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Research Paper

Role of Immobilization in Biodegradation of Surfactant Using Bacterial Whole Cell and Purified Enzyme for Water Treatment Planning

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A B S T R A C T

The widespread use of detergents increasing the global concern regarding its environmental pollution brought about by their active ingredients. Since detergents are pollutants, it is essential to degrade them by biological means. In the present study, we reported 16S rRNA gene sequencing-based *S. aureus* SS1 and *P. aeruginosa* SS2 able to degrade >90% of surfactant once immobilized in alginate and agar gel cubes evidenced by reduced total dissolved solids and chemical oxygen demand. Detergent free water received from *S. aureus*, and *P. aeruginosa* treatment also used to grow wheat seed and found to be promising for increasing vigour index.

***Key words*:** Immobilization, Detergent, Degradation, Plant growth

The toxic nature of surfactants making them potent pollutants. The detergent containing wastewater is rich phosphorous and can impact severely on the environment [1]. Detergents now widely concentrated in soil and water hence directly affects ecosystem nearby. Detergents can lead to foaming, eutrophication, and able to alter the pH, temperature and surface tension, and their negative effects need to be managed by various means [2]. Detergents are reported to severely affect fish proteomics, especially of serum, liver and heart tissues. Hence the presence of detergents in the aquatic ecosystem needs to be tackle by scientific approach [3]. In a natural remedy agent like bacteria now extensively studied to degrade detergents under various model studies. The bacterial species like *Pseudomonas sp.*, *Micrococcus luteus* and *Citrobacter sp.* noted as detergent degrader once isolated from freshwater bodies [4]. Sodium dodecyl sulphate degrader identified as *Bacillus cereus* strain reported for surfactant removal from water bodies [5]. In the present study efficiency of immobilized detergent degrader, bacterial species/derived enzyme noted for detergent degradation. Further treated water checked for wheat plant growth to record the overall performance of bacterial species under investigation and to note the role of immobilization carried out by alginate and agar.

MATERIALS AND METHODS

In the present study, detergent degrader bacterial species present in the contaminated water sampled and analyzed for their enzyme activity and water treatment possibility.

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Isolation of Bacterial species

The high concentration of detergent in water, creating an environment for the bacterial species to survive and able to degrade detergent. Hence attempt been made to sample water from Pune city and based on culturing to the basal medium broth added with 1% detergent, and isolates been enriched. The preparation kept static for 48 hours at 30°C during process. Upon incubation, 1 ml of sample inoculated on basal medium agar supplemented with 1% detergent and incubated at 30°C for 48 hours to record the bacterial growth.

Isolation of Bacterial species by 16S rRNA: The isolate obtained from the water able to grow in the presence of detergent further identified by 16S rRNA gene sequencing as per protocol reported by [6].

Immobilization of Bacterial cell and enzymes

As per 16S rRNA gene sequencing isolate SS1 identified as *Staphylococcus aureus* and SS2 as *Pseudomonas aeruginosa.* Both of the isolates able to express alkyl sulphatase enzyme capable of degrading alkyl sulphate (synthetic surfactant) majorly used in industry and categorized as a pollutant. In the study effect of immobilization on the degradation of 1% SDS by the enzyme/cells noted by MBAS assay. The detailed protocol has given below:

Immobilization with sodium alginate beads: In the preparation of 3% sodium alginate suspension weighed 0.9 g of sodium alginate dissolved in 30 ml boiling water and autoclaved at 121°C for 15 min. The cooled suspension then added with 47 µl cell suspension and kept stirring for 10 minutes. The mixture then transferred to a sterile syringe and added to chilled 0.2 M $CaCl_2$ solution from 5 cm height with constant stirring. Obtained beads then kept for curing at 4°C for 1h in the refrigerator. Beads then washed with sterile distilled water and kept preserving in 0.9% NaCl solution at 4°C.

Immobilization with agar blocks: In a 2% of molten agar about 47µl of cell suspension added at 40°C. The solution then poured in sterile flat-bottom Petri plate to solidify. Solidified agar blocks cut in equal size cubes and added to 0.1 M phosphate buffer (pH 7.0) and kept at 4°C.

Enzyme preparation: Similar to the cell suspension immobilization, the enzyme also been immobilized to get calcium alginate and agar blocks and further stored at 4°C till use.

Preparation of column and MBAS assay: Columns of alginate beads and agar blocks filled with 1% SDS for its degradation by saturating column for half an hour. After saturation, fractions were collected at an interval of 5 min till 90 minutes to record % degradation by MBAS assay (Protocol not shown).

Plant growth response of detergent-free water

Preparation of water samples: In order to prepare detergent-free water, contaminated water from household source divided into three parts.

In a first set, 100 ml of water loaded with 0.5 O.D. of *Staphylococcus aureus*, in a set 2 household water loaded with 0.5 O.D. of *Pseudomonas aeruginosa* and in control set 3 only household water used. All these sets kept incubating at 30°C for ten days. Upon incubation relative change in total dissolved solids, chemical oxygen demand initially recorded.

Plant growth in the presence of treated water

The detergent-free water of set 1 and set 2 along with control set (set 3) used for watering wheat seeds using cocopeat as organic manure. During plant growth, watering of all sets scheduled every fourth day per pot (vol. 20 ml) and overall growth performance recorded as shoot length (cm), root length (cm), chlorophyll content, % seed germination and vigour index.

RESULTS AND DISCUSSION

Isolation of detergent degrading bacteria

The contaminated water of Pune city once checked for detergent degrading bacteria on basal media containing 1% detergent content, two isolates gram stained Gram-positive and Gram-negative prominently able to utilize detergent as carbon source. Those are coded as isolate SS1 and SS2, as shown in (Fig 1).

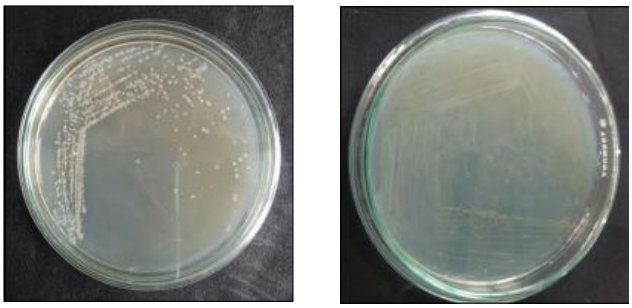

Fig 1 Growth of two detergent degrading bacteria on basal media supplemented with the 1% detergent

16S rRNA gene sequencing and homology

Based on the gene sequencing for the 16S RNA region, isolate SS1 and SS2 registered close homology with *Staphylococcus aureus* and *Pseudomonas aeruginosa* as represented by phylogram in (Fig 2-3).

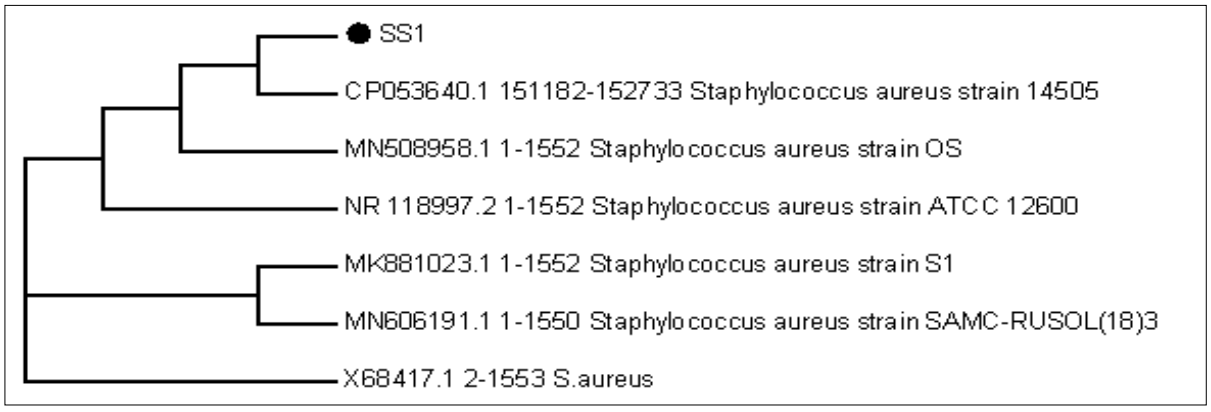

Fig 2 16S rRNA gene sequence homology confirmed isolate SS1 as *Staphylococcus aureus*

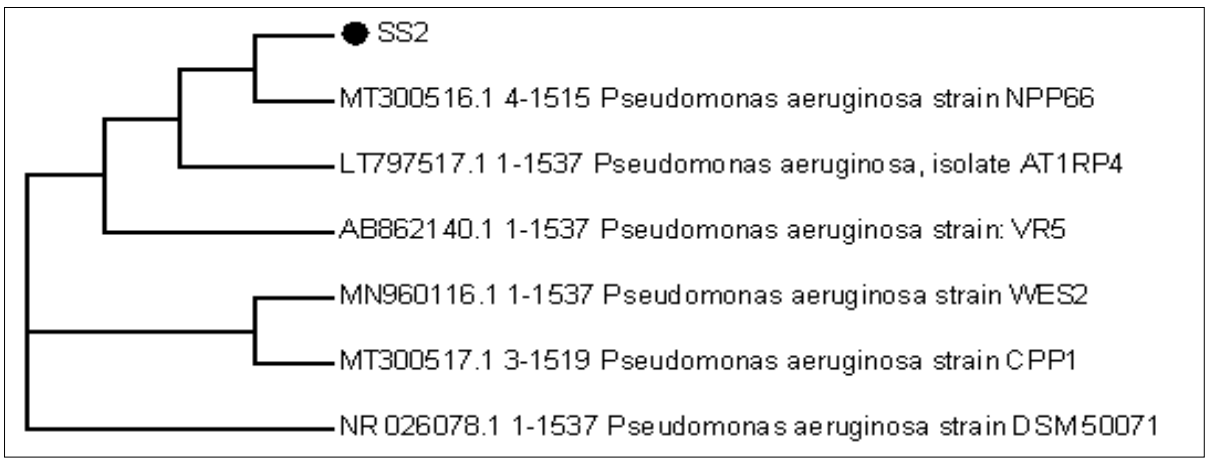

Fig 3 16S rRNA gene sequence homology confirmed isolate SS2 as *Pseudomonas aeruginosa*

Performance of immobilization of cells and enzymes for degradation

Staphylococcus aureus: *S. aureus* able to produce alkyl sulphatase once immobilized as the whole cell in alginate cells beads and agar cell block, noted maximum degradation of 1% SDS recorded up to 84.07% and 84.07%, respectively. Similarly, a purified fraction of alkyl sulphatase once immobilized by alginate beads and agar blocks, its improved degradation of 1% SDS noted with values reached at 96.83% and 95.45%, respectively (Table 1).

Pseudomonas aeruginosa: *P. aeruginosa* capable of producing alkyl sulphatase once immobilized as whole-cell beads in alginate and agar medium, obtained per cent degradation (1% SDS) noted as 92.37% and 96.81%, respectively. Further, purified alkyl sulphatase immobilization in alginate beads and agar gel blocks noted with improved per cent degradation of 1% SDS as 97.73% and 88.93%, respectively indicated that use of agar block for the whole cell and alginate beads for free enzyme performed well as shown in (Table 2).

Table 1 Degradation of 1% SDS performance recorded with whole cell immobilization of *S. aureus* and its purified alkyl sulphatase in alginate beads and agar gel blocks

S. No.	Time (min)	5	10	15	20	25	30	60	90
A	*Staphylococcus aureus* cell beads	80.97	82.3	81.85	83.62	83.62	84.07	81.85	81.05
B	*Staphylococcus aureus* cell agar block	80.08	80.53	80.53	81.41	83.41	84.07	81.85	81.85
C	*Staphylococcus aureus* enzyme beads	94.11	94.57	95.47	96.83	96.38	95.02	93.66	93.66
D	*Staphylococcus aureus* enzyme block	94.54	95.45	95.27	95.27	95.00	95.45	95.45	94.54

Table 2 Degradation of 1% SDS performance recorded with whole cell immobilization of *P. aeruginosa* and its purified alkyl sulphatase in alginate beads and agar gel blocks

S. No.	Time (min)	5	10	15	20	25	30	60	90
A	*Pseudomonas aeruginosa* cell beads	85.2	89.23	89.23	91.92	91.03	92.37	91.47	89.68
B	*Pseudomonas aeruginosa* cell agar block	93.18	94.18	93.18	95.45	96.81	93.18	94.54	94.09
C	*Pseudomonas aeruginosa* enzyme beads	95.94	95.94	96.84	97.73	97.29	96.84	95.94	95.94
D	*Pseudomonas aeruginosa* enzyme block	88.93	88.49	86.72	87.69	88.49	87.61	87.17	86.28

Treatment effect on detergent containing water

TDS: The alkyl sulphatase producing *P. aeruginosa* and *S. aureus* able to lower down total dissolved solids of detergent rich household water by more than 40% with ten days of incubation as compared to control shown in (Table 3).

Table 3 The bacterial presence reduced the total dissolved solids in the detergent containing water as compared to non-enriched water sample

Name	Total dissolved solid
Staphylococcus aureus	4620 ppm
Pseudomonas aeruginosa	4710 ppm
Control	9770 ppm

COD: As per chemical oxygen demand recorded for the detergent containing water treated with *S. aureus*, 5th day total COD demand reduced to 50%, i.e., 600 mg/lit while that of *P. aeruginosa* it remains at 800 mg/lit (33% reduction) and by the natural way it remains 1000 mg/lit (16%) as it has initially noted as 1200 mg/lit at 0th day. The reduction in COD value also been noted till 10th day where *S. aureus* treatment lowered the COD value up to 100 mg/lit (91.67%), and that of *P. aeruginosa* COD value recorded 400 mg/lit (66% reduction) where natural COD remained at 900 mg/lit (25% reduction) as given in (Table 4).

Table 4 Reduced COD demand noted with bacterial treatment

Group	COD value in mg/lit		
	0th day	5th day	10th day
Control	1200	1000	900
Staphylococcus aureus	1200	600	100
Pseudomonas aeruginosa	1200	800	400

Plant growth in the presence of treated water

The effect of detergent presence in the water indeed recorded to affect wheat seed growth negatively as compared to detergent reduced wastewater exposed seeds. Here the significant increase in shoot length, root length, chlorophyll content, % seed germination and vigour index recorded once *S. aureus* and *P. aeruginosa* treated water used for growth as compared to control (Table 5) and hence noted to be useful for reducing the load of detergent contamination in water.

Table 5 Wheat Plant growth recorded an increase in features once detergent reduced water given as compared to control

S. No.	Type of soil	Treatment	Shoot length (cm)	Root length (cm)	Chlorophyll content	% of seed germination	Vigour index
1	Natural	Wheat seed (10) control	6.95	17.34	0.009	20	485.8
2	Natural	Wheat seed (10) *Staphylococcus aureus*	15.5	34.9	0.02	80	4032
3	Natural	Wheat seed (10) *Pseudomonas aeruginosa*	14.75	30.05	0.02	80	3584

The presence of detergent in the wastewater proving to be the increasing ecological problem; since it affects normal clearance by biological and physiochemical functions [7-9]. The presence of detergent in wastewater creates an opportunity to the local microflora to sustain in its presence and to grow in the presence of surfactant as noted with various bacterial species able to degrade detergent once sampled from the contaminated region [10-12].

In the present study, it has believed that bacterial species localized in water body containing detergent residue must be able to degrade the same. As per finding contaminated water body found to be positive for *S. aureus* and *P. aeruginosa* capable of degrading SDS at 1% concentration once tested with the basal medium.

In the present study, detergent degrader *S. aureus* and *P. aeruginosa* once immobilized as a whole cell or in a

purified enzyme alkyl sulphatase form, both found to be effective in degrading 1% SDS up to 90% which justify the immobilization approach for industrial use. In a similar study, the success of immobilized *Pseudomonas sp.* reported with possible biodegradation of linear alkylbenzene sulfonate by immobilizing the cells in alginate and polyvinyl alcohol. They noted the better performance of immobilized cells to remove detergent than free cells [13].

In the present study, a load of detergent found to be lowered down by the *S. aureus* and *P. aeruginosa* once noted with low values of total dissolved solids; chemical oxygen demand indicates their potential in water treatment. The degradation of detergents has been well linked earlier with biological, chemical and physical process noted with reduced BOD, TDS and COD as recorded in the present study [14-15].

In the present study, a better effect of detergent degradation recorded once detergent free pure water able to improve overall growth of wheat plant as compared to detergent contaminated water treatment. This study put forward the success of *S. aureus*, and *P. aeruginosa* treated water beneficial for improving plant growth once treated water given as a water source.

In a similar study [16] reported the performance of alkyl sulphatase producers namely *Bacillus subtilis*, *Pseudomonas putida* and *Pseudomonas fluorescens* able to control detergent as a pollutant and assist in bioremediation of soil environment making it fertile for plant growth.

CONCLUSIONS

Increasing use of detergent in every sector; creating a complex micro-environment in receiving water bodies, soil, and air and cumulative response remains varied. Pollution always imparts a negative effect on the environment, which has short- or long-term effects. In the present study, we noted the potential of water-based isolates *S. aureus* and *P. aeruginosa* able to degrade surfactants efficiently once checked in immobilized form for whole-cell or as enzyme form. Not only is that they reduced the load for detergent in water bodies by lowering COD, TDS. Lastly, detergent-free water brings about positive changes in wheat growth and improved vigour index recorded with plants indicated the future use of the immobilized system with given bacterial species to control detergent pollution in water.

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