Application of rhamnolipid biosurfactant for bio-detergent formulation

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Abstract. Biosurfactants, also known as biological surface-active agents, are a group of surface-active agents, which are produced by a variety of microorganisms. Biosurfactants possess the characteristic property of lowering surface tension, increase the solubility of poorly soluble compound, low toxicity, non-allergenic, and biodegradable. Growing public awareness about the environmentally friendly health care and associated product has stimulated the search for eco-friendly compounds in laundry detergents. In this research, the application of biosurfactants in the formulation of a washing detergent was investigated. Variation of biosurfactants and its mixture with sodium tripolyphosphate as a builder and sodium sulfate as filler was applied to wash cotton fabric which was contaminated with known amounts of stain (chocolate milk). The effects of various bio-detergent formulations, including the ratio of biosurfactants and builder, was examined. The formulation presented in this study was also compared with some standard detergent for the stains removal efficiency. The results showed that the rhamnolipid biosurfactants have a promising as a substitute for its synthetic counterpart. Based on the colorfastness to wash, color strength (K/S), and color difference (ΔE) value, rhamnolipid based bio-detergent have similar washing effectiveness compared to the synthetic detergent. Research result also found that Na-bicarbonate and Na-citrate have the potential as a substitute for phosphate-based detergent builder.

1. Introduction
Nowadays, laundry detergent is becoming more popular due to the increasing demand and use in washing machines, where it provides rapid stain removal, softness, resiliency to fabric, can be dispersed in water, and is delicate for our skin. There are many different brands or types of detergents, and most of them claim some special qualities regarding their products. Concerns over the persistence of detergent chemicals in the environment and possible contamination of groundwater, other surface water sources also their subsequent health-related issues have raised speculation over biodegradability. Detergents on the market are generally products with active ingredients in the form of LAS surfactants (Linear Alkylbenzene Sulfonate) that derived exclusively from petroleum derivatives: benzene and linear paraffin. Problems arising from the use of LAS surfactants are biodegradability problem. During
its use, detergent is difficult to be degraded by bacteria in water, so detergent waste remains in the water; therefore the accumulation of the amount of detergent in water occurs. The accumulation of detergent in water can be a source of pollution in the water stream [1, 2].

The shift to an era of more environmentally friendly products has encouraged the industry to create environmentally friendly detergents. The solution to this problem can be overcome by using surfactants made from oleochemicals and other bio-based products. One example of a surfactant made from oleochemicals is Methyl Ester Sulfonate (MES) and Biosurfactants that produced from microbial origin. Biosurfactants are a group of surface-active agents, which are produced by a variety of microorganisms (bacteria, fungi). These materials are safer than their synthetic counterparts regarding environmental concerns [3].

Rhamnolipid is a biosurfactant produced by microorganisms. This compound has a hydrophilic group and a hydrophobic group so that rhamnolipid has diverse properties. In addition, rhamnolipid is much more easily degraded in nature (environmentally friendly) compared to synthetic surfactants. One of the advantages of rhamnolipid biosurfactants is that they are able to reduce surface tension and are able to form microemulsions between two different material phases. Besides that, the potential utilization of rhamnolipid is very broad i.e. as an emulsifier [4]; cosmetic mix ingredients [5]; controlling crop pests [6], and is used for bioremediation of land contaminated with hydrocarbon compounds [7].

This study aims to answer environmental problems caused by detergents, with detergent formulations using Rhamnolipid Biosurfactants which is more easily biodegraded, expected to create detergents that are environmentally friendly. In this research work, rhamnolipids biosurfactants were applied in the formulation of laundry detergent as a substitute for its synthetic counterpart (LAS) and their effectiveness in removing chocolate milk stains from cotton fabrics was examined.

2. Materials and Methods

2.1. Materials
Rhamnolipid biosurfactants with a purity greater than 90% were purchased from Pioneer Biotech, China. Sodium tripolyphosphate (STPP), Sodium bicarbonate, Sodium citrate, and Sodium sulfate obtained from Merck used as builder and filler, respectively. Sodium carboxymethyl cellulose from Bratachem and Lipase (64 LU/g) from DuPont.

2.2. Surface activity properties
Surface tension of aqueous solutions of Rhamnolipid was measured with a tensiometer (Fisher Sci. 21) by the duNouy ring method. Tests were performed at room temperature (25 °C) and pH 7.

2.3. Bio-detergent formulation
The making of bio-detergent in this study uses rhamnolipid as a substitute for its synthetic counterpart. The builders tested in the formulation were STPP, Na-bicarbonate, and Na-citrate. Na-sulphate as filler, Na- carboxymethyl cellulose as an anti-redeposition agent, and Lipase as enzyme additive. Water hardness before and after the washing test is not analyzed in this study. Composition of the bio-detergent formulations is shown in Table 1. Blank uses only water to wash the fabric, while control in this experiment uses only Rhamnolipid without the addition of other additives in order to know the strength of the Rhamnolipid alone in the washing performance.

2.4. Fabric washing test
Textile fabric washing efficiency was evaluated according to published guidelines [8] using the reference soap standard. Several washing baths containing rhamnolipid biosurfactants were investigated; STPP, Na-citrate, Na-bicarbonate; Na-sulphate; Na-carboxymethyl cellulose; Lipase also their mixtures with and without one or more of the other above ingredients. The white color fabric cotton samples were cut into 4x10 cm rectangle and each of them was stained with 2 ml of chocolate milk and dried overnight at room temperature. The stained fabric samples then treated in a Launder-
Ometer for 20 min at 90 °C with 5:1 v/w liquor to fabric ratio. Afterward, the fabric samples were removed from the washing bath, immersed in 100 ml deionized water at 30 °C for 20 min, removed, and allowed to dry at room temperature. Indicators of washing efficiency were the color fastness, color strength (K/S) and the color difference (∆E). Colorfastness to wash was measured using greyscale for staining method as per SNI ISO 105-C06:2010 standard.

The stained fabric samples were assessed for the depth of color strength (K/S) and color difference value using a spectrophotometer. The K/S is a function of color depth and calculated by the Kubelka-Munk equation [9, 10].

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\frac{K}{S} = \frac{(1-R)^2}{2R}
\]

Where R is the reflectance at complete opacity, K is the absorption coefficient, and S is the scattering coefficient.

3. Results and Discussion

3.1. Bio-detergent formulation

Detergents are associated with cleaning solid objects, removing unwanted objects from the surface. This cleaning process can be done by various methods, including simple mechanical separation (e.g., rubbing and dipping the cloth into water), separating with solvents (e.g., the addition of organic solvents), and separation by adding water and chemicals such as surfactants. Washing system with detergent consists of objects to be cleaned called substrate, dirt and stains that will be cleaned through a washing process, and liquid bath (liquid containing water and surfactant to clean impurities). The results of washing will depend on the interaction of the elements and the washing conditions used, such as temperature, time, given mechanical energy, and hardness of water used. Detergents have a formula for cleaning dirty substrates under various washing conditions. Some detergents, such as toilet soap, only consist of one component. Some other detergents have more than one component. In general, detergent formulas contained more than one material consist of surfactants, builders, fillers and additives. Complementary materials such as water softeners/chelating agents, anti-redeposition agents, enzymes, bleaching agents, dispersing agents, perfumes, and other minor additives are also included in the commercial formulations [11, 12].

In this study, several washing baths containing surfactant (rhamnolipid biosurfactants); builders (STPP, Na-citrate, Na-bicarbonate); Na-sulphate (filler); Na-carboxymethyl cellulose (anti-redeposition); Lipase (additive) also their mixtures were investigated (Table 1). Knowing the relationship between the increase in phosphorus input into water bodies which has caused the phenomenon of eutrophication of water bodies has further raised public concern over the last few decades. This has led to actions by several countries, especially developed countries such as Germany, the Netherlands, Italy, the United States, and Japan to reduce the phosphorus content, especially those from urban and industrial sources [13]. An attempt to replace the role of STPP as a builder was tried by reducing and replacing it with several compounds, namely Na-bicarbonate and Na-citrate as well as a combination of both.

| Materials                  | Unit | Blank | Control | Var-1 | Var-2 | Var-3 | Var-4 |
|----------------------------|------|-------|---------|-------|-------|-------|-------|
| Rhamnolipid                | %    | 0     | 20      | 20    | 20    | 20    | 20    |
| STPP                       | %    | 0     | 0       | 30    | 10    | 0     | 0     |
| Na-bicarbonate             | %    | 0     | 0       | 0     | 10    | 15    | 15    |
| Na-citrate                 | %    | 0     | 0       | 0     | 10    | 15    | 15    |
| Na-sulphate                | %    | 0     | 0       | 0     | 10    | 15    | 15    |
| Na-carboxymethyl cellulose | %    | 0     | 0       | 0     | 0     | 0     | 0     |
| Lipase                     | %    | 0     | 0       | 0     | 0     | 0     | 2     |
| Total                      |      |       | 100     | 100   | 100   | 100   | 100   |
Biosurfactants, derived from microbial extracellular are comparable with its synthetic counterpart in terms of reducing the surface tension of liquids. These amphipathic molecules have a hydrophilic head which is normally a simple sugar or peptide, and a hydrophobic tail which is normally a lipid. Rhamnolipids are a group of biosurfactants which are generally produced by *Pseudomonas aeruginosa* strains of bacteria. The critical micelles concentration (CMC) value of Rhamnolipids biosurfactants used in this study are shown in Table 2. CMC value was determined by measuring the surface tension at different concentrations [14, 15]. Below the CMC, the surface tension decreases with increasing biosurfactants concentration as the number of the active agent at the interface increases. While above the CMC, the surface tension of the solution is constant because the interfacial biosurfactant concentration does not change anymore. Compared with other surfactants, rhamnolipids have a much lower CMC value of 20 mg/l, as shown in Table 3.

**Table 2.** Surface tension value of various concentration of rhamnolipid biosurfactants and CMC value measurement (inset graph)

| Rh (mg/l) | ST (mN/m) |
|----------|-----------|
| 0        | 71.42 ±0.52 |
| 2        | 49.85 ±0.21 |
| 4        | 46.20 ±0.26 |
| 6        | 43.60 ±0.10 |
| 8        | 40.03 ±0.22 |
| 10       | 38.23 ±0.17 |
| 20       | 35.20 ±0.14 |
| 30       | 34.03 ±0.22 |
| 40       | 33.43 ±0.21 |
| 50       | 33.00 ±0.10 |
| 60       | 32.58 ±0.17 |
| 70       | 31.95 ±0.07 |
| 80       | 31.45 ±0.13 |
| 90       | 30.80 ±0.10 |
| 100      | 30.17 ±0.25 |
| 110      | 29.57 ±0.15 |
| 120      | 29.00 ± 0.10 |

**Table 3.** Nature and properties of various surfactants

| Surfactant                          | Nature    | CMC value, mg/l | Ref.   |
|-------------------------------------|-----------|-----------------|--------|
| Sodium dodecyl sulphate             | Anionic   | 2380            | [16]   |
| Tween 20                            | Non-ionic | 140             | [16]   |
| Linear alkylbenzene sulfonate       | Anionic   | 120             | [17]   |
| Rhamnolipid                         | Anionic   | 20              | This study |

3.2. *Washing performance*

Detergency is the process of cleaning the surface from undesirable foreign objects by using washing / soaking liquid in the form of a solution containing surfactants. The detergency process occurs through the formation of micelles by surfactants that are able to form globules of impurities through a decrease in interface tension and with the help of electrostatic interactions between charges [12]. The result of the formulated bio-detergent washing test in removing the stain, visually can be seen in Figure 1.

Surfactants or surface-active agents are organic compounds that act as active ingredients in detergent formulations. Surfactants can reduce surface tension thereby allowing particles to stick to the fabric to be washed free from fibers and float or dissolve in water. The presence of active ingredients in detergent formulations is often associated with the ability of its detergency. Detergency is a specific
property possessed by surfactants to clean a surface from impurities [12]. However, surface-active compounds cannot completely clean dirt from the surface without the presence of other additional compounds as support, namely builders, anti-redeposition, enzymes, and other additives. The test results in this study confirm this, where the washing results in controls that contain only 20% active ingredient in the form of rhamnolipid biosurfactant are visually not good enough to clean stains (mild stain can be seen visually on the fabric). The use of Na-bicarbonate and Na-citrate is intended to replace the role of STPP as a phosphate-containing builder, where the use of phosphate compounds in detergents began to be limited and avoided due to eutrophication issues [13,18,19]. Washing result indicates that both Na-bicarbonate and Na-citrate are suitable as a builder in replacing STPP. The color difference (ΔE) using greyscale for staining method shows the performance of each bio-detergent formulation used in this study (Table 4).

![Figure 1](image_url)

**Figure 1.** A-1: initial white cotton fabric; A-2: stained fabric with chocolate milk; A-3: washing result of standard detergent. Washing performance of various bio-detergent formulation: Blank (only water); Control; Var-1; Var-2; Var-3; and Var-4.

| Variation                        | Color Fastness | (K/S)  | (AE)   | Remark     |
|----------------------------------|----------------|--------|--------|------------|
| Initial fabric                   | 1-2            | 0.068  | -      | Very good  |
| Stained fabric                   | 5              | 2.088  | 32.75  | Very bad   |
| Wash with standard detergent     | 1-2            | 0.076  | 1.36   | Very good  |
| Blank (wash with water only)     | 4-5            | 0.321  | 12.94  | Bad        |
| Control (wash with Rhamnolipid)  | 4              | 0.085  | 1.78   | Fairly bad |
| Var-1                            | 3-4            | 0.066  | 0.47   | Fairly good|
| Var-2                            | 3-4            | 0.066  | 0.45   | Fairly good|
| Var-3                            | 3-4            | 0.061  | 0.49   | Fairly good|
| Var-4                            | 3-4            | 0.086  | 1.46   | Fairly good|

In detergent industry, enzymes are used enormously in terms of quantity and economic value. Lipase enzyme catalyzes the hydrolysis of triglycerides into diglycerides and monoglycerides by releasing fatty acid. Lipase (Triacil glycerol acyl hydrolase) is a group of enzymes that catalyze the hydrolysis of long chains of triglycerides. Lipase is a lipolytic enzyme that dissolves in water and can
work in oil-in-water emulsions. This enzyme catalyzes the hydrolysis of fats and oils into glycerol and fatty acids in the presence of water. During hydrolysis, lipase takes the acyl group from the glyceride to form the acyl lipase complex; then the acyl group is transferred to the OH group from water. Commercial lipase enzymes generally are extracellular lipases produced from microbes [20]. This is because the production of enzymes using microbes has several advantages, i.e., microbes can be cultured quickly in a small space to produce enzymes in large quantities and a short time, the enzyme content produced is more easily controlled and predicted, the composition of media and other components can be arranged and costs of its production is cheaper compared to synthesizing enzymes using sources from plants or animals.

4. Conclusion
The results of the bio-detergent washing test with lipase enzyme additive did not show a significant difference compared to the performance of other bio-detergent formulations. This is because the types of stains tested in this study did not contain significant amounts of fat, so the role of the lipase enzyme was not seen. The use of lipase as a detergent additive is beneficial in terms of economy and environment. Normally fat or oil stains are difficult to remove in low-temperature conditions if using conventional detergents. But by using lipase as detergent, fat and oil stains are easily removed. This is a saving in energy use. In addition, the use of detergent lipases reduces the impact of chemical pollution on the environment because lipases can be decomposed naturally in the environment, are non-toxic, and do not leave harmful residues [20].

References
[1] Mousavi S A and Khodadoost F 2019 Effects of detergents on natural ecosystems and wastewater treatment processes: a review Environ Sci Pollut Res Int. doi: 10.1007/s11356-019-05802-x
[2] Anwar A F 2011 Effect of greywater irrigation on soil characteristics 2nd Int. Conf. on Env. Sci. and Dev. IPCBEE pp 15–18
[3] Helmy Q, Kardena E, Funamizu N and Wisnuprapto 2011 Strategies toward commercial scale of biosurfactant production as potential substitute for it's chemically counterparts Int. J. Biotechnology 12 66-86 doi: 10.1504/IJBT.2011.042682
[4] Muthusamy K, Gopalakrishnan S, Ravi T K and Sivachidambaram P 2008 Biosurfactants: Properties, commercial production and application Current Science 94 736-47
[5] Lourith N and Kanlayavattanaku M 2009 Natural surfactants used in cosmetics: glycolipids International Journal of Cosmetic Science 31 255–61
[6] Vatsa P, Sanchez L, Clement C, Baillieul F and Dorey S 2010 Rhamnolipid biosurfactants as new players in animal and plant defense against microbes International Journal of Molecular Science 11 5095-108
[7] Kaskatepe B and Yildiz S 2016 Rhamnolipid biosurfactants produced by Pseudomonas species Braz. arch. biol. technol. 59 1-16 doi: 10.1590/1678-4324-2016160786
[8] SNI ISO 105-C06 2010 Test for Colour Fastness-Part C06: Colour Fastness to Domestic and Commercial Laundering
[9] Kubelka P and Munk F 1931 Ein beitrag zur optik der farbanstriche Z. Techn. Phys. 12 593-601
[10] Hossain Md F, Asaduzzaman Md, Kamruzzaman Md, Talukder Md E, Miah M R and Quan H 2016 Application of new synthetic fifth generation thickeners for printing cotton fabric with reactive dyes American Journal of Polymer Science & Engineering 4 123-32
[11] Smulders E 2002 Laundry Detergents (Weinheim: Wiley-VCH Verlag)
[12] Lynn J L 2009 Detergency and detergents Kirk-Othmer Encyclopedia of Chemical Technology (New York: John Wiley & Sons)
[13] Glennie E B, Littlejohn C, Gendeiben A, Hayes A, Palfrey R, Sivil D and Wright K 2002 Phosphates and alternative detergent builders EU Environment Directorate Report No. UC 4011 WRec plc Swindon
[14] Arsyah D M, Karden E and Helmy Q 2018 Characterization of biosurfactant produced by petrofilic bacteria isolated from hydrocarbon impacted soil and its potential application in bioremediation *IOP Conf. Ser.: Earth Environ. Sci.* 106 012101 doi: 10.1088/1755-1315/106/1/012101

[15] Effendi A J, Karden E and Helmy Q 2018 *Biosurfactant-Enhanced Petroleum Oil Bioremediation* In: Kumar V, Kumar M, Prasad R (eds) Microbial Action on Hydrocarbons (Singapore: Springer) doi: 10.1007/978-981-13-1840-5_7

[16] Singh R P, Rawat J P and Kumar R 2000 Effect of cationic, non-ionic and anionic surfactants on the adsorption of carbofuran on three different types of indian soil *Adsorption Science & Technology* 18 333-46

[17] Smith D L 1997 Impact of composition on the performance of sodium linear alkylbenzenesulfonate (NaLAS) *JAOCS* 74 837-45

[18] Pattusamy V, Nandini N and Bheemappa K 2013 Detergent and sewage phosphates entering into lake ecosystem and its impact on aquatic environment *International Journal of Advanced Research* 1 129-33

[19] Kundu S, Coumar M V, Rajendiran S, Rao A and Rao A S 2015 Phosphates from detergents and eutrophication of surface water ecosystem in India *Current Science* 108 1320-5

[20] Amara A A, Salem S R and Shabeb M S A 2009 The possibility to use bacterial protease and lipase as biodetergent *Global Journal of Biotechnology & Biochemistry* 4 104-14