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FORMULATION OF LIQUID BIODETERGEN FROM COMBINATION OF STARFRUIT (*Averrhoa bilimbi*) AND LAURIL-DEA SURFACTANT WITH THE ADDITION OF PROTEASE

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ABSTRACT

The use of detergents in Indonesia continues to increase significantly, in line with the increase in Indonesia's population and rapid industrial development. Detergents that contain active ingredients such as branched alkylbenzene sulfonates (BAS) or linear alkylbenzene sulfonates (LAS) surfactants, which are derived from petroleum, can hurt the environment and living creatures because they can pollute the environment. This observation aims to create a bio-detergent formulation with protease enzyme additives, combining the green surfactant Lauril-DEA and saponin biosurfactant from starfruit extract (Averrhoa bilimbi). Biodetergent is formulated with 12 variations in the concentration of saponin extract (ratio of saponin extract and distilled water 35%:40%, 30%:45, 25%:50%, 20%:55%) and enzyme (3%, 4%, and 5%). Biodetergent analysis is carried out by testing pH, density, and detergency power to see the conformity of the product with Standar Nasional Indonesia (SNI). Analysis of waste contamination from biodetergent products (pH of waste, levels of COD, BOD, and TSS of waste) was observed to determine the impact of the product on the environment. The biodetergent product produced from this research has a pH of 10-12 and a density of 1.031-1.07 G/mL. All observations have complied with SNI 06-476-1996 concerning liquid washing detergents. The detergency power was 30.3-63.8 NTU, with the best detergency power obtained in the third observation. The best experimental biodetergent waste had a pH of 7, BOD 21.21 mG/L, COD 104.53 mg/mL, and TSS 30 mg/L. These characteristics meet the waste water quality standards for laundry industry businesses and activities and are as good as commercial detergents. Therefore, the biodetergent product produced in this research can be an alternative product that supports environmental sustainability.

Keywords: biodetergent, detergency power, lauril-DEA, saponin, surfactant.

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INTRODUCTION

The problem with using detergents that contain synthetic surfactants is that they can pollute the environment. Therefore, environmentally friendly surfactants are needed. The use of detergent, apart from helping with washing activities, also has a polluting effect on the environment. Detergents containing active ingredients, such as BAS or LAS surfactants derived from petroleum, can hurt the environment and living creatures because they are difficult to break down by microorganisms and pollute the environment [1, 2].

The use of detergent in Indonesia itself began to experience a significant increase in the 1990s, in line with the increase in Indonesia's population and rapid industrial development. In recent years, detergent use has increased drastically, reaching around 10% annually. In 2007, detergent use in Indonesia reached 500,000 tons per year, and only 62% of that was met from local production [3].

Detergents contain three main ingredients: surfactants, builders (phosphate compounds), and additives (bleach and fragrance). The most significant components of detergent are building materials, which are between 70-80%; essential ingredients, which are around 20-30%; and relatively few additives, which are between 2-8% [4]. Surfactants, usually used as detergents, are generally anionic and toxic and can cause destabilization for living things. Apart from that, surfactants with polar and nonpolar groups can unite a mixture of oil and water. Active detergent compounds include branched alkylbenzene sulfonates (BAS) or linear alkylbenzene sulfonates (LAS) surfactants. Because BAS compounds are difficult to decompose, LAS compounds are predominantly used to replace BAS compounds [5, 6].

Using detergent will produce waste because, after use, the used washing water containing detergent is disposed of in the environment. Early detergent formulations contained non-biodegradable surfactants. Detergent wastewater is a pollutant for the environment because it contains BAS, which is relatively complex. Surfactants, as the main component in detergents, have chemical chains that are difficult for nature to degrade. Therefore, alternative surfactants from natural ingredients are needed; one example is saponin [4, 7, 8].

Saponin is a non-ionic surfactant found in natural ingredients and can be a cleaning agent. One of the plants that contains saponin is starfruit (*Averrhoa bilimbi*). This plant contains many compounds, including saponins, flavonoids, and polyphenols. Saponin, one of the secondary metabolites of starfruit, is a glycoside composed of sugar linked to an aglycone. Aglycones have a structure consisting of triterpenoid or steroid chains, which are nonpolar. The saponin structure causes saponin to act like a detergent or detergent, which is called a natural surfactant [9-12].

However, the performance of saponins is not as optimal as general surfactants. Therefore, it is necessary to add surfactants derived from natural components (plants or microbiology) or chemically synthesized from natural raw materials, usually called biosurfactants. An example of a biosurfactant is alkanolamide surfactant. Alkanolamides can be produced from the reaction between alkanol amines and fatty acids in vegetable oils and are widely used in food, cosmetics, and medicines. This surfactant has good characteristics. Namely, it is easily decomposed in nature, does not damage the skin, and has a low level of toxicity [13, 14].

Detergents with the addition of enzymes are widely used today for various types of liquid, paste, and powder detergents [15]. Enzymes are one of the additives in making detergent. 1-2% of detergent-making additives contain enzymes, bleach, brighteners, perfumes, and dyes. One enzyme that can be used is protease. Protease enzymes function to hydrolyze protein stains on clothing so that dirt containing protein, such as blood, mucus, and sweat, will be easily washed. Besides that, other impurities bound to protein are also easier to remove. The proteases found in detergents usually work at alkaline pH and relatively high temperatures. This alkaline protease is used as an additive in detergents because of its biodegradable ability and can improve the work of detergents in general [16-18].

From the background description above, this research aims to make a detergent by combining starfruit extract, which produces saponin, with a biosurfactant derived from alkanol amide, namely lauryl diethanolamine, and also additional protease enzymes as additives. From these observations, we can produce detergent products that can reduce environmental problems, with quality equivalent to commercial detergents.

THEORETICAL BASIS

Detergent

Detergent is a cleaning product generally used in the laundry process, with the main ingredients being surfactants and phosphates in builders. This causes laundry waste to have high surfactant and phosphate content [4, 19]. Detergent waste is a pollutant that can endanger the life of organisms in water because it causes the supply of oxygen from the air to be very slow due to the foam covering the surface of the water. Detergents are generally composed of three main components, namely, surfactants as the essential detergent ingredient) which ranges from 22-30%, builders (phosphate compounds), and additives (bleach and fragrance. Detergents on the market are generally detergents with surfactant active ingredients LAS. LAS is the most widely consumed detergent ingredient. LAS is a biodegradable compound usually contained in wastewater at around 1–20mG/L. LAS can decompose under aerobic conditions (enough oxygen and microorganisms), but this degradation is rapid. Naturally, it takes a long time, around nine days, and only reaches 50%. LAS cannot decompose under anaerobic conditions (no air), so LAS cannot decompose in the conditions of rivers in Indonesia, which are primarily murky [20, 21].

Surfactant

Surfactants are molecules with polar groups that like water (hydrophilic) and nonpolar groups that like oil (lipophilic) simultaneously, so they can unite mixtures consisting of oil and water. Surfactants are surface-active materials which work to reduce the surface tension of liquids. This active property is obtained from the dual nature of the molecules. The polar part of the molecule can be positively, negatively, or neutrally charged; the neutral part, the polar part, has a hydroxyl group, while the nonpolar part is usually a long alkyl chain. Surfactants are generally synthesized from petroleum derivatives, and their waste can pollute the environment because they are difficult to degrade [20, 22].

Starfruit

Starfruit A. bilimbi has various benefits because this plant contains many compounds, including saponins, flavonoids, and polyphenols. Saponin, one of the secondary metabolites of starfruit, is a glycoside composed of sugar linked to an aglycone. Aglycones (sapogenins) have a nonpolar structure consisting of triterpenoid or steroid chains. The saponin structure causes saponin to act like a detergent or detergent, which is called a natural surfactant. The saponin in starfruit is triterpene saponin with brown rings produced in the saponin color test with LB reagent [10]. Starfruit contains 6.8-8.4% saponin after extraction with solvent [9].

Protease Enzyme

Protease is a group of enzymes that are widely used in industry. Protease is an enzyme that hydrolyzes peptide bonds in proteins into oligopeptides and amino acids. Proteases (serine protease, cysteine/thiol protease, aspartate protease, and metal protease) are enzymes widely used in industries such as the pharmaceutical, leather, detergent, food, and waste processing industries - proteases used in industry account for around 60% of enzyme sales worldwide. Proteases can be isolated from 44.78% bacteria, 43.85% plants, and 11.15% animals. Proteases from bacteria are the most abundant compared to other sources, namely from plants and animals [23].

Protease and lipase can be used as stand-alone biodetergents without using additional chemicals. Protease enzymes function to hydrolyze protein stains on clothing so that dirt containing protein, such as blood, mucus, sweat, and so on, will be easily washed. Besides that, other impurities bound to protein are also easier to remove. The proteases found in detergents usually work at alkaline pH and relatively high temperatures. This alkaline protease is used as an additive in detergents because of its biodegradable ability and can improve the work of detergents in general. The protease enzyme is now used as a bird's nest wash to replace the chemical hydrogen peroxide (H_2O_2), which is known as a bleaching agent that is natural and safe for the body [16].

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MATERIALS AND METHODS

Materials

The materials that will be used in this research activity are starfruit (*Averrhoa bilimbi*), Ethanol of 96%, HCl of 37%, concentrated H_2SO_4, Lauric Acid, Diethanolamine (DEA), Hexane, 2-propanol, Ferroin indicator, perfume, and aquades. Purification materials in acetone and analytical materials in the form of potassium hydroxide (KOH), phenolphthalein, isopropanol, and hydrochloric acid (HCl) were obtained from E Merck.

Experimental Design

The experimental design for biodetergent formulation is given in Table-1. Making biodetergent is done by mixing the constituent ingredients until they are homogeneous.

Table-1. Experimental design for biodetergent formulation.

No.	Surfactants: Aquadest (%)	Protease (%)	Aquadest (mL)	pH	Density (G/mL)	Detergency Power (NTU)
1	35:40	3	20	11	1.070	77.6
2		4	20	10	1.061	91.3
3		5	20	10	1.055	101.2
4	30:45	3	22.5	11	1.051	101.5
5		4	22.5	10	1.053	97.2
6		5	22.5	11	1.058	96.6
7	25:50	3	25	12	1.050	96.9
8		4	25	11	1.044	89.9
9		5	25	11	1.048	98.3
10	20:55	3	27.5	12	1.040	107.1
11		4	27.5	12	1.023	118.5
12		5	27.5	11	1.031	120.6

Experimental Procedure

Foam and color test

Foam Test. 0.5 grams of simplicia was put into a test tube containing 10 ml of distilled water, shaken, and one drop of 2 N hydrochloric acid solution was added. The sample contains saponin if a stable foam forms with a 1-3 cm height for 30 seconds.

Color Test. 0.5 grams of simplicia was put into a test tube containing 10 ml chloroform and heated for 5 minutes in a water bath while shaking. Next, a few drops of LB reagent were added. If a brown or violet ring forms, it indicates the presence of triterpene saponins, while the green or blue color indicates the presence of steroid saponins.

Biosurfactant from starfruit extract

Extraction of starfruit simplicia is carried out as follows [9]. Star fruit samples were washed thoroughly, sliced thinly, and dried in the sun for three days. Once dry, grind with a chopper and filter through a 20 mesh sieve. The starfruit Simplicia was placed in an Erlenmeyer flask, and then 120 mL of ethanol solvent was added (to vary the ratio 1:6) and covered with aluminum foil. Extraction was carried out for 30 minutes in an Ultrasonic water bath. After obtaining the extract, evaporation occurs until a thick macerate is obtained.

Biodetergent

The procedure for making our biodetergent is based on previous research with several modifications [1]. Making biodetergent starts from making solution A by homogenizing the biosurfactant with distilled water with variable concentration ratios (35%:40%, 40%:35%, 45%:30%, and 50%:25%) of the biodetergent volume. Solution B is made from a mixture of 20% Lauryl Diethanolamine and protease enzymes of 3%, 5%, and 7% of the biodetergent volume. Solutions A and B are dissolved, and an additive, namely 1% perfume, is added. The solution mixture was heated and stirred at a temperature of 50℃ and a stirring speed of 250 rpm for 1 hour and then left for 12 hours at room temperature.

RESULTS AND DISCUSSIONS

Saponin in Starfruit Extract

Saponin is a secondary metabolite compound contained in plants. The molecular structure of saponin, consisting of a series of C and H atoms, makes this compound have biological activity as an antibacterial, which is generally applied in making detergents. Saponins can be developed in various fields, such as agriculture, the cosmetics industry, shampoo, food, and medicine [24].

Starfruit contains saponins, flavonoids, ascorbic acid, tannins, glucosides, citric acid, and minerals, especially potassium and calcium. Saponins have a

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triterpenoid or steroid chain structure, which is nonpolar, so it acts like a surfactant. This saponin content is found not only in the fruit but also in the leaves and leaf stalks. The starfruit plant's saponin, flavonoid, and tannin content can be antibacterial and antifungal against gram-positive and gram-negative bacteria and fungi [9]. The saponin content in starfruit is 3.582% [10].

Based on the foam test results, it can be seen that the sample contains saponin with the formation of stable foam with a height of 1-3 cm for 20 seconds. Simplicia is put into a test tube containing 10 ml of distilled water, shaken, and then one drop of 2 N hydrochloric acid is added. The basis of the foam test reaction is the nature of the saponin compound, which dissolves easily in water and creates foam when shaken. Water is a solvent, while HCl 2 N is a reagent [10].

The color test was carried out with 0.5 grams of Simplicia, put into a test tube containing 10 ml of chloroform, and heated for 5 minutes in a water bath while shaking. Next, a few drops of LB reagent were added. If a brown or violet ring forms, it indicates the presence of triterpene saponins, while the green or blue color indicates the presence of steroid saponins. The qualitative color test results showed that the sample contained triterpene saponins with the formation of brown rings [10].

Effect of Formulation on pH

The pH value is obtained by measuring the biodetergent solution on all runs on a pycnometer that has been cleaned and dried and then filled with distilled water at a temperature of 25°C. The pH measurement results for each run can be seen in Figure-1.

The higher the concentration of starfruit extract, the pH of the biodetergent produced will decrease. This is because the pH of the star fruit extract obtained is 6.

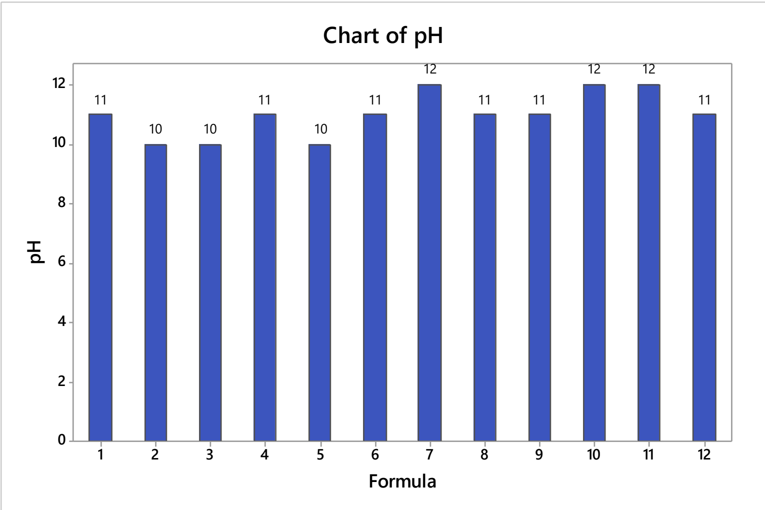

Figure-1. Results of pH measurements for each observation.

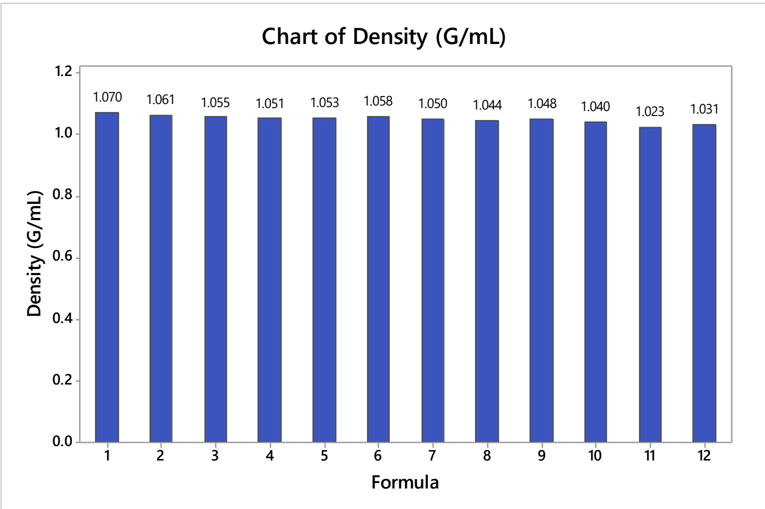

Figure-2. Effect of variations in detergent formulation on bio detergent density.

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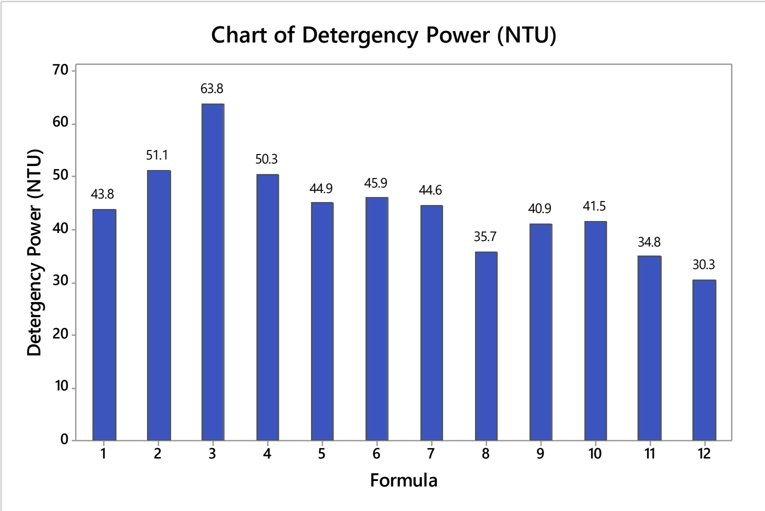

Figure-3. Effect of variations in detergent formulation on the detergency power of detergent.

Furthermore, the resulting pH is higher at the Lauril-DEA surfactant. This can happen because the pH of the Lauril-DEA surfactant is 12. If you look at the protease enzyme content, the higher the protease enzyme content, the lower the resulting pH. This is because the pH of the protease enzyme obtained is 7. According to previous research, the optimum biodetergent pH is 11 [1]. Detergents work actively in alkaline conditions because they can neutralize dirt and help the dirt remain suspended in the solution.

Based on SNI 06-4075-1996, the pH value of liquid detergent ranges from 10.0 to 12.0. In this study, the pH value of each variation of detergent was obtained, including variations in the concentration of biosurfactant n, distilled water, and the concentration of protease enzymes. Based on SNI 1996, it can be concluded that all detergents have a pH value that is by SNI because the pH obtained from this research is around 10-12.

Effect of Formulation on Density

Density is a measure that describes the extent to which the mass of an object is concentrated in a specific volume. In other words, density measures how dense or dense an object is. A physical quantity describes how mass is distributed in the available space. The effect of variations in detergent formulation on the density of the detergent can be seen in Figure-2. The density of the liquid detergent will affect the ability of the detergent to dissolve in water and the stability of the liquid detergent emulsion. The more significant the difference in density of the detergent components will cause a decrease in the emulsion stability of the liquid detergent [25].

The density of the liquid detergent obtained will decrease as the operating temperature increases. The liquefaction process of the ingredients used and the increase in speed will accelerate the homogeneity of the mixture. This occurs because the mixture's uniformity level increases as the temperature increases. The speed of the melting process of the ingredients used will accelerate the achievement of mixture uniformity. Decreasing the detergent density value results in its density getting closer to the density of water, thereby improving the quality of liquid detergent so it can dissolve well in water [1]. Based on SNI 06-4075-1996, the density of liquid detergent products ranges from 1.0–1.3 G/mL. The density value of the liquid detergent produced ranges from 1.031-1.07 G/mL and meets SNI quality requirements.

Effect of Formulation on Detergency Power

Detergency is the specific property possessed by surface-active substances to clean a surface of dirt. However, surface active agents cannot clean dirt from surfaces thoroughly without other supporting substances, so detergency is defined more specifically as specific properties possessed by surface-active substances that increase the cleaning power of a washing solution [26].

The effect of variations in detergent formulations on the detergency power of detergents can be seen in Figure-3. The detergent power value of the liquid biodetergent produced in this study was between 30.3-63.8 NTU. Detergent power testing is used to assess the effectiveness of foam in cleaning dirt stuck to fabric. The mechanism for removing dirt by detergent is by reducing surface tension to form an emulsion and binding the dirt in suspension form so that the dirt can be removed.

Figure-3 shows that the best detergency power is in run 3, where the concentration of starfruit leaf extract is the highest in this run. The more starfruit the extract added, the more alkaline the formula will be due to the increase in the OH- group, which comes from saponin. Addition of extract and pH can influence increasing detergency power. The more starfruit extract is added, the pH formed also increases. This is because high pH shows lots of OH- ions. The more OH groups there are, the more nonpolar groups in clothing fat will be removed, so the percentage of detergent power will be higher.

The presence of OH- ions in triterpenoid saponins influences the value of the detergency power produced.

OH- ions will bind to nonpolar (positively charged) groups in fats/fatty acids. The more OH- ions produced, the more nonpolar groups will be bound by OH- ions, so more fat will be released from the white cloth, meaning that the greater the pH value, the greater the % detergency power [26]. It is also supported by another active substance, the protease enzyme, which is helpful as an active antibacterial ingredient. Protease enzymes function to increase the effectiveness of detergent cleaning power by accelerating the degradation of dirt in the form of proteins and their derivatives [1].

The liquid biodetergent product from this research has been tested to clean stains on clean white fabrics with satisfactory results. In the washing test procedure, only one formulation was used, namely the run three formulation, which had the best detergency power compared to the other run formulations.

Descriptive Statistic

Descriptive of pH

Descriptive analysis needs to be carried out to describe and conclude observational data. Table-2 shows the data output interpretation to analyze the substrate ratio and enzyme amount effect on detergent pH. Table-2 shows that the biodetergent formulated at substrate ratio 1 has an average pH of 10.333 with a variance of 0.333. At substrate ratio 1, the minimum pH is 10, and the maximum is 11. Compared to detergents using substrate ratio 1, the average pH at substrate ratio two is slightly higher, namely 10.667, with a minimum pH of 10 and a maximum of 11.

If the substrate ratio is increased to 3 or 4, the same variance is found as the previous ratio, 0.333, but the minimum pH becomes 11, and the maximum pH becomes 12. Judging from the shape of the distribution, detergents using a substrate ratio of 1 and 3 have a skewness of 1.73, which is of value. Positive so that the shape of the distribution is tailed and deviates to the right. The distribution form for biodetergent uses a substrate ratio of 2 and 4 and has a tail that deviates to the left (skewness 1.73).

Table-2 also illustrates the effect of the amount of protease enzyme on the pH of biodetergent. If 3% enzyme is used, the biodetergent pH will have an average of 11.500, but if the amount of enzyme is increased to 4%, the average biodetergent pH will be smaller, namely 10.750. Increasing the addition of protease enzymes to 5% does not increase the average pH; namely, the average pH remains 10.750. However, increasing the amount of protease enzyme reduces the maximum pH of the biodetergent from pH 12 to 11.

Descriptive of density

Interpretation of the data output to analyze the effect of substrate ratio and number of enzymes on biodetergent density is shown in Table-3. Table 3 shows that for detergents formulated at substrate ratio 1, the average density is 1.0620 with a maximum density of 1.0700 G/mL. Increasing the substrate ratio of the raw materials used reduces the average density of detergent, respectively 1.0540, 1.0473, and 1.0313 G/mL. The same decrease was also found in the minimum and maximum density of biodetergent.

An illustration of the influence of the amount of protease enzyme on the density of biodetergent is shown in Table-3. Three variations in the amount of enzyme were used, namely 3%, 4% and 5%. Increasing the use of protease enzymes appears to reduce the maximum density of detergent from 1.0700 G/mL, 1.0610 G/mL, and 1.0580 G/mL, respectively. Meanwhile, the lowest minimum density of the three enzyme uses was obtained using a 4% protease enzyme, 1.0230 G/mL. Likewise with the average density, the lowest density was obtained using 4% enzyme, 1.0453 G/mL.

Descriptive of detergency power (NTU)

The Detergency Power (NTU) of the biodetergent produced was observed for its effect on the substrate ratio and the amount of enzyme. The results obtained are given in Table-4. Interpretation of the data output for biodetergent formulated at a substrate ratio 1 has an average density of 52.90. Increasing the substrate ratio from 1 to 4 reduces the average NTU value by 52.90, 47.03, 40.40, and 35.53. As the average NTU, the maximum NTU also decreases with increasing substrate ratio. So, a substrate ratio of 1 provides the highest NTU value of the observation range.

The amount of enzyme used also affects detergency power. Table-4 shows that increasing the number of enzymes from 3% to 4% to 5% will reduce the minimum value of NTU but increase the maximum number of NTU from 48.87, 49.55, to 59.32. These different results also cause the average NTU to vary, where the maximum average is found when using an enzyme amount of 5%.

Table-2. Interpretation of data output to analyze the effect of substrate ratio and enzyme amount on detergent pH.

Substrate Ratio	Mean	Variance	Minimum	Median	Maximum	IQR	Skewness
1	10.333	0.333	10	10	11	1.000	1.73
2	10.667	0.333	10	11	11	1.000	-1.73
3	11.333	0.333	11	11	12	1.000	1.73
4	11.667	0.333	11	12	12	1.000	-1.73
Enzyme Amount (%)	Mean	Variance	Minimum	Median	Maximum	IQR	Skewness
3	11.500	0.333	11	11.5	12	1.000	0.00
4	10.750	0.917	10	10.5	12	1.750	0.85
5	10.750	0.250	10	11.0	11	0.750	-2.00

Table-3. Interpretation of data output to analyze the effect of substrate ratio and enzyme amount on biodetergent density.

Substrate Ratio	Mean	Variance	Minimum	Median	Maximum	IQR	Skewness
1	1.0620	0.00006	1.0550	1.0610	1.0700	0.0150	0.59
2	1.0540	0.00001	1.0510	1.0530	1.0580	0.0070	1.15
3	1.0473	0.00001	1.0440	1.0480	1.0500	0.0060	-0.94
4	1.0313	0.00007	1.0230	1.0310	1.0400	0.0170	0.18
Enzyme Amount (%)	Mean	Variance	Minimum	Median	Maximum	IQR	Skewness
3	1.0528	0.00016	1.0400	1.0505	1.0700	0.0228	1.03
4	1.0453	0.00027	1.0230	1.0485	1.0610	0.0308	-1.01
5	1.0480	0.00015	1.0310	1.0515	1.0580	0.0220	-1.35

Table-4. Interpret data output to analyze the effect of substrate ratio and enzyme amount on the detergency power of biodetergents.

Substrate Ratio	Mean	Variance	Minimum	Median	Maximum	IQR	Skewness
1	52.90	102.43	43.80	51.10	63.80	20.00	0.78
2	47.03	8.25	44.90	45.90	50.30	5.40	1.50
3	40.40	19.99	35.70	40.90	44.60	8.90	-0.50
4	35.53	31.76	30.30	34.80	41.50	11.20	0.58
Enzyme Amount (%)	Mean	Variance	Minimum	Median	Maximum	IQR	Skewness
3	45.05	13.98	41.50	44.20	50.30	6.80	1.25
4	41.63	60.73	34.80	40.30	51.10	14.53	0.51
5	45.23	195.65	30.30	43.40	63.80	26.37	0.73

Boxplot analysis

A boxplot summarizes the sample distribution presented graphically, which describes the shape of the data distribution (skewness), a measure of central tendency, and a measure of the spread (diversity) of the observed data. Boxplots help summarize more detailed information regarding the distribution of observed data values. The Boxplot can also show whether the observation data has outlier and extreme values.

Effect of substrate ratio and amount of enzyme on pH

The results of box plot observations of substrate ratio on biodetergent pH are shown in Figure 4. Substrate ratios 1 and 2 have a minimum pH value of 10 and a maximum of 11. Meanwhile, if substrate ratios 3 and 4 are used, it will produce the smallest observed value of 11 and the largest of 12. The entire observation data in the Boxplot in Figure-4 does not have outliers, extreme values, medians, or wishers. The whiskers line is an extension line from the box, and the whiskers line connects the box, both upwards and downwards.

The length of the box depicted in Figure-4 corresponds to the interquartile range (IQR), which is the difference between the highest quartile (Q3) and the lowest quartile (Q1). IQR describes a measure of data dispersion. The longer the IQR field indicates the more spread out the data is. So, referring to Figure-4, because all substrate ratios used have the same IQR field length, it can be concluded that the overall biodetergent pH observations have the same distribution in all substrate ratios applied.

Figure-5 shows the results of boxplot observations of the amount of enzyme against pH. The most extended box size is at 4% enzyme amount, which means the distribution of pH data at 4% enzyme amount is the largest. The median data for 3% enzyme content is 11.5, while the median for 4% is 10.5. However, if the amount of enzyme is 5%, then the median pH is 11, and the maximum is 11.

Whiskers are lines above the most significant value and below the smallest value of the observed data. The smaller whiskers' length equals 1.5 times the IQR value. Each whisker line starts from the end of the IQR box and ends at a data value that is not categorized as an outlier. Figure- 5 shows that if a 4% enzyme quantity is used, top whiskers will be found, indicating a higher pH value than the data set within the IQR. If an enzyme amount of 5% is used, there are bottom whiskers, meaning a pH value is lower than the IQR data set.

Effect of substrate ratio and amount of enzyme on density

Values above or below the whiskers are called outlier or extreme values. Outlier values are data values located more than 1.5 x the length of the box (IQR). In Figure- 6, no outlier values were found when observing the substrate ratio's effect on density. However, of the four substrate ratios used, all have median values of 1.0610, 1.0530, 1.0480, and 1.0310, respectively. At a median value of 50% of the observation data is less than or equal to this value. Besides that, the four median values are not in the middle of the box; this indicates that the data is not symmetrical (skewed).

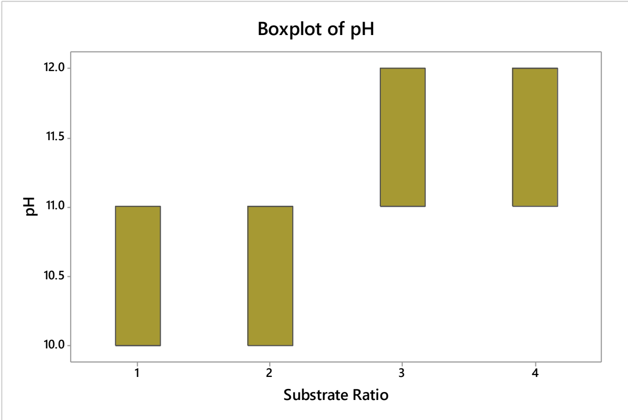

Figure-4. Boxplot observation results of substrate ratio against bio-detergent pH.

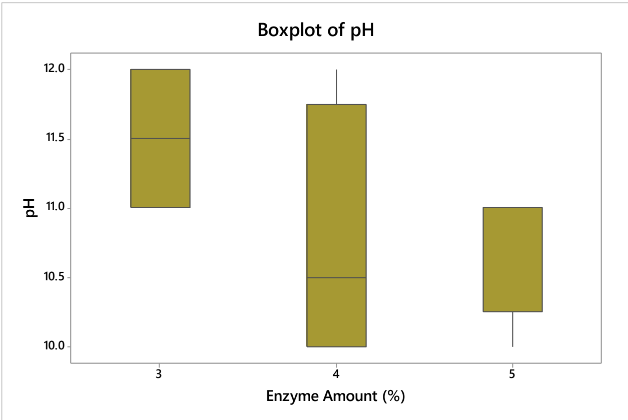

Figure-5. Boxplot observation results of the amount of enzyme against the pH of the biodetergent.

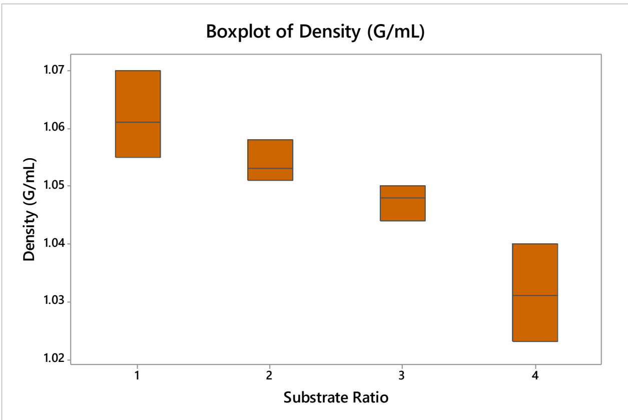

Figure-6. Boxplot observation results of substrate ratio against detergent density.

The length of the box describes the level of distribution or diversity of observation data, and in Figure-6, the most extended box is if a substrate ratio of 4 is used. Substrate ratio 1 has the most extended and maximum distribution of data. This indicates that if a substrate ratio of 1 is used, it will produce the maximum biodetergent density compared to the other three ratios. A substrate

VOL. 19, NO. 7, APRIL 2024 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

ratio of 4 will produce a good density distribution but with a minimum value.

A boxplot illustration of the influence of the amount of enzyme on density is given in Figure-7. The most extended box was found for the amount of enzyme 4%, with an IQR of 0.0308, while the other two uses of the amount of enzyme had almost the same IQR, namely 0.0228 and 0.0220.

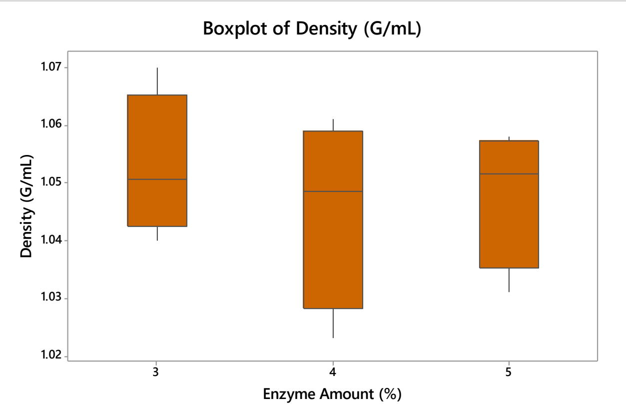

Figure-7. Boxplot observation results of the amount of enzyme against the density of biodetergent.

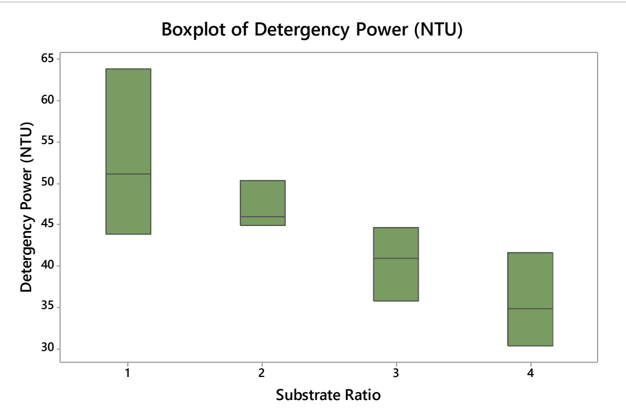

Figure-8. Boxplot observation results of substrate ratio on the detergency power of biodetergent.

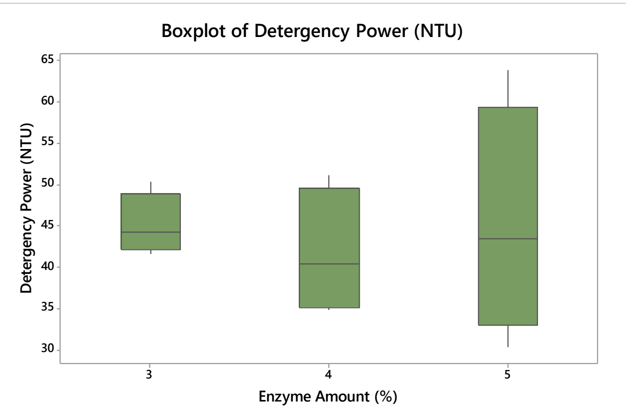

Figure-9. Boxplot observation results of the amount of enzyme on the detergency power of biodetergent.

In the Boxplot in Figure-7, all three have whiskers. However, the output data is not symmetrical (skewed), indicated by the median not being in the middle of the box and one of the whiskers being longer than the others. If the data is symmetrical or comes from a normal distribution, then the median line will be in the middle of the box, the top and bottom whiskers will have the same length, and there will be no outliers or extreme values.

If a 3% enzyme is used, the whiskers that appear have a longer top, which shows that the data distribution tends to lean towards the right (positive skewness). Meanwhile, the whiskers obtained have a longer bottom for 4% and 5% enzyme amounts, which shows that the data distribution is tilted towards the left (negative skewness).

Effect of substrate ratio and amount of enzyme on NTU

Substrate ratio and amount of enzyme were also varied to observe their effect on detergency power (NTU). The effect of substrate ratio on NTU is given in Figure-8. One of the advantages of box plots is that they can compare the distribution of several groups of data simultaneously. When compared, all four boxplots produced do not have whiskers, and the median value is not in the middle. This shows that the distribution of NTU data needs to be symmetrical. The most extended box is found at a substrate ratio of 1, so the distribution of NTU values, if this ratio is used, is the highest. For substrate ratios 1 to 4, IQR values were obtained at 20.00, 5.40, 8.90, and 11.20.

The minimum value is the smallest observation value produced- and the maximum value is the most significant observation value shown by the Boxplot. Figure-9 shows the effect of the amount of enzyme on the NTU value of biodetergent. At 3%, 4%, and 5% enzyme amounts, the minimum NTU values are 41.5, 34.80, and 30.30, respectively. Meanwhile, the maximum NTU values are 50.30, 51.10, and 63.80, respectively. So, the difference between the maximum and minimum values will produce an IQR of 6.80, 14.53, and 26.37, respectively. The largest IQR and the most extended box were found if 5% enzyme was used. The Boxplot presents the interquartile range (IQR) plane, where 50% of the observation data lies in the box.

In Figure-9, there are no outliers, but whiskers are found in the three boxplots obtained. Similar to the effect of the amount of enzyme on density in Figure-7, the Boxplot in Figure-9 also has whiskers that are not the same length between the top and bottom whiskers. Also, the median value of the three boxplots is not in the middle. From these two facts, it can be concluded that NTU's output data is not symmetrical or does not come from a normal distribution. The skewness of the data distribution for the three amounts of enzyme used is positive (positive skewness), indicated by the whiskers having a longer top. The skewness values for the enzyme amounts of 3%, 4%, and 5% are 1.25, 0.51, and 0.73, respectively.

The detergency power variable provides an overview of the effectiveness of the biodetergent

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produced. For this reason, Figure-10 shows the relationship between the interaction of substrate ratio and enzyme amount on detergency power. The contour plot in Figure-10 illustrates that the best plot to obtain the highest NTU value is when the enzyme quantity interacts with 5%, and the substrate ratio is 1. In this condition, the NTU value reaches >60. The interaction that produces the minimum NTU is estimated to be found if an enzyme amount of 4% to 5% is used and a maximum substrate ratio of 3 to 4 is used. Medium detergent power is found at a substrate ratio of less than two, but the range of enzyme amounts is broad, from 2% to 5%.

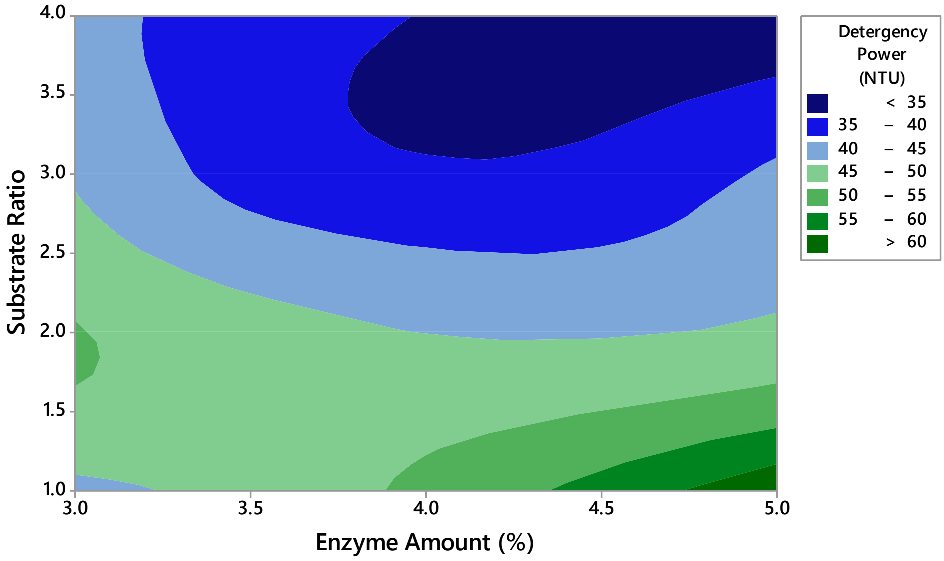

Figure-10. Interaction of substrate ratio and enzyme amount on detergency power.

Analysis of Biodetergent Contamination Waste

pH value

A pH value that is too acidic or alkaline is not good; it can damage and disturb the aquatic environment. The pH value of washing water waste produced from the results of this research was obtained at 7. Meanwhile, commercial detergent as a comparison was obtained at 6. Based on the regulation of the Minister of Environment and Divinity of the Republic of Indonesia No. 5 of 2014, the pH value of waste quality standards is 6-9. The resulting washing waste is safely disposed of directly into the water. Compared with commercial detergents, this research biodetergent produces equally good waste.

BOD (Biological Oxygen Demand)

The organic material that is decomposed in BOD is organic material that is ready to decompose (readily decomposable organic matter). BOD measures the amount of oxygen microbial population's use in waters in response to the entry of decomposable organic matter. From this understanding, although the BOD value indicates the amount of oxygen, for simplicity, it can also be interpreted as a description of the amount of readily decomposable organic material in the waters [27].

As a result of this research, the BOD concentration in washing water waste from biodetergent products is still within the safe range, namely 21.21 mg/L. Based on the regulation of the Minister of Environment and Divinity of the Republic of Indonesia No. 5 of 2014, The BOD value of waste quality standards is 75 mg/L. Compared with commercial detergent products of 18.74 mg/L, this research's BOD value of biodetergent waste is just as good.

COD (Chemical Oxygen Demand)

The COD parameter is the amount of oxygen microorganisms consume during the oxidation of organic materials that can be oxidized with the help of solid prooxidants. The COD measurement value determines the amount of organic matter in water [28].

The concentration of COD contained in washing water waste from biodetergent products as a result of this research is still within the safe range, namely 104.53 mg/L. Based on the regulation of the Minister of Environment and Divinity of the Republic of Indonesia No. 5 of 2014, The COD value of waste quality standards is 180 mg/L. Compared with commercial detergent products of 90.27 mg/L, the COD value of this research's biodetergent waste is just as good.

TSS (Total Solid Suspension)

Total Solid Suspension content analysis aims to determine the amount of suspended substances in liquid waste in mg/L units [29]. Based on the regulation of the Minister of Environment and Divinity of the Republic of Indonesia No. 5 of 2014, the waste quality standard TSS value is 60 mg/L. This study's TSS value obtained from detergent was 30 mg/L, which is still safe. Compared with commercial detergent products of 38 mg/L, the TSS value of biodetergent waste from this research is better.

CONCLUSIONS

The conclusions that can be obtained from this research are as follows:

The combination of three active components, which include lauryl-DEA surfactant, saponin extract from star fruit, and protease enzyme, together with the addition of aroma, can produce a detergent that meets SNI 06-476-1996 standards regarding liquid washing detergents in terms of physical appearance, acidity level (pH), and density.

The best results were found using a specific composition, namely 20% saponin extract, 20% lauryl-DEA surfactant, and around 5% protease enzyme. The biodetergent product produced has a cleaning ability of 63.8 NTU, while the commercial product has a cleaning ability of 65 NTU. The cleaning ability of this biodetergent product can remove various types of stains on fabric with satisfactory results and is almost equivalent to commercial detergent.

The density of detergent products that meet the requirements of SNI 06-476-1996 concerning liquid washing detergents is around 1.0-1.3 g/mL. Where the test results obtained density numbers ranged from 1.031-1.07 g/mL and met SNI quality requirements.

Waste-water produced by detergent also meets the requirements stipulated in the Minister of Forestry and Environment Regulation Number 5 of 2014 concerning wastewater quality standards for the laundry industry. This includes the pH value (7), TSS (30 G/mL), BOD (21.21 G/mL), and COD (104.53 G/mL). Biodetergent also has better washing quality and lower pollution characteristics when compared to commercial detergents, where the pH value (6), TSS (38 G/mL), BOD (18.74 G/mL), and COD (90.27 G/mL)

The main parameter, namely cleaning ability, shows satisfactory results when using the three active ingredients, compared to a combination of one or two active ingredients alone.

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REFERENCES

[1] Maranggi I. U., Rahmasari B., Kania F. D., Fadarina, Yuniar, Purnamasari I. and Meidinariasty A. 2020. Aplikasi biosurfaktan dari daun sengon (Albizia falcataria) dan kulit buah pepaya (*Carica papaya L.*) sebagai detergen ramah lingkungan. Politeknik Negeri Sriwijaya, Prosiding Seminar Mahasiswa Teknik Kimia. 1(1): 11-19.

[2] Wetwitayaklung P., Limmatvapirat C. and Phaechamud T. 2013. Antioxidant and anticholinestease activities in various parts of *Sonneratia caseolaris (L.).* Indian Journal of Pharmaceutical Sciences. 75(6): 649-656.

[3] Rachmawati P. A. 2019. Biodegradable detergen dari saponin daun waru dan ekstraksi bunga tanjung. Indonesian Chemistry and Application Journal. 2(2): 1-4. doi: 10.26740/icaj.v2n2.p1-4

[4] Apriliyani N. 2017. Penurunan kadar surfaktan dan sulfat dalam limbah laundry. Media Ilmiah Teknik Lingkungan. 2(1): 37–44.

[5] Fernianti D., Mardwita M. and Suryati L. 2017. Pengaruh jenis detergen dan rasio pengenceran terhadap proses penyerapan surfaktan dalam limbah detergen menggunakan karbon aktif dari ampas teh. Jurnal Distilasi. 2(2): 10-14. DOI: 10.32502/jd.v2i2.1147.

[6] Rufchaei R., Abbas-Mohammadi M., Mirzajani A. and Nedaei S. 2022. Evaluation of the chemical compounds and antioxidant and antimicrobial activities of the leaves of *Eichhornia crassipes* (water hyacinth). Jundishapur J Nat Pharm Prod. 17(1): 111.

[7] Masyithah Z., Amelia A. R. and Hafsyah. 2023. Synthesis and characterization of n-acyl alkanolamide surfactant from fatty acids and alcohol amines using sodium methoxide catalyst. ARPN Journal of Engineering and Applied Sciences. 18(2): 55-63.

[8] Masyithah Z., Purba S. O. and Rajagukguk D. 2019. A two-step catalytic production of coco fatty acid amide from coconut oil using a metal catalyst. ARPN Journal of Engineering and Applied Sciences. 14(11): 2100-2105.

[9] Adiwibowo M. T., Herayati, Erlangga K. and Fitria D. A. 2020. Pengaruh metode dan waktu ekstraksi terhadap kualitas dan kuantitas saponin dalam ekstrak buah, daun, dan tangkai daun belimbing wuluh *(Avverhoa bilimbi L.)* untuk aplikasi detergen. Jurnal Integrasi Proses. 9(2): 44-50.

[10] Fahrunnida and Pratiwi R. 2015. Kandungan saponin buah, daun dan tangkai daun belimbing wuluh (Averrhoa bilimbi L.). Prosiding KPSDA. 1(1): 220224.

[11] Aras N. R. M., Santi, Nur R. A., Is-ma and Yusriadi. 2023. Antiseptic liquid soap from corn oil (Zea mays L) and aloe vera extract with the variation of SLS (sodium lauryl sulfate). Indonesia Chimica Acta. 16: 23-31.

[12] Lawan R. and Idris A. 2021. Effects of some locally made antiseptic soaps on some skin microbes. International Journal of Scientific Research in Science, Engineering and Technology. 8(3): 339-341.

[13] Masyithah Z., Simanjuntak L. and Yudhika S. R. 2019. Simultaneously trans-esterification and amidification of coconut oil into cocamide-DEA using heterogeneous catalyst. ARPN Journal of Engineering and Applied Sciences. 14(11): 2111-2117.

[14] Sanharis A. and Wahyuningtyas D. 2018. Modifikasi sabun antiseptik alami dari virgin coconut oil (VCO) - minyak sereh dengan penambahan minyak zaitun sebagai antioksidan. Jurnal Inovasi Proses. 3(2): 46-52.

[15] Ilmiah S. N., Mubarik N. R. and Wahyuntari B. 2018. Characterization of protease from *Bacillus licheniformis* F11.1 as a bio-detergent agent. Makara Journal of Science. 22(3). DOI: 10.7454/mss.v22i3.8809.

[16] Soeka Y. S. and Sulistiani. 2014. Karakterisasi protease *Bacillus subtilis* A1 InaCC B398 yang diisolasi dari terasi samarinda. Berita Biologi. 13(2): 203–212. DOI: 10.14203/ beritabiologi.v13i2.694.

[17] Rochester J., Bolden A., Pelch K. E. and Kwiatkowski C. F. 2017. Potential developmental and reproductive impacts of triclocarban: a scoping review. Journal of Toxicology. DOI: 10.1155/2017/9679738

[18] Wetwitayaklung P., Limmatvapirat C. and Phaechamud T. 2013. Antioxidant and anticholinesterase activities in various parts of *Sonneratia caseolaris (L.).* Indian Journal of Pharmaceutical Sciences. 75(6): 649–656.

[19] Sahambangung M., Datu O., Tiwow G. and Potolangi N. 2019. Formulasi sediaan sabun antiseptik ekstrak daun pepaya. Jurnal Biofarmasetrikal. 2(1): 43-51.

[20] Bt Yaser N. M., Che Mat F., Rashid T. and Thanabalan M. 2015. Degradation of aqueous diethanolamine (DEA) solutions using UV/H_2O_2 process. Chemical Engineering Transactions. 43: 2263-2268. DOI: 10.3303/CET1543378.

[21] Yim H. S., Chye F. Y., Rao V., Low J. Y., Matanjun P., How S. E. and Ho C. W. 2013. Optimization of extraction time and temperature on antioxidant activity of *Schizophyllum commune* aqueous extract using response surface methodology. Journal of Food Science and Technology. 50(2): 275-283.

[22] Masyithah Z. and Alfizah D. 2022. Optimization of octadecanoic conversion to stearoyl lysine surfactant applying box-behnken design. ARPN Journal of Engineering and Applied Sciences. 17(16): 1543-1535.

[23] Pamaya D., Imam Muchlissin S., Tri wahyuni M. E., Darmawati S. and Norma E. S. 2018. Isolasi bakteri penghasil enzim protease *bacillus amyloliquefaciens* irod2 pada oncom merah pasca fermentasi 48 jam. Seminar Nasional Edusainstek FMIPA UNIMUS. 40--46.

[24] Ngginak J., Apu M. T. and Sampe R. 2021. analisis kandungan saponin pada ekstrak seratmatang buah lontar (*Borassus flabellifer linn*). Bioedukasi (Jurnal Pendidikan Biologi). 12(2): 221-228. DOI: 10.24127/bioedukasi.v12i2.4451

[25] Hendra H., Barlian E., Razak A. and Sanjaya H. 2016. Photo-degradation of surfactant compounds using uv rays with addition of TiO2 catalysts in laundry waste. Sainstek : Jurnal Sains Dan Teknologi. 7(1): 59. DOI: 10.31958/js.v7i1.126.

[26] Yuliyanti M., Husada V. M. S., Fahrudi H. A. A. and Setyowati W. A. E. 2019. Optimasi mutu dan daya detergensi sediaan detergen cair ekstrak biji mahoni (*swietenia mahagoni*). Jurnal Kimia Dan Pendidikan Kimia. 4(2): 65-79.

[27] Andika B., Wahyuningsih P. and Fajri R. 2020. Penentuan nilai BOD dan COD sebagai parameter pencemaran air dan baku mutu air limbah di pusat penelitian kelapa sawit (PPKS) Medan. Jurnal Kimia Sains Dan Terapan. 2(1): 14-22.

[28] Atima W. 2015. BOD dan COD sebagai parameter pencemaran air dan baku mutu air limbah. Biosel: Biology Science and Education. 4(1): 83. DOI: 10.33477/bs.v4i1.532.

[29] Khofifah K. and Utami M. 2022. Analisis kadar total dissolved solid (TDS) dan total suspended solid (TSS) pada limbah cair dari industri gula tebu. Indonesian Journal of Chemical Research. 7(1): 43-49. DOI: 10.20885/ijcr.vol7.iss1.art6