassium both for growth except for strain N1 (Requires only sodium) and strain N2 (Requires only potassium). Based on tests strains S1, S2, S5, N2 & N3 are assumed to be *Planococcus halophilus*; strain S4 *Lactobacillus halotolerance* and strains S3 & N1 *Bacillus subtilis*. Maximum SDS degradation efficiency was obtained with strain S1 and S3 and maximum NIRMA degradation was obtained with strain N. Better degradation was obtained using consortium of NIRMA degraders and SDS degraders i.e. 19% and 16% respectively. SDS degradation was obtained at 1% concentration and NIRMA degradation was obtained at 05% & 1% concentrations. 100% degradation of washing textile effluent was obtained after 15 days of treatment with SDS and NIRMA consortia. Simultaneously dye degradation was also obtained that was confirmed using Azo Reductase activity. FTIR (Fourier-transform infrared spectroscopy) results also seconded the biodegradation process whereby toxic surfactants and dyes were converted to non-toxic form which are safe to be released into the environment.

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