

## BIO-DETERGENT FROM *BRASSICA JUNCEA* (MUSTARD) SEED OIL AND STUDY OF ITS QUALITY PARAMETERS & ENVIRONMENTAL INDEXES

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### ABSTRACT

Bio-detergent was prepared using *Mustard Oil*. Its detergent properties were studied and compared with those of different brands of commercially available detergents. Several parameters such as surface tension, pH, solubility, foamability, wettability, emulsification, cleaning action and hemolytic activity on RBCs were determined. The synthesized detergent is found to be eco-friendly, has low bio-toxicity and shows good surfactants properties like low surface tension, high wettability, high foaming ability with rapid collapse and high solubility. The detergent is found to be more biodegradable when compared to a commercial detergent. And also, a study of cytotoxic activity on RBCs reveals that mustard oil detergent is less toxic as compared to commercially available detergent thereby showing the potential of mustard oil for the production of high quality bio-detergent.

**KEYWORDS:** Bio-Detergents; *Brassica Juncea* (Mustard) Seed Oil; Cytotoxic Activity; Eco-Friendly

### INTRODUCTION

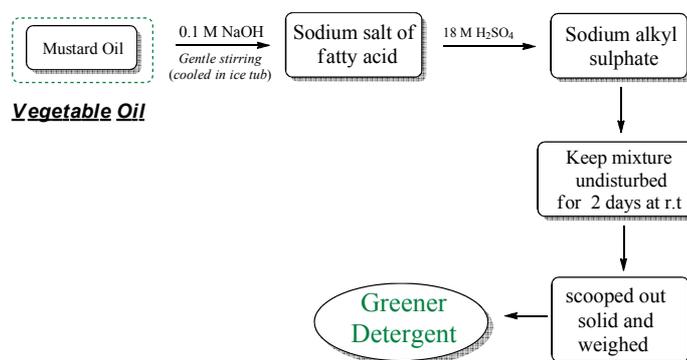
Large amount of detergents<sup>1-3</sup> are used these days for laundering, dishwashing, leather cleaning etc. and their efficiency improved by the addition of foaming agent, water softeners, bleaching agent, brighteners and fragrance. Sodium triphosphate is added as water softener in many commercial detergents<sup>4</sup>, runoff the phosphates reached into water bodies leads to an exponential growth of algae which compete with other aquatic organism for sunlight and oxygen, thus causing the aquatic organism die<sup>5,6</sup>. As per Indian's Environment protection law, phosphates in detergent have been classified as toxic chemicals<sup>7</sup>. However, there is no regulation which restricts the use of phosphates in detergents. Thus, in order to protect the environment from there toxic detergents, the development of bio-detergents<sup>8</sup> is of utmost importance. The detergents prepared from vegetable oil are generally linear alkyl sulphonates<sup>9,10</sup> which can be easily digestible by many of the microorganism and hence are biodegradable. In the present work, a greener detergent is developed from mustard oil and its detergent properties were studied in detail<sup>11</sup>.

### MATERIALS AND METHODS

#### Production of Detergent

To 150 mL of 0.1M NaOH solution taken in a flask, 100 g of mustard oil was added with gentle stirring. The reaction mixture was kept in an ice bath, as the process was exothermic. 18 M conc. H<sub>2</sub>SO<sub>4</sub> was then added to the reaction mixture so that the pH was maintained at 9.3 and the reaction mixture was kept undisturbed at room temperature for two days.

After the completion of two days, the yellow coloured solid was scooped out and dried at room temperature. The dried detergent was crushed out to a fine powder (figure 1).



**Figure 1: General Scheme for the Synthesis of Greener Detergents from Mustard Oil**

### Calculation of % Yield of Mustard detergent

The percentage yield was determined by using the formula:

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

### Determination of pH

pH of aqueous mustard detergent solution (5%) was recorded by using pH paper as well as pH meter (Systronics, MK5).

### Solubility Test

100 mL of 10% solution of detergent was taken in a 250 mL beaker and left undisturbed for 5 minutes. Using a vacuum pump, the detergent solution was filtered on a pre-weighed Whatmann filter paper. After the filtration, the filter paper with residue was carefully picked out. It was dried in oven at a temperature of 100 °C until the constant mass was not obtained.

### Determination of Surface Tension

0.1% detergent solution was taken and its surface tension was determined by using drop number method<sup>12</sup>. Surface tension of water was taken as a reference.

### Foamability Test

Rose and Miles<sup>13</sup> method was used to check the foamability test of the prepared detergent. 10 mL of detergent solution was added in the test tube and shaken for 10 times. The foam was formed in a test tube and the time for disappearance of foam by the length of 2mm was recorded.

### Hard Water Test

20 mL of 1 % detergent solution was taken in three labeled test tubes. Then 0.5 mL of 5% MgCl<sub>2</sub>, 5 % CaCl<sub>2</sub> and 5% FeCl<sub>3</sub> was added into the respective test tubes. The mixture was kept undisturbed till the precipitates were formed. Observed and compared the amount of scum formed in each test tube.

### Emulsification Test

This test was carried out by taking 5 mL of 1 % detergent solution in a test tube. Then 0.5 mL of mustard oil was added to the detergent solution and vortexed for 1 minute. Emulsion was formed, so time was recorded till the solution became clear. For comparison water was taken as reference.

### Wetting Ability Test

Draves method was used to determine the wetting ability test for the prepared detergent. 1 g cotton thread was placed over the surface of 200 mL of 0.1 % detergent solution. The time taken by the thread to be completely immersed into the detergent solution was noted.

### Beetroot Test of Detergents

The Kirb method was used for this assay. One fresh tuber of beet root was taken and sliced in to 1 inch pieces. These beet root pieces were thoroughly washed with running water so that the excess stain was removed. Now 2 pieces of beet root were added in a test tube containing 5 mL of 0.1 % detergent solution and kept undisturbed for 30 minutes at room temperature. 1% methanolic solution of HCl was used as the reference as that caused 100% disruption of the cells. After the completion of the incubation period, the absorbance (OD) was taken at 535 nm using UV spectrophotometer.

### Hemolytic Effect of Detergent on RBCs

Deh-Nod<sup>14</sup> method was used to study RBC hemolysis assay. Centrifuged the heperanized blood cells at 4000 rpm for 10 minutes. The pallet was formed at the bottom of the centrifuged tube, then resuspended this pallet in 10 mL of PBS (phosphate buffer saline) and centrifuged again for 10 minute at 4000 rpm. Finally recollected the pallet and suspended it in the same volume of PBS to get suspended RBCs.

### Test

Labeled three eppendorfs as control 1, control 2 and last for detergent sample respectively. As a reference for 100% cell lysis, 20  $\mu$ L of RBC and 980  $\mu$ L of PBS were added in control 1. 20  $\mu$ L of RBC and 980  $\mu$ L of Triton X100 (1%) were added in control 2. To the third eppendorf, 100  $\mu$ L of the detergent, 20  $\mu$ L of RBC and 880  $\mu$ L of PBS were added. Absorbance for each eppendorfs was recorded at 540 nm.

### Biodegradability Test

BOD<sub>5</sub> test was performed with 100 mg/L of prepared detergent solution by keeping them in a dark for 5 days at 20 °C. For better result, 5 mL of inoculum of water from the local drain were added. In the end, amount of oxygen used initially and after five days was measured using oxygen meter<sup>15,16</sup>.

## RESULTS AND DISCUSSIONS

From the Kendriya Bhandar located at Delhi University, Utility Centre, North Campus, New Delhi, mustard oil was purchased. The biodegradable detergent was synthesized in laboratory from this oil and studied for its cleaning performance and biotoxicity assay as demonstrated in Table1.

**Table 1: Qualitative Parameters Checked for Synthesized Detergent and Compared with another Laundry Detergent Brought from Delhi University Cooperative Store Ltd. as Control**

S. No.	Parameters Studied	Detergent from Mustard Oil	Control
1	Solubility in cold water	100%	78%
2	pH	11.12	9.65
3	Surface tension	40.48	61.20 dyne/cm
4	Foaming ability	11 minutes	22.40 minutes
5	Wettability	3 minutes	48 mints 42 seconds
6	Emulsion stability	23 minutes	32 minutes
7	Biodegradability	>60 %	<38 %
8	Beet root assay OD <sub>535</sub>	0.409	0.490
9	Cytotoxic test	62.32 %	75.37 %

The obtained % yield of synthesized detergent was found to be almost 97% thus the opted method used for the preparation of detergent was quite satisfactory. The prepared detergent was found to be completely soluble in cold water as compared to the commercial detergent, which helps in conservation of water and energy. The pH value was found in preferable range as it is nonhazardous to the skin and fibers. The results reported in Table 1 shows that the detergent obtained from the mustard oil has highest energy efficiency as the surface tension found for the detergent (40.48 dyne/cm) was quite lower, this suggests that surface tension of water used is dramatically reduced with the addition of prepared detergent. The persistence of foam for the detergent was very short as compared to the control detergent, thus favoring the trend towards washing with minimum water. The BOD<sub>5</sub> value for detergent is more than 60%, thereby indicating that microbes can easily degrade the detergent and hence, if the detergent is discharged in the ecosystem it would be environment friendly since it is easily decomposed into simpler molecules. Beet root assay test favors the detergent from mustard oil over the commercially available detergent because it causes less haemolysis and it is thus, less toxic. Cytotoxic activity study on RBC's also proves that the detergent from mustard oil has lesser toxicity. Therefore mustard oil can be used for producing high quality biodetergent.

## CONCLUSIONS

The preparation of biodegradable detergent from mustard oil is first time reported and the detergent is eco-friendly, with low bio-toxicity and shows good surfactant properties like low surface tension, high wettability, good foaming with rapid collapse and high solubility as compared to some commercially available detergents, 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.

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