

SYNTHESIS OF DETERGENT FROM RICE BRAN OIL AND STUDY OF ITS QUALITY PARAMETERS INCLUDING CYTOTOXIC ACTIVITY

MEENA KHETRAPAL¹, PADMSHREE MUDGAL², LATA³, AYUSHI⁴, DEEPIKA⁵ & CHARU⁶

^{1,3,4}Department of Chemistry, Daulat Ram College, University of Delhi, Delhi, India

^{2,5,6}Department of Biochemistry, Daulat Ram College, University of Delhi, Delhi, India

ABSTRACT

In this research paper, biodegradable detergent was synthesized from *Oryza Sativa* (rice) bran oil. The prepared detergent was studied for several parameters such as % yield, surface tension, foaming ability, wettability, emulsion stability, biodegradability and cytotoxic activity. The foaming ability test for synthesized detergent showed the persistence of foam only for a very short time which favoured the trend towards washing with minimum water. The synthesized detergent was found to be more biodegradable when compared to standard detergents. A study of cytotoxic activity on RBCs reveals that rice bran detergent is less toxic as compared to commercially available detergents thereby showing the potential of rice bran oil for the production of high quality biodetergent. The efficiency of the synthetic detergent was improved with the addition of foaming agents, bleaching agent and fragrance.

KEYWORDS: Biodegradable, Cytotoxic Activity, Detergents, Emulsion Stability, *Oryza Sativa* (Rice), Bran Oil and Surface Tension

INTRODUCTION

Detergents^{1,2} are chemicals used for dishwashing, laundry, leather cleaning, degradation of organic waste from exhaust pipes and many other industrial purposes. Chemically, detergent is a mixture of surfactants with cleaning properties even in dilute solutions. The commercial detergents, in addition to surfactants, usually contain builders like polyphosphates, bleach additives like fabric brighteners and anti-redeposition agents³. There is a growing concern about the huge environmental impact of the increasing use of these detergents. The surfactants in the detergents form a micelle, which surrounds the dirt particles and moves it away. It is the same micelle which makes surfactants toxic to fish. It gets into fish gills and impairs their ability to breathe⁴. Surfactants like linear alkyl benzene sulfonates^{5,6} (LABS) and nonylphenol ethoxylates^{7,8} (NPE) are highly toxic to aquatic organisms and persist in the environment for a long time and are even more toxic.

One of the most common builder/ water softener used in detergents is sodium triphosphate. Run off of phosphates into water streams poses significant eutrophication risks⁹. Phosphates cause exponential growth of algae. The algae take sun light and oxygen away from other aquatic life and cause fish and animals to die. Such environmental concerns shifted focus on Green cleaning practices. So development of new Green detergents started, which had increased biodegradability^{10,11}, less toxicity, were environmentally safe and had increased cleaning efficiency to remove all types of stains without harsh treatment. Major sources of biodegradable detergents are vegetable oils, fats and bio-polymers. A research into biodegradability of alkyl based detergents showed that branched molecules are indigestible to bacteria as compared to the linear chain detergents. Mostly, vegetable oil derived detergents possess straight chain linear alkyl

sulfonate, LAS, making them suitable for production of greener detergent¹². India has become the second largest producer of rice bran oil. As such large quantity of oil (nearly 54%) can be obtained from rice husk¹³. Detergent from rice bran oil contains ingredients which have antifungal and bactericidal effect^{14,15}. The main fatty acids¹⁶ present in Rice bran oil are Palmitic acid (18.50 %), Stearic acid (2.50 %), Oleic acid (43.0 %), Linoleic acid (32.0 %), Linolenic acid (2.20 %), Arachidic acid (1.80 %).

This work is aimed at producing biodegradable detergent from *Oryza Sativa* bran oil and comparing the results with some standard detergent available in the market. Production of environmentally friendly synthetic detergents from rice bran oil has the dual advantage as the residue obtained during refining is a rich source of many nutrients like oryzanols, tocopherols, vitamin E, lecithin, phytic acid, inositol, ferulic acid and wax¹⁷.

MATERIAL AND METHODS

Preparation of Detergent

50 g of rice bran oil was taken in a 250 mL beaker and 100 mL of 0.1 M NaOH was added to it with gentle stirring. As the process was exothermic, the reaction mixture was cooled using an ice tub. Initially the pH measured was above 10, therefore 18M sulphuric acid was added into the beaker containing the mixture till the pH was adjusted to ~9.3. Then the mixture was kept undisturbed for 2 days at room temperature. After 2 days the precipitate (solid part) was scooped out and weighed. Then it was crushed and converted into the powdered form. The % yield of detergent was calculated using the formula:

$$\% \text{ Yield of detergent} = \frac{\text{Biodegradable detergent}}{\text{Rice bran oil taken}} \times 100$$

Solubility

2% solution of detergent was prepared in a 100 mL conical flask. It was then left undisturbed for 2 minutes. The solution was filtered on a preweighed Whatman filter paper using vacuum pump. The filter paper with residue was carefully picked up and dried in oven at a temperature of $100 \text{ }^{\circ}\text{C} \pm 5 \text{ }^{\circ}\text{C}$ until a constant mass was obtained.

Determination pH

0.1% solution of detergent was taken. A pH meter was calibrated using buffers of pH 4, 7 and 10. Using the calibrated pH meter, pH of the detergent was measured.

Determination of Surface Tension

Stalagmometer was used for calculating surface tension of the detergent using drop number method. Stalagmometer was fitted in a clamp stand. Two marks A and B were marked above and below the bulb at equal distances. Using water, rate of flow was regulated (10-15 drops/30 seconds) by making suitable adjustment with the pinch cork. After this, the number of drops was counted when the water/detergent solution level inside stalagmometer decreased from point A to point B.

Foaming Ability Test

10 mL of 0.1% detergent solution was taken in a test tube and shaken 10 times. The time for disappearance of 2 mm foam was recorded and compared.

Wetting Performance Test

1 gram cotton thread was weighed and placed in 200 mL of 0.1 % detergent solution. The time taken by the thread to be completely inside the detergent solution was noted.

Emulsion Stability Test

5 mL of 1% solution of detergent was taken in a labeled test tube and 0.5 mL mustard oil was added to it. The mixtures were vortexed for 1 minute. A white emulsion was formed and the time was recorded when the solution became clear.

Biodegradability Test

BOD₅ test was carried out simultaneously with 100 mg of synthetic detergent and control detergent¹⁸⁻²¹. The amount of oxygen taken up initially and after five day was measured using oxygen meter.

Beet Root Assay

0.1 inch beetroot slices (uniformly sliced) were placed in water to wash off its excess color, replacing the water till the water became colorless. Then beetroot slices were placed in a test tube containing 5mL of 0.1% detergent solution and left undisturbed for 1 hour at room temperature. After 1 hour the absorbance was recorded at 535 nm using spectrophotometer. For reference, 1% HCl in methanol was used that caused 100% disruption of the cells.

Cytotoxic Test: *Hemolytic activity*

Preparation of RBC: Heparinized blood was centrifuged at 4000 rpm for 10 minutes. The pellet was then resuspended in same amount of PBS and centrifuged again at 4000 rpm for 10 minutes. The pellet obtained was once more resuspended in PBS and centrifuged at 4000 rpm for 10 minutes. Then supernatant liquid was discarded and the pellet was suspended in equal volume of PBS and is the required RBC.

Method: Three eppendorfs were taken with two of them labeled as control 1 and 2 respectively and the third for the detergent sample. In control 1, 20 μ L of RBC and 980 μ L of PBS were added (as a reference for 100% lysis). In control 2, 20 μ L of RBC and 980 μ L of Triton X100 (1%) were added. To the last eppendorf, 20 μ L of RBC, 880 μ L of PBS and 100 μ L of the detergents were added. Absorbance was then taken at 540 nm.

RESULTS AND DISCUSSIONS

The rice bran oil was purchased from Kendriya Bhandar located at Delhi University, Utility Centre, North Campus, New Delhi and the biodegradable detergent prepared in laboratory from this oil. The prepared detergent was studied for several quality parameters. The results of various parameters studied reflecting the characteristics of the synthesized detergent are demonstrated in Table1.

Table 1: Qualitative Parameter Checked for Rice Bran Oil Detergent, Compared with a Laundry Detergent Brought From Delhi University Cooperative Store Ltd. as Control

| S. No. | Parameters Studied | Detergent from Rice Bran Oil | Control |
|--------|--------------------------|--------------------------------|----------------|
| 1 | Solubility in cold water | 100% | 74% |
| 2 | pH | 9.22 | 10.45 |
| 3 | Surface tension | 39.18 dyne/cm | 56.054 dyne/cm |
| 4 | Foaming ability | Foam disappeared in 45 seconds | 19.53 minutes |

Table 1: Contd.,

| | | | |
|---|-----------------------------------|-----------------------------|-----------------------|
| 5 | Wettability | 1 minutes 15 seconds | 47 minutes 63 seconds |
| 6 | Emulsion stability | stable for more than 1 hour | 30 minutes |
| 7 | Biodegradability | >60 % | <43 % |
| 8 | Beet root assay OD ₅₃₅ | 0.303 | 0.472 |
| 9 | Cytotoxic test | 44.41 % | 72.82 % |

The calculated % yield of pure detergent was found to be almost 95% (47.4 g of detergent from 50 g rice bran oil) so the modified method used for the synthesis of detergent was quite satisfactory. The detergent is completely soluble in cold water as compared to the control detergent which helps in conservation of water and energy. The pH test showed that the prepared detergent was basic in nature. The pH range is most preferable as it is non corrosive to the skin and fibers. The result presented in **Table 1** showed that, the detergent synthesized from rice bran oil has high enough efficiency. As the surface tension obtained for the detergent (39.18 dyne/cm) was quite lower than that of the control detergent, it reveals that surface tension of water is dramatically reduced with addition of detergent. Also the foaming ability test of synthesized detergent shows the persistence of foam for a shorter time thus favoring the trend towards washing with minimum water. The result obtained for the biochemical oxygen demand (BOD) indicates that micro-organisms can easily decompose the detergent (BOD value more than 60%). Thus, this detergent if discharged in the ecosystem would be environment friendly since micro-organisms can easily decompose it down into simpler compounds as compared to the other standard detergents tested. Beet root assay test also favors the biodegradable detergent over the commercially available detergent as it causes less membrane disruption and hence is less toxic. The study of cytotoxic activity on RBC also suggests that detergent from rice bran oil has less toxic effect as compared to commercially available detergents; so this can be utilized for the production of high quality bio detergent.

CONCLUSIONS

The preparation of biodegradable detergent from rice bran oil is first time reported and tested for several quality parameters such as surface tension, foaming ability, wettability, emulsion stability, biodegradability and cytotoxic activity which suggest it to be suitable for the production of high quality detergent.

ACKNOWLEDGEMENTS

The authors are thankful to University of Delhi for providing funds for the completion of Innovation Project 2013-2014.

REFERENCES

1. Bhairi, S. *Calbiochem-Novabiochem Corporation*, **2001**, 3-29.
2. *Source Category Survey: Detergent Industry*, **1980**, EPA Contract No. 68-02-3059.
3. Sekhon, B. S., Sangha, M. K. *Resonance*, **2004**, 08, 0035-0045.
4. Byrne, D., Speare, H. W., Ferguson, *Dis. aquat. Org.*, **1963**, 6, 185-196.
5. Leon, V.M., Lopez, C., Lara-Martin, Prats D., Varo, P., Gonzalez-Mazo, P., *Chemosphere*, **2006**, 64, 1157-1166.
6. Zinqin, O., Ayfer, Y., Yaowu, H., Antonius, K., Tieheng, S., *Chemosphere*, **1995**, 30(2), 313-325.

7. Rice, C.P., Schmitz-Afonso, I., Loyo-Rosales, J.E., Link, E., Thoma, R., Fay, L., Altfater, D., Camp, M.J. *Environmental Science and Technology*, **2003**, 37, 3747-3754.
8. Vazquez-Duhalt, R., Marquez-Rocha, F., Ponce, E., Licea, A. F., Viana, M.T. *Applied Ecology and Environmental Research*, **2005**, 4(1), 1-25.
9. Whiteside, M. C. *Hydrobiologia*, **1983**, 103, 107-150.
10. Matthew, J.S., Malcolm, N.J. *Biochimica et Biophysica Acta*, **2000**, 1508, 235-251.
11. Jan, W.G. *Encyclopedic Dictionary of Polymers*, New York, Springer Science, **2007**, 108-109.
12. Isah, A.G. *Leonardo Electronic Journal of Practices and Technologies*, **2006**, 9, 153-160.
13. Patel, M., Naik, S. N. *Journal of Scientific & Industrial Research*, **2004**, 63, 569-578.
14. Ley, A., Jain, P., Singh, A. *Recent Research in Science and Technology*, **2013**, 5(2): 18-19.
15. Almel Ltd., Beaurline, D. J. Patent No: WO 2003047537 A1.
16. Faria, S. A. S., Bassinello, P. Z., Penteado, M. V. C. *Brazilian Journal of Pharmaceutical Sciences*, **2012**, 48, 4.
17. Sharma, A.R. *Saarc Oils and Fats Today*, **2002**, 25-6.
18. Winkler, L. W. *Berichte der Deutschen Chemischen Gesellschaft*, 1888, 21(2), 2843-2854.
19. Lenore, S., Clescerl, A. D., Eaton, E. W. *Rice*, **2005**. Standard Methods for Examination of Water & Wastewater (21st ed.). Washington, DC: American Public Health Association, American Water Works Association, and the Water Association.
20. <http://www.cwc.gov>
21. <http://water.usgs.gov>

