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Research Article

# DEVELOPMENT OF RHAMNOLIPID BASED WHITE BOARD CLEANER

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## Abstract

In the present study biosurfactant based white board cleaner was formulated, using column purified rhamnolipid produced by *Pseudomonas aeruginosa*. A rhamnolipid concentration of 0.01% was finalized, as it was efficiently able to remove fresh and aged white board marker stains on white board. The rhamnolipid formulation was found to be stable over a tested span of 6 months and it did not exhibit any cytotoxicity on Human Lung Epithelial Cell Line (L132) and Normal Human Skin Fibroblast (NHSF) cell line. The cleaning efficiency was equivalent to conventional synthetic surfactants, but the biosurfactant based approach is more environment friendly and biocompatible.

**Keywords**: Human Lung Epithelial Cell Line; Normal Human Skin Fibroblast; Rhamnolipid; White board

## Introduction

White boards are used in various industrial and academic organizations, cleaning and maintaining them is to be performed suitably or else will lead to scouring of surface, ghosting and clinging problem, it is especially prevalent when static charges are generated, on wiping it no longer removes the dust, but instead redeposits it to other portions of the board. This makes the white board blotchy with dust, and very difficult to read. A white board, after being exposed to the chemical solvents present in most of the marker pen inks and different types of chemical cleaners used to clean the board, gradually goes from a non-porous glazed surface to a more open porous surface. This leads to a condition known as ghosting. Ghosting occurs as marker pen ink flows down into the porous surface and dries up. Due to ghosting on white board only the surface dust is removed when it is erased conventionally (Illnois tool works Inc., 2006). Chemically synthesized surfactants are derived from petrochemical sources and these compounds have been extensively developed for large scale industrial applications, mainly in the area of products such as detergents and surface cleaners. The dynamic current movement for industrial sustainability has developed an active interest in biosurfactants as possible replacements for at least some of these chemical surfactants (Roger and Ibrahim, 2012). Being amphiphilic in nature rhamnolipids tend to partition preferentially at the interface between the phases of different degrees of polarity and hydrogen bonding. The formation of such an ordered molecular film at the interface lowers the surface and interfacial tensions. This phenomenon helps dispersion of hydrophobic substances in aqueous media. The biosurfactants have advantage over their chemical counterparts because of higher biodegradability, surface activity, emulsifying properties and biological activity, and lower toxicity and critical micelle concentration (CMC) (Satpute *et al.*, 2010; Banat *et al*., 2010). Up till now, no study has been reported on the use of rhamnolipid surfactants for use as white board cleaner. We have formulated a simple ecofriendly white board cleaning formulation using rhamnolipid and tested its stability cytotoxicity and cleaning efficiency.

## Materials and Methods

### *Product Formulation*

Various concentrations (0.005%-0.01%) of column purified rhamnolipid obtained from *Pseudomonas aeruginosa* (data not shown) were prepared using sterile alkaline distilled water (pH: 8.0) as diluent. The formulations were tested for their ability to efficiently remove fresh and aged white board marker stains (0-7-15 day old), ghosting phenomenon and formation of soapy residue after cleaning white board.

### *Comparison of cleaning efficiency*

Comparison of cleaning efficiency of Rhamnolipid based white board cleaner and two synthetic surfactant based white board cleaner in market was done by testing their ability to efficiently remove fresh and aged white board marker stains (0- 7 -15 day old), ghosting phenomenon and formation of soapy residue after cleaning white board.

***Toxicity Studies (Mosmann, 1983)***

Human lung epithelial (L-132) cell line and Human skin fibroblast (NHSF cell line were cultured in Dulbecos Modified Eagels Medium (DMEM) supplemented with high-glucose, l-glutamine, 10% fetal bovine serum (Sigma-Aldrich), 100 U/ml penicillin, 100g/ml streptomycin (Himedia). Culture incubator was maintained at $37^0$C in an atmosphere of 5% $CO_2$ and 95% relative humidity, after 48 h. of incubation 70–80% confluency was reached, and the cells were detached with 0.1% trypsin–Ethelyne Diamine Tetra Acetic acid (Himedia) and used for cytotoxicity studies. The cytotoxicity of rhamnolipid at the concentrations (0.01%-0.04%) on the L-132 and NHSF was evaluated by the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5diphenyltetrazolium bromide) (Sigma-Aldrich) colorimetric assay). Briefly, 200µl of $l\times10^5$ cells/ml was seeded into 96-well plates (Tarson). After 48 h culture with DMEM, the cells were further incubated with different concentration of surfactants for 2 h. Then, incubation medium was withdrawn and the cells were washed twice with Phosphate Buffered Saline. Aliquots (100 µl) of MTT solution (1.0 mg/ml) and 100 µl DMEM were added to each well. After 4 h of incubation, the supernatant was discarded and formazan crystals were dissolved in Dimethyl Sulfoxide followed by vigorous mixing. Control wells were incubated with DMEM only without surfactant, and were treated similarly as above. The optical density was determined by microplate reader at 560 nm (Emax, Molecular Devices Co., Sunnyvale, CA, U.S.A.). The percent viability of the cells was determined from the absorbance values considering that of the control as 100%.

***Stability Studies***

The ability of Rhamnolipid based white board cleaner to effectively remove fresh and aged white board marker stains (0-7-15 day old), ghosting phenomenon and formation of soapy residue after cleaning white board were tested for a time period of 6 months.

## Result and Discussion

***Product Formulation***

Rhamnolipid concentration of 0.01% was finalized for product development, it effectively removed white board marker stains from white board without leaving spots, ghost and soapy residue on white board, lower concentrations had less cleaning efficiencies and exhibited ghosting phenomenon and concentrations above 0.3% lead to soapy residue formation on white board (Table 1).

***Toxicity Studies of the Formulation***

To study the cytotoxic effects of the formulation on human cell line, *in-vitro* cytotoxic studies were performed on Human Lung Epithelial Cell Line (L-132) and Normal Human Skin Fibroblast (NHSF) cell line to ensure no harmful side effects are caused by the use of the formulation. The above cell lines were selected because skin and lung regions are exposed to surfactant while using it. The rhamnolipid formulation did not show any cytotoxic effects on the tested cell lines. Hence on the basis of the results obtained it can be concluded that the formulation did not exhibit any harmful side effects and is safe to use (Fig. 1 and 2).

**Table 1**: White board cleaner formulation

| Sr. No. | Concentration of Rhamnolipid | Cleaning efficiency | | | Ghost formation | | | Soapy residue formation | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 7 | 15 | 0 | 7 | 15 | 0 | 7 | 15 |
| 1 | 0.005% | +++ | - | - | + | +++ | +++ | - | - | - |
| 2 | 0.01 % | +++ | +++ | +++ | - | +++ | +++ | - | - | - |
| 3 | 0.02 % | +++ | +++ | +++ | - | +++ | +++ | - | - | - |
| 4 | 0.04 % | +++ | +++ | +++ | - | +++ | +++ | - | - | - |
| 5 | 0.06 % | +++ | +++ | +++ | - | +++ | +++ | - | - | - |
| 6 | 0.08 % | +++ | +++ | +++ | - | +++ | +++ | - | - | - |
| 7 | 0.1 % | +++ | +++ | +++ | - | +++ | +++ | - | - | - |
| 8 | 0.2 % | +++ | +++ | +++ | - | +++ | +++ | - | - | - |
| 9 | 0.3 % | +++ | +++ | +++ | - | +++ | +++ | + | + | + |
| 10 | 0.4 % | +++ | +++ | +++ | - | +++ | +++ | ++ | ++ | ++ |
| 11 | 0.5 % | +++ | +++ | +++ | - | +++ | +++ | ++ | ++ | ++ |
| 12 | 0.6 % | +++ | +++ | +++ | - | +++ | +++ | ++ | ++ | ++ |
| 13 | 0.7 % | +++ | +++ | +++ | - | +++ | +++ | ++ | ++ | ++ |
| 14 | 0.8 % | +++ | +++ | +++ | - | +++ | +++ | ++ | ++ | ++ |
| 15 | 0.9 % | +++ | +++ | +++ | - | +++ | +++ | ++ | ++ | ++ |
| 16 | 1.0 % | +++ | +++ | +++ | - | +++ | +++ | ++ | ++ | ++ |
| 17 | Control | +++ | - | - | - | - | - | - | - | - |

Key: + Positive; - Negative

To avoid adverse reactions like skin or eye irritation, the concentrations of components used in commercial formulations must be carefully controlled. Biosurfactants are generally considered as the low or nontoxic products and therefore are suitable for pharmaceutical, food and cosmetic industries (Muthusamy *et al.*, 2008). A study by Poremba K (1991) suggested that synthetic anionic surfactant displayed an LC50 (concentration lethal to 50% of test species) against *Photobacterium phosphoreum* ten times lower than rhamnolipids, demonstrating the higher toxic nature of chemical-based surfactants. Various studies also reported higher EC50 (effective concentration to decrease 50% of test population) value for biosurfactants than synthetic surfactants Ozdemir *et al.* (2004) investigated the adsorption characteristics of keratin-rhamnolipid (R-Keratin) and keratin-SDS (SDS-Keratin) at the air and liquid interface. Tests results showed weaker interactions for R-Keratin than for SDS-Keratin.

### *Comparison of Cleaning Efficiency (Fresh Stains and Aged Stains)*

The cleaning efficiency of rhamnolipid formulation was compared with two synthetic surfactant. All three surfactants exhibited equivalent cleaning efficiency, no ghosting and soapy residue formation. Hence rhamnolipid based formulation can be used to replace synthetic chemical based white board cleaners as they are biocompatible and green alternative of toxic and environmental hazardous synthetic surfactants (Table 2).

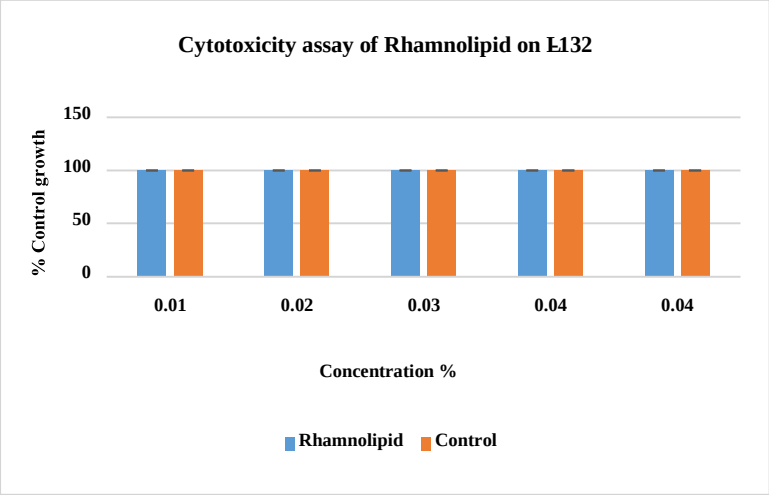


**Fig. 1:** Cytotoxicity assay of Rhamnolipid on L-132

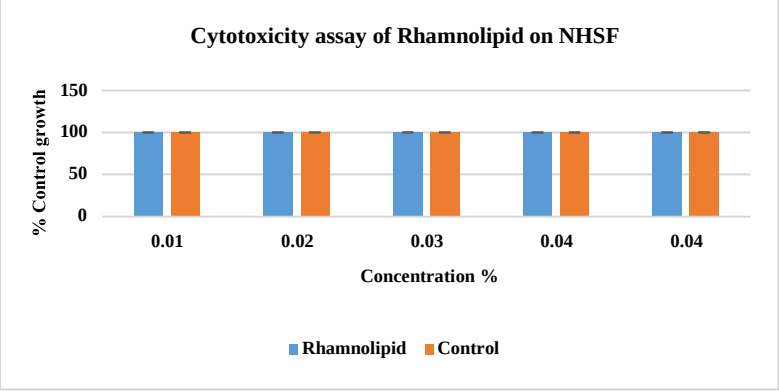


**Fig. 2:** Cytotoxicity assay of Rhamnolipid on NHSF

**Table 2**: Comparison of cleaning efficiency

| Sr. No. | Surfactant | Cleaning efficiency | | | Ghost formation | | | Soapy residue formation | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 7 | 15 | 0 | 7 | 15 | 0 | 7 | 15 |
| 1 | RLF | +++ | +++ | +++ | - | - | - | - | - | - |
| 2 | Synthetic surfactant 1 | +++ | +++ | +++ | - | - | - | - | - | - |
| 3 | Synthetic surfactant 2 | +++ | +++ | +++ | - | - | - | - | - | - |

**Key:** +++: complete stain removal; - no ghost formation / soapy residue formation

**Table 3:** Stability studies of Surfactants

| Sr. No. | Months | Cleaning efficiency | | | Ghost formation | | | Soapy residue formation | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 7 | 15 | 0 | 7 | 15 | 0 | 7 | 15 |
| 1 | 1 | +++ | +++ | +++ | - | - | - | - | - | - |
| 2 | 2 | +++ | +++ | +++ | - | - | - | - | - | - |
| 3 | 3 | +++ | +++ | +++ | - | - | - | - | - | - |
| 4 | 4 | +++ | +++ | +++ | - | - | - | - | - | - |
| 5 | 5 | +++ | +++ | +++ | - | - | - | - | - | - |
| 6 | 6 | +++ | +++ | +++ | - | - | - | - | - | - |

**Key:** +++: complete stain removal; - no ghost formation / soapy residue formation

***Stability Studies of Rhamnolipid Formulation***

Stability of the rhamnolipid formulation was tested for a span of 6 months, it was observed that its efficiency of removing fresh and aged white board marker stains on white board remained stable in the tested time frame (Table 3).

## Conclusion

Rhamnolipid based white board cleaner was formulated, and its efficiency of cleaning was found similar to the chemical surfactants currently used in market, hence it can be used to replace synthetic surfactant based cleaners. Biosurfactants has distinctive advantages over synthetic surfactants such as their biodegradable nature, exhibits no or very low toxicity.

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