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Formulation of disinfectant liquid cleaning detergent using violacein as antibacterial agent for household applications

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ABSTRACT

Synthetic antibacterial agents and surfactants have been used widely in detergent formulation. These synthetic antibacterial agents and surfactants have major drawbacks in both environment and health. Synthetic surfactants and antibacterial agents are non-biodegradable, toxic to aquatic life and can cause skin irritation to human. Thus, violacein pigment, a natural antibacterial agent obtained from *Chromobacterium violaceum* UTM5 and a plant-based surfactants such as sodium laureth sulphate (SLES), coconut fatty acid diethanolamide (CDE) and cocomidopropyl betaine (betaine) were used in this study. Two types of builder which are plant-based, (sorbitol) and synthetic-based, (ethylenediaminetetraacetic acid) were added. The builder act as anti-redeposition agent and help to soften the hard water, thus improving the washing performance of the surfacants. The physical properties of the detergent formulation were tested based on the following properties: pH, foaming ability, viscosity and washing performance. Six formulations of the same ratio of surfactants (SLES:CDE:betaine;8.31:1:2) but different ratio of sorbitol and EDTA were prepared. Five different concentrations of violacein pigment was added in the formulation which contained 5% sorbitol as it give the best physical performance with 12.36 cm^3 volume of foam, with the viscosity 3.02 mm^2s^{-1}, washing performance of 97.94% and pH of 8.80. Then antibacterial test was performed using disc method. The antibacterial test performed showed the detergent formulation with different concentrations of violacein pigment have different antibacterial properties which were determined by the inhibition zone. The formulation with high concentration of violacein pigment have high antibacterial properties than others. Comparison study with commercial detergent products in the market show a comparable physical performance and antibacterial properties although it only use three surfactant, one builder and an antibacterial agent in the formulation.

Keywords: plant-based surfactant, violacein pigment, antibacterial activity.

1. INTRODUCTION

Detergents are formulation consisting of surface-active agents (surfacants), builders, fillers, boosters,and auxiliary compounds [1] that are used as cleaning agents for laundry, household goods, cosmetic cleaners and industrial applications. The development of detergent formulations introduce the use of antibacterial agents, thus variety of antibacterial detergent and soaps are being produce. Antibacterial soaps can remove 65% to 85% bacteria from human body [2], therefore it become popular among the consumers.

The problem faced in the antibacterial detergent formulation is the use of synthetic surfactants and antibacterial agents. Sodium dodecylbenzenesulfonates as shown in Figure 1 is an example of synthetic surfactant [3] majorly used in the laundry detergent while triclosan, Figure 2 is widely used as a synthetic antibacterial agent [4]. The use of these compounds in detergent formulation have raise a concern because both can cause health problem and harmful to the environment [1,5]. Triclosan is quite toxic to aquatic animals [5] and non-biodegradable.

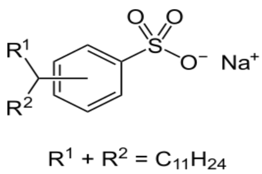

Figure 1 Chemical structure of sodium dedocylbenzenesulfonates

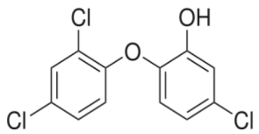

Figure 2 Chemical structure of triclosan

In this project, a natural antibacterial agent, violacein pigment and plant-based surfactants, sodium laureth sulphate (SLES), coconut fatty acid diethanolamide (CDE) and cocomidopropyl betaine (Betaine) were used to replace the synthetic compounds. Two different builders which are plant-based, sorbitol and ethylenediaminetetraacetic acid, synthetic builder also added in the formulations. Plant-based surfactants and natural antibacterial agents are bio-friendly and less harmful compared to synthetic compounds. Six formulations with the same ratio of surfactants but different ratio of builders were prepared. Then, violacein pigment was added in the formulation that gives the best physical performance which are evaluated based on the foaming ability, viscosity, pH and washing performance. Antibacterial test was conducted using disc method. The comparison study with commercial detergent product was carried out.

2. EXPERIMENTAL

2.1. Preparation of Violacein Pigment

Violacein pigment was prepared by using a spray drying method from *Chromobacterium violaceum* [6]. There are four steps of preparing a violacein pigment. Firstly, the culture condition of the bacteria, extraction of the pigment, preparations of microcapsules and the last steps is spray dry.

Step 1: Bacteria and culture condition

The culture of *C. violaceum* UTM 5 [7] was maintained on Nutrient agar (NA sterilized at 121 °C for 15 min) medium containing (g/L): Peptone 5, Beef extract 1, Yeast extract 2, NaCl 5, Agar 15 at 4 °C in the laboratory and sub cultured every month. For batch fermentation, 7 L bioreactor (BiotronLiFlus SP 7.5 L, Korea) was used. 4.5 L of nutrient broth was inoculated with 500 mL seed culture (*C. violaceum* UTM5) in its late exponential phase. The temperature, pH, stirring rate and air flow rate were controlled at 30 °C, 7, 200 rpm and 2 L/min respectively for 24 h.

Step 2: Extraction of the pigment

Pigment was extracted using ethyl acetate (125 mL) and placed in the fume hood to remove the solvent until 50 mL of concentrated pigment was obtained. The extract stored in a single container under refrigeration till analysis.

Step 3: Preparation of the microcapsules

Carrier agent solution: Gum Arabic was used. The solution was prepared in a final concentration of 10% (w/v) prior to the addition of the pigment. The concentrated pigment extract was added to the carrier agent with one part of the concentrated pigment to three parts of the carrier solution (v/v). The mixtures were homogenized in a magnetic stirrer, maintained at room temperature and subjected to spray drying.

Step 4: Spray drying

The feed mixtures were fed in a spray dryer. The atomizing air and temperature feed rate were kept constant at 1.15 kg/cm^3 and 30 °C respectively. The air flow was set at 60 m^3/h and the liquid feeding pump flow-rate was set at 2 mL/min. The inlet and outlet temperature were set at 180 °C and 85 °C respectively. Single standard nozzle (0.7 mm) was used to performed the atomization process. Glass cyclone used in this process as drying chambers have a size of ID; 12 cm, OD: 14 cm and length (41 cm) respectively. The spray dryer was run with water at the fixed operating conditions for 10 min before and after the spray drying process. The powders collected from the collecting chamber and cyclone were then weighed and stored at room temperature.

2.5 Preparation of Detergent Formulation

Six detergent formulation with the same ratio of surfactants (SLES:CDE:betaine;8.31:1:2) and different ratio of builders were prepared as shown in Table 1. Distilled water was added until it reach 100% wt. The detergent solution was stirred and mixed well using magnetic stirrer. The formulations was stored in sample bottle and labelled.

Table 1 Liquid Detergent Formulation

Detergent Formulation	Weight (g)				
	CDE	SLES	Betaine	Sorbitol	EDTA
1	1.00	8.31	2.00	5.00	0
2	1.00	8.31	2.00	3.00	0
3	1.00	8.31	2.00	1.00	0
4	1.00	8.31	2.00	0	5.00
5	1.00	8.31	2.00	0	3.00
6	1.00	8.31	2.00	0	1.00

2.6 Study of Formulated Detergent Performance

The performance of the formulated detergents were determined based on the physical properties : pH, foaming ability, washing performance, and antibacterial activities.

2.6.1 pH

The pH meter were calibrated before the performing the pH test. Three readings were taken to obtain the average pH value for each detergent formulation [8].

2.6.2 Foaming Ability

The foaming ability was measured based on the height of the foam formed in a beaker. A ratio of 1:10 of detergent and distilled water is used. 1 mL of detergents were mixed with 10 mL of distilled water in a beaker. The initial height of the solution is recorded and the solution are stirred using mechanical stirrer (500rpm) for 30 minutes to produce the foam. Then, the height of the solution and foam is recorded once again and the volume of foam was determined by calculating the difference between final and initial height. The volume of the foam was calculated by using equation below [8].

$$\text{Volume of foam} = \pi r^2 h \qquad \text{(Eqn. 1)}$$

where *r* is the radius of the beaker and *h* is the height of the foam in centimeters.

2.6.3 Washing Performance

The washing performance of the formulated detergents was tested using weighed soil before and after washing. The soil used in this research is cooking oil. The weight of a white cloth (approximately 2.5 cm × 2.5 cm), W_1 was determined using analytical balance and the the value was recorded. The white cloth was immersed in soil for 2 seconds and left the cloth for 10 minutes and the weight, W_2 . was recorded. Then the cloth was in a beaker containing the formulated detergent of ratio 1:10 of detergent and water and stirred using mechanical stirrer for 30 minutes. After washing, the cloth was dry in the oven and the final weight, W_3 of the cloth was measured [8,9]. Calculate the washing performance by using equation below.

$$\text{Washing performance} = \frac{(W2-W1)-(W3-W1)}{(W2-W1)} \times 100\% \qquad \text{(Eqn. 2)}$$

2.6.4 Viscosity

The viscosity of the formulated detergents was determined by using a 50mL burette. The burette was filled with the 50mL detergent and ensured the meniscus of the solution is slightly above 0mL mark. A beaker was placed below the burette to collect the solution. Then, the stopcock was open all the way and the stopwatch was started immediately. The stopcock was closed when the minuscus hit the 20mL mark and simultaneously stop the stopwatch. The time taken for 20mL of solution to flow through a burette was recorded. The weight of collected solution was determined using analytical balance and the value was recorded. The viscosity of the solution was calculated using equation below

$\eta = \rho \times v$ (Eqn. 3)
$v = c \times s$ (Eqn. 4)

where, η is dynamic viscosity, ρ is density, v is kinematic viscosity, c is flow rate of burette, and s is time in second.

2.6.5 Antibacterial Activities

2.6.5.1. Disc Method

A loophole of *Staphylococcus aureus* was streak on the Mueller-Hinton agar and incubated for 24 hours at 37°C. 500 µL of the bacteria, sub-culture Mueller-Hinton Broth for 1hour and 30 second was spread over the MH agar plate using sterile cotton swab. Sterilized disc (whatman no.5, 6 mm diameter) are impregnated with 10 µL of different concentration of violacein (0.02 g/ml, 0.04 g/ml, 0.06 g/ml, 0.08 g/ml and 0.1 g/ml) and place on the agar surface. The petridish was sealed and incubated at 37°C for 24 hours [10]

2.7 Comparison between the Prepared Detergent Formulation with Existing Commercial Detergents Product

The performance of the formulated detergent will be compared with the existing commercial detergent products in the market. The commercial detergents are randomly selected and compared in terms of foaming ability, viscosity washing performance and antimicrobial activities using the same method as described in section 3.3.

3. RESULTS AND DISCUSSION

3.1. Physical Performance of detergent formulation

Table 2 and Figure 3 represent the result of washing performance, volume of foam, viscosity and pH of six detergent formulation. From the table below, it can be seen that all formulations have washing performance more than 95% in removing the soil (cooking oil) from cloth. Detergent formulation 3 has the lowest percentage of washing performance, 95.99% and volume of foam, 10.14 cm^3 compared to other formulations. Formulation 1 has the highest volume of foam and 98.83% washing performance. Based on this result, it shows that the foam produced help to trap the soil before it is rinsed away to remove the soil. Although formulation 4 has the highest washing performance, 99.03 %, it only has 10.78 cm^3 volume of foam. Thus it proven, EDTA is the most efficient builder that improve the washing performance of detergent as it holds the soil to prevent it from re-deposited back on the surface. According to Table 2, the effect of EDTA is more significant for viscosity compared to sorbitol. The viscosity of formulation with 5%wt of EDTA is 10.78 which is three times larger than formulation 1, 5%wt of sorbitol. Besides that the formulation with a high amount of EDTA have a high pH.

Table 2 Physical performance of detergent formulation.

Detergent Formulation	Washing Performance (%)	Volume of Foam (cm^3)	Viscosity (mm^2/s)	pH
1	98.83	12.36	3.02	8.80
2	97.94	10.14	1.39	9.06
3	95.99	10.14	1.32	9.04
4	99.03	10.78	10.78	10.12
5	97.80	11.09	2.82	10.04
6	97.24	10.78	1.23	9.94

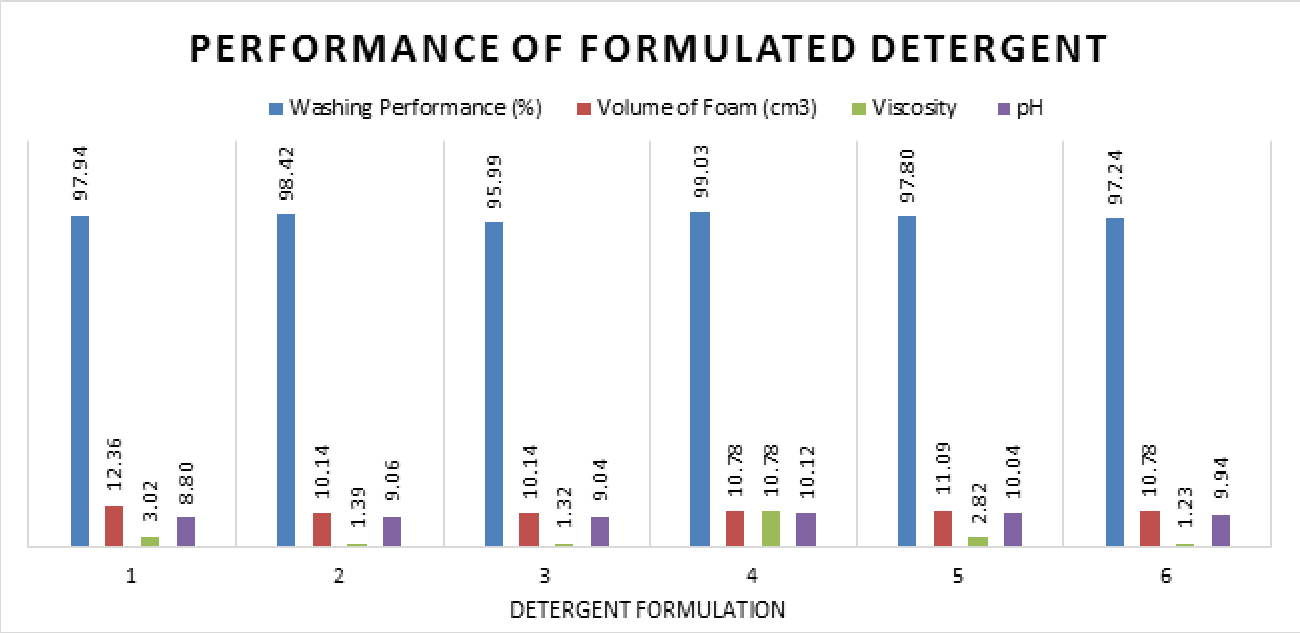

Figure 3 Physical performance of formulated detergent

3.2 Antibacterial Test

Five different concentration of violacein pigment as shown in Figure 4 was added in detergent formulation 1, because it has the best physical performance. In addition, formulation 1 used a plant-based surfactants and builder. These surfactants and builder are less harmful for both environment and human. Natural-based compound are easily biodegraded compared to synthetic compound.

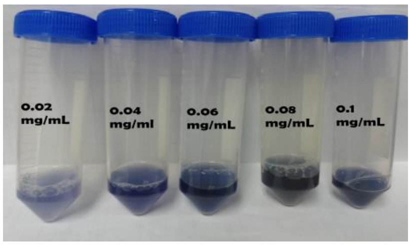

Figure 4 Antibacterial detergent formulation with different concentration of violacein (from left to right;0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, 0.1 mg/mL

The antibacterial activity of formulated detergent was determined by using a disc method. Different concentration of violacein pigment used in the formulation shows different inhibition zone of bacteria. The zone of inhibition was greater at maximum concentration while it was minimum at lower concentration. From Table 3, the minimum inhibitory concentration of detergent formulation for S.*aureus* is 0.02 g/mL and 0.04 g/mL.

Table 3. Inhibition zone formed on agar plate.

Concentration of violacein (g/mL)	Inhibition Zone(mm)
Blank (distilled water)	0
0.02	9
0.04	9
0.06	12
0.08	13
0.1	15

3.3 Comparison of the Formulation with Commercial Antibacterial Detergent Product

Four types of commercial antibacterial detergent product, which are handsoap, laundry, dishwash and floor are tested. Two different brand labelled as A and B for each types of commercial antibacterial product was selected randomly. The result of each types are recorded in the Table 4.

Table 4 Result of Physical Performance and Antibacterial test of Commercial Product

Product	Washing Performance (%)	Volume of Foam (cm3)	Viscosity (mm2/s)	pH	Inhibition Zone (mm)
Laundry A	95.38	10.14	7.73	8.78	16
Laundry B	96.44	10.14	6.08	8.53	15
Handsoap A	98.59	11.09	16.92	5.98	14
Handsoap B	95.21	11.09	69.83	4.92	18
Dishwash A	97.66	12.68	17.02	4.46	19
Dishwash B	97.27	10.14	20.97	8.32	35
Floor A	91.67	9.19	1.40	6.24	10
Floor B	92.68	10.78	1.31	1.62	12

From the table above, the pH for each products are varied according to their types and applications. The pH of commercial product are lower than formulation. The lowest pH is 1.62, which is used as a floor cleaning and both laundry detergent A and B have a high pH, 8.78 and 8.53 respectively. The volume of foam formation for all commercial products also are almost similar to the formulation. However the viscosity of the commercial detergent are higher compared to the formulated detergent except floor cleaning detergents as they have a lot of additives in their formulation. Besides that, the washing performance of handsoap, dishwash and laundry detergent are almost the same with the formulated detergent. Dishwash B has a high antibacterial properties as there are large inhibition zone around observed on the agar plate. Antibacterial properties for laundry B and formulation with 0.1 g/mL of violacein are the same. The floor cleaning products has the weakest antibacterial properties as it contain less component compared to other commercial products.

The formulated detergents used a small amount of surfactants, builder and antibacterial agent compared to commercial product and it gives the same performance and antibacterial properties with most of the product tested. The main compounds used in this study are obtained from natural sources, thus the cost of production are a lot cheaper than commercial detergent that use a synthetic compounds. The natural surfactants used in this study are obtained from the waste of palm tree oil and violacein pigment are obtained from C.*violaceum.* These natural sources are biodegradable and less toxic.

4. CONCLUSION

Disinfectant liquid cleaning detergent using violacein pigment as antibacterial agent was successfully prepared. The formulation also used plant-based surfactants which are sodium laureth sulphate (SLES), coconut fatty acid diethanolamide (CDE) and cocomidopropyl betaine (betaine), and sorbitol as a builder. These formulation are introduced to replace the synthetic detergent in the market. In addition, the natural-based formulation also give similar performance as the synthetic product. The formulated detergent gives 12.36 cm^3 volume of foam, with the viscosity 3.02 mm^2s^{-1}, washing performance of 97.94%. The minimum inhibitory concentration of violacein in the formulation is 0.02 and 0.04 g/mL. The maximum concentration of vioacein pigment in formulation gives high antibacterial properties which are almost the same as the commercial products.

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