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Starch nanoparticle (SNP) bioflocculant from breadfruit (*Artocarpus altilis*) for the treatment of river water

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ABSTRACT

This study aimed to develop a biodegradable flocculant from breadfruit starch *(Artocarpus altilis)* as an eco-friendly alternative to chemical flocculants for river water treatment. Breadfruit starch was isolated, acetylated using acetic anhydride, and ultrasonicated at 140 W for 75 minutes to produce Starch Nanoparticle (SNP) bioflocculant, reducing particle size from 6,270.80 nm to 407.20 nm. The acetyl percentage and degree of substitution of the synthesized SNP bioflocculant were 9.78% and 0.41, respectively. Despite a low yield of 1.27%, the SNP showed promising flocculation performance. Water from the Ngalan River exceeded national standards during inclement weather, highlighting the need for treatment. Jar test experiments were conducted using 1 liter of river water to evaluate both the synthesized bioflocculant and the commercial flocculant. ANOVA results show a significant difference in color and turbidity removal but no significant difference in Total Suspended Solids (TSS). It was revealed that the 9 mL dose of 0.1 wt. % SNP bioflocculant was the minimum effective amount, achieving the lowest recorded values for color, turbidity, and TSS which consequently resulted to the highest removal efficiencies. A T-test further reveals that the SNP bioflocculant is more effective than the commercial flocculant in color removal, while both flocculants perform similarly in turbidity and TSS reduction. The SNP bioflocculant outperformed the commercial flocculant in color removal and showed comparable effectiveness in reducing turbidity and TSS, though it required a higher dose. Despite this, it achieved higher overall removal rates and shows promise as an effective, eco-friendly alternative for water treatment.

KEYWORDS

acetylation, flocculant, potable, turbidity, ultrasonic

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INTRODUCTION

About 17% of the global population currently lack access to safe and sufficient water. This issue is expected to worsen within 25 years, half of the world's population may face difficulties in securing adequate freshwater due to increasing pollution of both surface and groundwater sources (Asia Society 2009).

One potential solution is bulk water production from surface waters, such as rivers, which due to its massive volume, can help meet the rising demand for a reliable and consistent water supply in the urban areas (OpenWASH, 2016). However, before river water can be used as a public supply, it must undergo a series of treatment processes such as coagulation, flocculation,

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sedimentation, filtration and disinfection to improve its quality and remove pollutants (Toprak 2004). In coagulation and flocculation, chemicals are added to neutralize the charges of particle impurities and aid in the clumping of destabilized particles and facilitate the sedimentation process (OpenWASH, 2016). While chemical flocculants are widely used due to their affordability and long shelf life, these often have negative environmental impacts and produce by-products that require complex handling and costly treatment. This concern has led to growing interest in natural flocculants as an alternative to synthetic flocculants. (Zaman et al., 2020)

Starch is a natural flocculant that can serve as an alternative to these synthetic flocculants, as it is both biodegradable and eco-friendly (Lekniute – Kyzike et al. 2023). The disadvantage of using starch derived bioflocculants is its rapid tendency to degrade and its brief shelf life (Wang et al. 2015). To address this issue and preserve starch, it is subjected to acetylation, a process in which new functional groups are incorporated into its molecular structure. It is widely used among various starch modification methods to improve the physicochemical and functional properties of starch. (Subroto et al. 2023). Moreover, it also enhances the flocculation of starch by increasing its swelling power and solubility (Posada – Velez, et al. 2023)

Breadfruit “kulo” *Artocarpus altilis* is one of the many starch resources in many Asian countries yielding abundant fruit each year. In 2022, Harsanto et al. conducted experiments using Breadfruit as a source material for nanoparticle production. Breadfruit was chosen primarily because of its abundant starch content and soft tissues that facilitate extraction (Harsanto et al. 2022).

This research explores the potential of breadfruit starch, in the form of nanoparticles, as a natural flocculant to replace synthetic flocculants for the treatment of river water for municipal use.

The study primarily aims to produce a starch nanoparticle (SNP) bioflocculant from breadfruit. Specifically, it seeks to calculate the yield and determine its characteristics after acetylation. It also evaluates the quality of the Ngalan River by assessing the removal of turbidity, color, and total suspended solids after treatment with the bioflocculant. Furthermore, the study identifies the optimum or lowest effective dosage of the bioflocculant for river water treatment and examines whether there is a significant difference in the performance between the bioflocculant and the commercial flocculant used by the water treatment plant at its respective operational dose. The comparison was made based on the percentage reduction of color, turbidity and total suspended solids.

MATERIALS AND METHODS

Breadfruit was sourced locally within the province of Negros Occidental. Only breadfruit meat was used for starch extraction; seeds and peels of the fruit were discarded. The purity and proximate analysis of extracted starch were not addressed in this study. Natural starch was extracted from breadfruit, subjected to chemical modification using acetylation, and then processed into bioflocculant nanoparticles by the physical method of ultrasonication. The resulting acetylated Starch Nanoparticle from breadfruit underwent a series of jar tests experiments to evaluate its performance as a bioflocculant and determine the lowest dosage with the highest effectivity for treating river water during extreme weather conditions.

Ngalan river is the freshwater source for the water treatment plant, located in Brgy. Granada, Bacolod City. River water used for the study was taken from the same intake point via sampling line to simulate the coagulation – flocculation process of the bulk water treatment plant that supplies municipal water to the city of Bacolod. Water quality parameters of Ngalan river were determined before and after the coagulation – flocculation treatment. The efficiency of the bioflocculant was measured by its ability to remove color, turbidity and total suspended solids from the water. Simultaneously, acetylated SNP bioflocculant was characterized using Particle Size Analyzer to examine its chemical structure on a molecular level. Success of acetylation was verified by titration tests to measure the Acetyl percentage and Degree of Substitution.

The general flowchart for the research procedure is shown in the figure below.

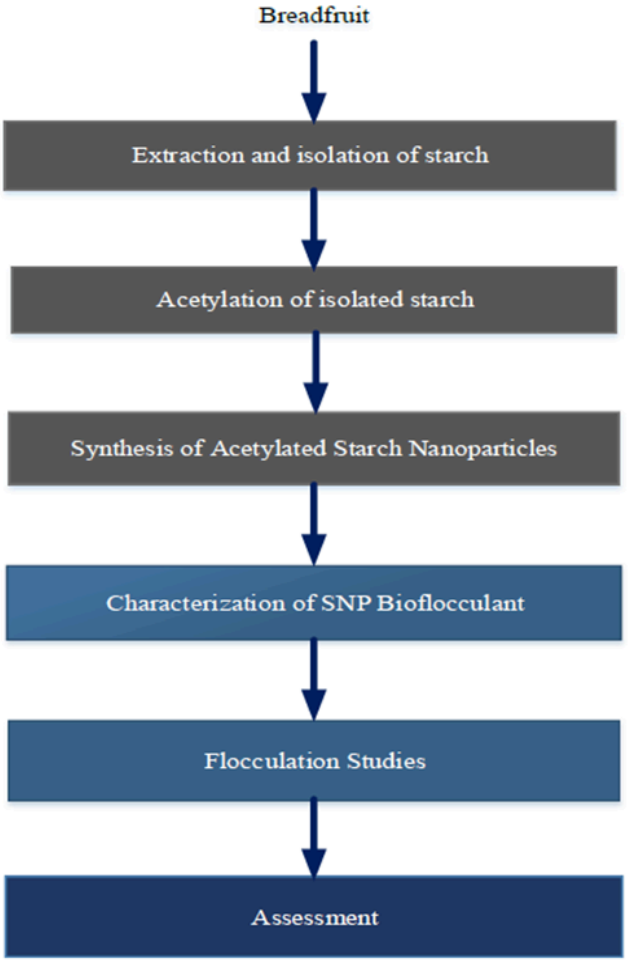

Figure 1. General workflow of this study.

Extraction of Starch from Breadfruit

Matured breadfruits grown for 15-20 weeks were harvested, peeled, cut, and rinsed to obtain breadfruit meat, which was processed into a puree using a food processor (Anwar et al. 2016). A starch slurry was created by adding 5 parts water to 2 parts of the extracted breadfruit puree. The slurry was sieved using a muslin cloth to remove the fibers and allowed to settle for 4 hours. The supernatant was decanted off, and the starch granules were dispersed again in the same volume of water. This sedimentation process was repeated thrice, discarding the liquors each time (Adebowale et al. 2005; Noorfarahzilah et al. 2020).

The starch milk slurry from the final stage of sedimentation was centrifuged in batches and spread thinly in petri dishes for moisture removal. Dehydration was carried out in a drying oven at 45℃ for a couple of hours until constant weight is achieved (Adebowale et al. 2005; Harsanto et al. 2022). The resulting dried substance was scraped from the petri dishes and pulverized by a mortar and pestle to yield native starch granules (Anwar et al. 2016).

Figure 2 shows the process of starch isolation from breadfruit.

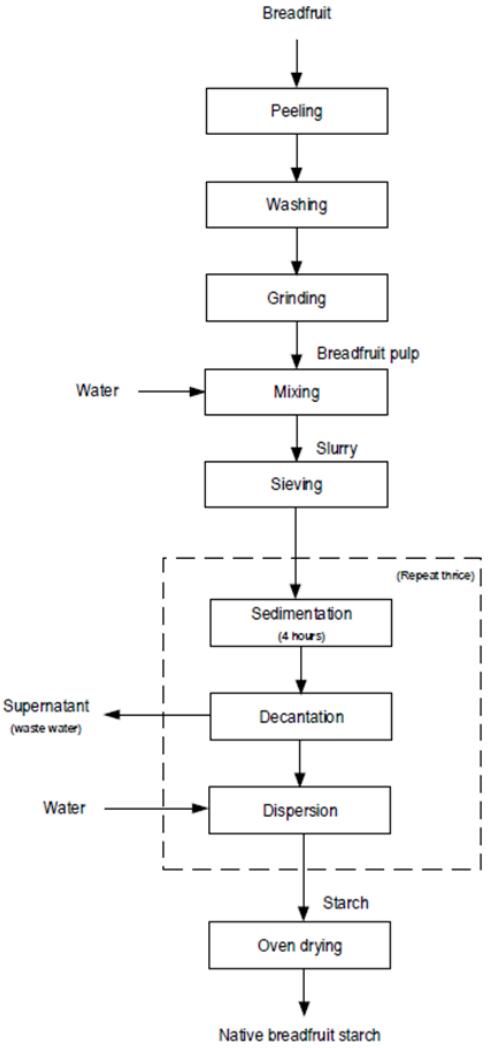

Figure 2. Wet milling process for starch isolation from breadfruit (Adapted from: Adebowale et al. 2005).

Acetylation of Isolated Starch

The isolated breadfruit starch was dissolved in water at a ratio of 100 g to 225 ml and stirred constantly for one hour. The pH of the solution was then adjusted to 8.0 using 3% (w/v) NaOH before adding 8 mL dropwise of analytical reagent grade Acetic anhydride while maintaining the pH within 8.08 – 8.4 with the prepared NaOH solution. (Phillips et al. 1999; Ogundipe et al. 2019). The resulting solution was heated in a water bath at 45°C and left to soak for 90 minutes (Zuhra et al. 2018). Drops of 0.5 M HCl were added to adjust the pH to 4.5. The recovered precipitate represents acetylated starch. It was washed to neutral pH, and oven-dried at 40°C until a constant weight is achieved. The dried starch was pulverized and sieved through a 100-mesh screen (Singh et al. 2012).

Synthesis of Starch Nanoparticles

The physical method of Haaj et al. (2012) employing high intensity ultrasound treatment was used for the synthesis of nanoparticles. Acetylated starch suspension with 1.5% solids content was prepared by thoroughly mixing 1.5 g of acetylated starch with 100 ml distilled water. A magnetic stirrer was used to agitate the solution.

Figure 3 shows the 50 Hz TUE-500 Toption Ultrasonic Homogenizer equipped with a 6 mm diameter horn tip used for the ultrasonication of the resulting starch suspension. Ultrasonic power rating is at 500 W.

Figure 3. Ultrasonic homogenizer used in the ultrasonication of starch suspension (TUE-500 Toption).

The ultrasonic treatment of the 1.5% w/v starch suspension was carried out in a glass beaker immersed in a basin containing ice to maintain a constant temperature of 8±1. The machine was set to 28% power rating, dissipating approximately 140 W ultrasonic power within a 15-minute processing time. Ultrasonication was repeated five times under the same conditions which results to accumulated treatment duration of 75 minutes (Haaj et al. 2012).

After ultrasonication, the slurry was diluted from 15,000 ppm (1.5% w/v) to 1000 ppm for use in the flocculation tests and based on the requirement of the testing center. A sample was sent to an external laboratory for Particle Size Analysis.

Characterization of Synthesized Starch Nanoparticle (SNP) Bioflocculant

(a) Particle Size

The size and distribution of synthesized starch nanoparticles were determined using a Particle Size Analyzer, which employed Dynamic Light Scattering. Nanoparticle samples was sent for analysis to the Center for Advanced New Materials, Engineering and Emerging Technologies (CANMEET) Facility at the University of San Agustin, Iloilo City.

(b) Structure and Surface Modification

Acetyl percentage and Degree of Substitution were determined titrimetrically, based on the study of Khurshida et al. (2021), as a measure of the efficiency of acetylation. A gram of Acetylated breadfruit SNP was placed in a 250 mL flask, followed by the addition of 50 mL of 75% ethanol in distilled water. The flask was loosely sealed and agitated, warmed to 50℃ in water bath for half an hour, and subsequently cooled. Afterward, 40 mL of 0.5 M KOH was added. Any excess alkali was neutralized by back titrating the solution with 0.5 M HCl using phenolphthalein as indicator. The solution was left to stand for 2 hours, during which any additional alkali that may have leached from the sample will be titrated. The same procedures were repeated using the isolated, unmodified breadfruit starch.

The volume of HCl titrant resulting from the acetylated starch and the unmodified starch was used to calculate for the acetyl percentage and degree of substitution (Khurshida et al. 2021).

Flocculation Studies

Grab samples were collected from the Ngalan river, specifically at the intake point of the submersible feed pump during inclement weather conditions. The samples were collected in clean, one-gallon containers and analyzed in three trials, limited to Total Suspended Solids,

turbidity and color since the coagulation – flocculation process primarily involves the removal of solids and suspended matter. Turbidity was analyzed on site using the calibrated Eutech TN-100 turbidimeter. Samples for apparent color were evaluated using the Hach DR3900 spectrophotometer, and total suspended solids were analyzed by gravimetric method in the USLS chemical laboratory. These values serve as the initial measurements for river water quality.

One liter of river water sample was transferred to a glass beaker and placed on the multiple spindle stirrer equipment, with the paddle stirrer submerged toward the center of the beaker. The paddle stirred the mixture for 2 minutes at 200 rpm to homogenize the samples. Then, 0.1 mL of 25 wt. % Polyaluminum Chloride which serves as the coagulant, was injected into the mixture while it continues to stir for an additional 2 minutes. The speed was lowered to 100 rpm before injecting 3 mL of the synthesized SNP bioflocculant with a concentration of 0.1 wt. %. The stirrer equipment was switched off after another 2 minutes, allowing a settling time of 15 minutes. These procedures were based on company manual and the actual practice of the water treatment plant. The existing Polyaluminum Chloride (PAC) used by the water treatment plant was applied as coagulant for the study.

Approximately 900 mL of the solution was collected for analysis of turbidity, color and total suspended solids. These values serve as the final measurements after the flocculation treatment. The same procedures were repeated while changing the volume of SNP bioflocculant to 5 mL, 7 mL, 9 mL and 11 mL, each conducted in three trials.

Three flocculation set-ups were established based on the procedures above:

a. Control set-up: Coagulant alone, without any flocculant
b. Coagulant + commercially available flocculant
c. Coagulant + breadfruit SNP bioflocculant

Jar test was conducted for both the synthesized natural flocculant and the commercial flocculant. By employing the same dosage, procedures, and conditions stipulated in the work instructions, the performance of the synthesized bioflocculant can be compared against the existing commercial grade flocculant used by the water treatment plant. This was measured by the reduction of turbidity, color and total suspended solids in river water.

Statistical Treatment

Statistical analysis was conducted to measure and analyze the data collected during the jar test experiments. A one-way Analysis of Variance (ANOVA) was used to determine the lowest effective dosage (optimum dose) of the synthesized acetylated SNP bioflocculant for treating river water. The T-test was the statistical tool employed to relate the performance of the optimum dose of the breadfruit SNP against the optimum dose of the commercial grade flocculant established by the water treatment plant. To assess this, the experiment compared the lowest effective dose, determined by ANOVA and post-Hoc tests, against the 3 mL dosage of commercial flocculant, according to standard procedures for water treatment.

There were no human or animal engagements involved during the conduct of this study. Therefore, ethical approval and informed consent were not required. Nevertheless, this research underwent an ethics review and received ethics clearance through a full board evaluation.

RESULTS

Extraction of Starch from Breadfruit

Matured breadfruit weighing 736 g and 568 g was used in the study, which returned 436 g and 352 g of breadfruit meat, respectively, after the removal of peels and core. Only 17.95 g of breadfruit starch was recovered from 788 g of breadfruit meat. Based on the given data and the formula:

$$\%yield = \frac{weight\ of\ breadfruit\ starch}{weight\ of\ breadfruit\ meat} \times 100$$

Yield of breadfruit starch from meat was calculated to be 2.28%. This value is relatively low compared to the 3.19% and 10% yields reported in the studies by Awokoya et al. (2018) and Ningsih Nst et al. (2024), respectively. Both studies also utilized the same extraction method described by Adebowale et al. (2005). Additionally, breadfruit starch isolation was carried out by Akanbi et al. (2009) using the same procedure, resulting to 14.26% yield.

The fruit determines the yield since starch content varies with the maturity of the breadfruit (Ningsih Nst et al., 2024). As the fruit ripens, a portion of its starch is converted into sugar, resulting in a lower starch yield. To maximize starch extraction in this study, breadfruit at the earliest stage of maturity should be used. At this stage, the fruit exudes a white milky sap that runs over the surface. The fruit remains hard and green but is already mature but not yet ripe (Healthy Pacific Lifestyle Section). Aside from the maturity stage, the yield can also be attributed to the climatic conditions and seasonal effects (Bezerra et al., 2019).

Other than the nature of the fruit, several factors in starch processing likely contributed to the low recovery. During sedimentation, a yellow mucilage formed, causing starch to adhere and decanted along with the solvent. Further losses occurred from residues retained in vessels and removed with the impurities, particularly during centrifugation, which required multiple test tubes. Residual starch was left in the tubes and the manual removal of impurities also reduced yield. Moreover, repetition of the sedimentation, decantation and centrifugation process increased the probability of wastage and contributed significantly to the decline in recovered starch. These combined factors during extraction and purification explain the reduction in starch recovery (Akanbi et al., 2009). To minimize starch loss, a more cautious handling is recommended and a centrifuge with larger capacity should be used.

The breadfruit starch slurry was subjected to starch confirmation test. It turned a blue-black color upon contact with the Iodine solution, indicating the positive presence of starch. Figure 4 shows the isolated starch.

Figure 4. Isolated breadfruit starch.

Acetylation of Isolated Starch

Breadfruit starch soaked with acetic anhydride for 90 minutes at 45°C resulted to acetylated starch. About 10.0 g of acetylated starch was produced through the chemical modification process from 13.4 g of the synthesized breadfruit starch. Some losses and product accumulation were encountered along the process.

Figure 5 illustrates the starch after the acetylation process.

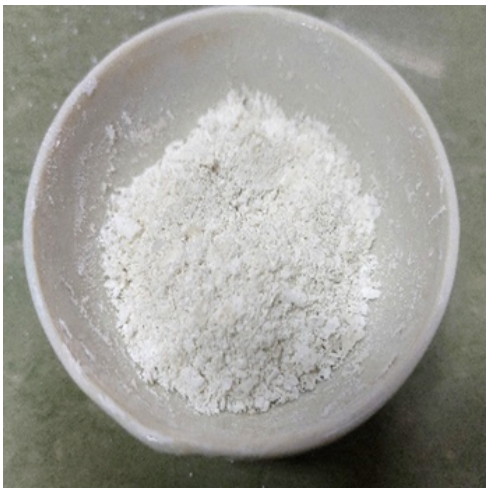

Figure 5. Acetylated breadfruit starch.

Synthesis of Starch Nanoparticles

The SNP bioflocculant produced, as seen in Figure 6, is in solution form. However, it can be assumed that all of the starch is converted to nanoparticles since ultrasonication of acetylated starch at the right level of ultrasound energy yields almost 100% conversion, close to perfect (Haaj et al. 2012). The yield of SNP bioflocculant produced after the breadfruit starch extraction, acetylation and ultrasonication processes was calculated from the formula, wherein the weight of acetylated starch can be substituted for the weight of the SNP:

$$\%yield = \frac{weight\ of\ SNP}{weight\ of\ breadfruit\ meat} \times 100$$

About 10.0 g of acetylated starch is expected to produce the same amount of starch nanoparticles, which conversely result in a yield of 1.27% SNP bioflocculant.

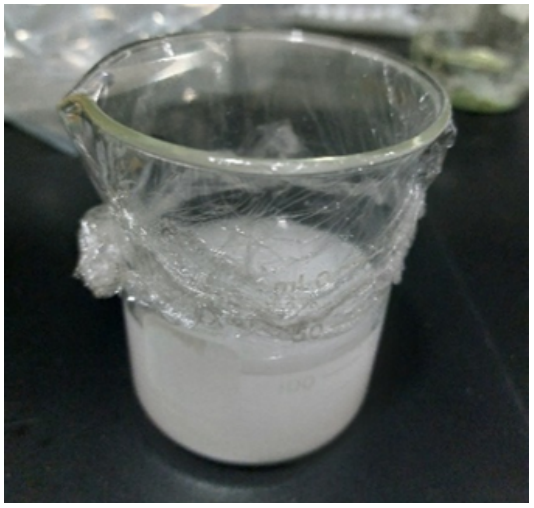

Figure 6. Starch nanoparticle (SNP) bioflocculant.

Characterization of Synthesized Starch Nanoparticle (SNP) Bioflocculant

(a) Particle Size

Unmodified breadfruit starch and the acetylated SNP, both with a concentration of 1000 ppm, were subjected to three trials of DLS analysis. Results reveal that the mean size diameter of the isolated breadfruit starch and SNP bioflocculant is at 6,270.80 nm and 407.20 nm, respectively. The size comparison between the isolated breadfruit starch and the starch nanoparticle is demonstrated in Figure 7.

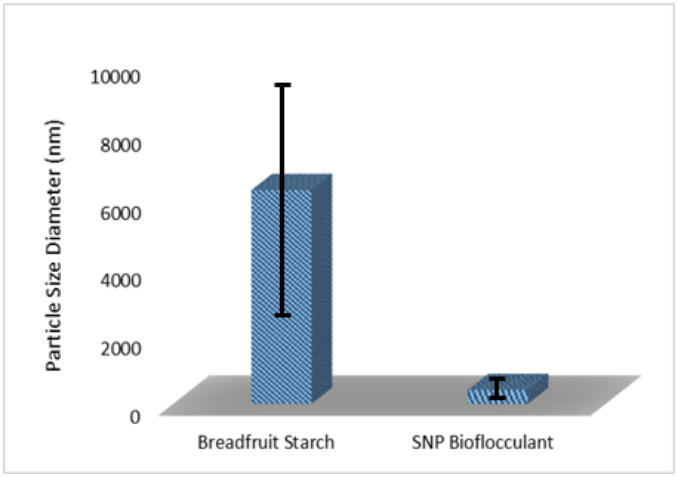

Figure 7. Particle sizes of breadfruit starch and SNP bioflocculant.

The particle size distribution of breadfruit starch before and after ultrasonication are given by the histogram for breadfruit starch and SNP bioflocculant in Figures 8 and 9, respectively.

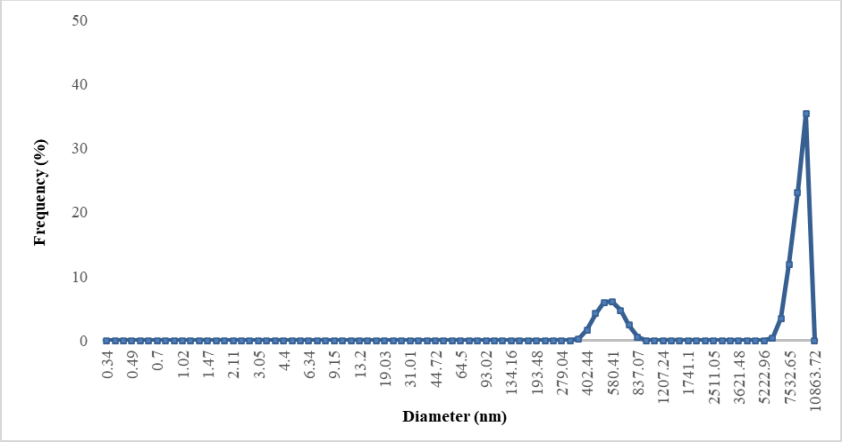

Figure 8. Histogram of breadfruit starch before ultrasonication.

The largest portion, accounting for 35.39% of the isolated breadfruit starch particles, has a diameter of 9,615.42 nm.

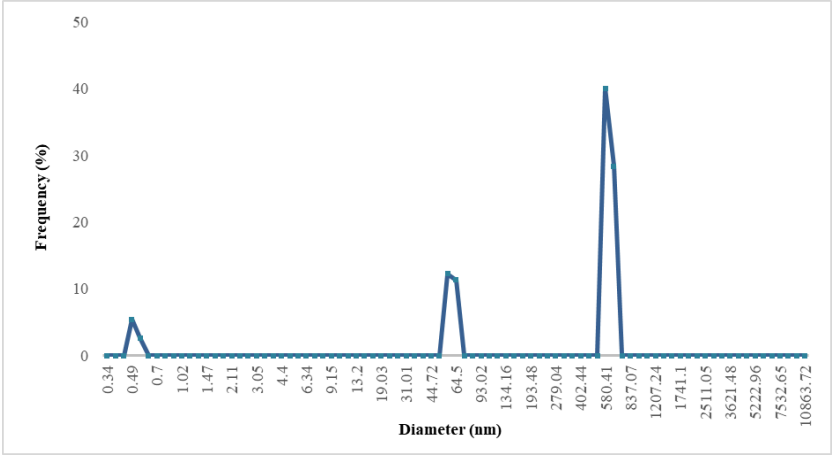

Figure 9. Histogram of breadfruit starch after acetylation and ultrasonication.

About 40.02% of the SNP particles has a diameter of 580.41 nm.

(b) Structure and Surface Modification

The effectiveness of surface modification was assessed by measuring the acetyl percentage and degree of substitution, using unmodified breadfruit starch as the blank. Titration experiments were conducted in triplicate, with the starch products serving as the analyte. From the three trials, the average volume of HCl titrant used for the Isolated Breadfruit starch was 16.88 mL, and 12.33 mL for the Acetylated Breadfruit starch.

The calculation for Acetyl percentage and Degree of Substitution is given by the following formulas:

$$Acetyl\% = \frac{(Blank - Sample) \times Molarity\ of\ HCl \times 0.043 \times 100}{Sample\ weight\ (g)}$$

$$Degree\ of\ Substitution\ (DS) = \frac{(162 \times Acetyl\%)}{[4300 - (42 \times Acetyl\%)]}$$

The formula shows that the acetyl percent was used to calculate the Degree of Substitution. Acetylated breadfruit starch was found to have an average Degree of Substitution (DS) of 0.41 and an acetyl percentage of 9.78, as determined by the titration method.

Quality of River Water

Water quality samples for Ngalan River were taken during inclement weather conditions. Average color, turbidity, and total suspended solids are at 2,092.67 PtCo CU, 116.60 NTU and 58.87 mg/L, respectively. The river water conditions are presented in Table 1, alongside the national standards.

Table 1. Water quality of Ngalan River in Bacolod City, Philippines

	PNSDW/ DAO 2016-08	Untreated River Water	Remarks
Turbidity (NTU)	5	116.60	Failed
Apparent Color (PtCo CU)	10	2,092.67	Failed
Total Suspended Solids (mg/L)	50	58.87	Failed

For color, turbidity and TSS, the untreated river water falls short of the standard parameters for drinking water.

Flocculation Studies

Flocculation experiments were conducted using a multiple-spindle stirrer equipment, both without any flocculant, with a commercial flocculant and with varying concentrations of the synthesized breadfruit SNP bioflocculant.

The graph in Figure 10 correlates bioflocculant dose to the color of river water.

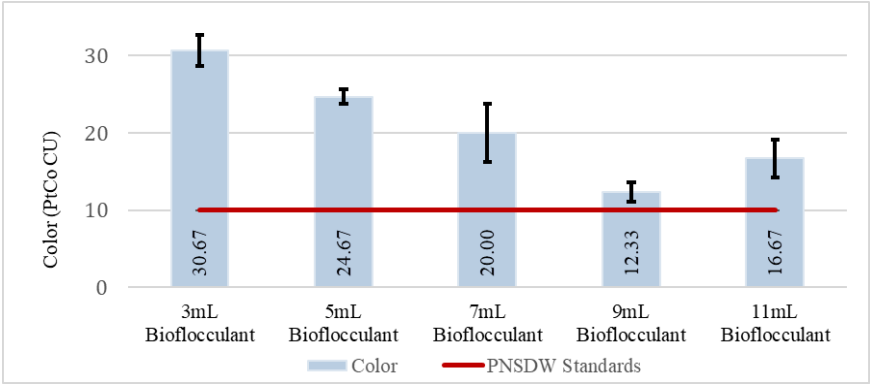

Figure 10. Bioflocculant effect on color of river water. Bars represent turbidity of river water according to volume of bioflocculant. Red line represents PNSDW standards on turbidity.

The color of river water after treatment has significantly decreased from 2,092.67 PtCo CU. However, the resulting water quality for all five doses of bioflocculant did not pass the national standard of 10 PtCo CU (Department of Health 2017). Further treatment after the flocculation process is necessary to make the water safe for drinking. Visual inspection of the graph shows that the lowest color was achieved at a bioflocculant dose of 9mL.

Figure 11 shows the effect of bioflocculant concentration on the turbidity of river water.

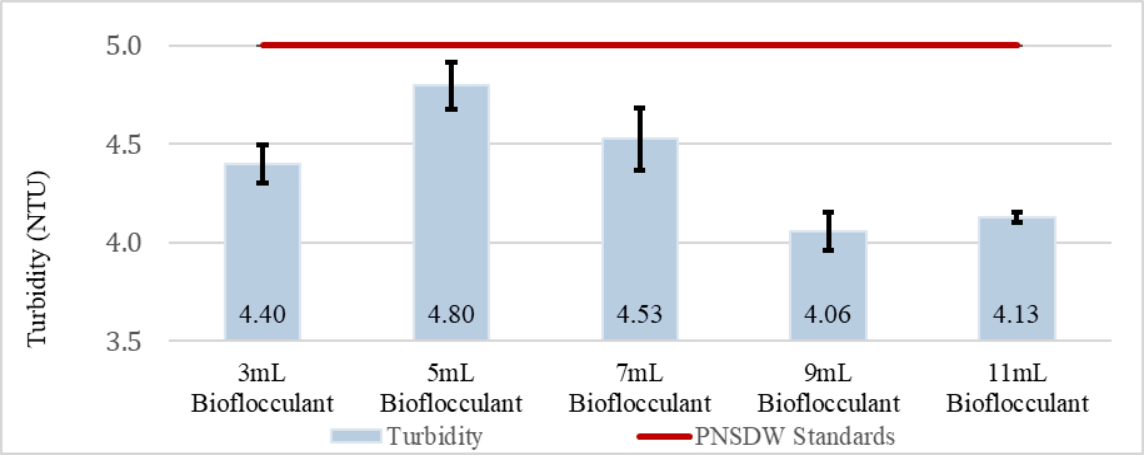

Figure 11. Bioflocculant effect on turbidity of river water. Bars represent turbidity of river water according to volume of bioflocculant. Red line represents PNSDW standards on turbidity.

The turbidity resulting from flocculation tests using the synthesized SNP bioflocculant has met the national drinking water standard of 5 NTU (Department of Health 2017). The lowest turbidity of 4.06 NTU was achieved using 9 mL of bioflocculant.

The impact of bioflocculant concentration on total suspended solids is illustrated in Figure 12.

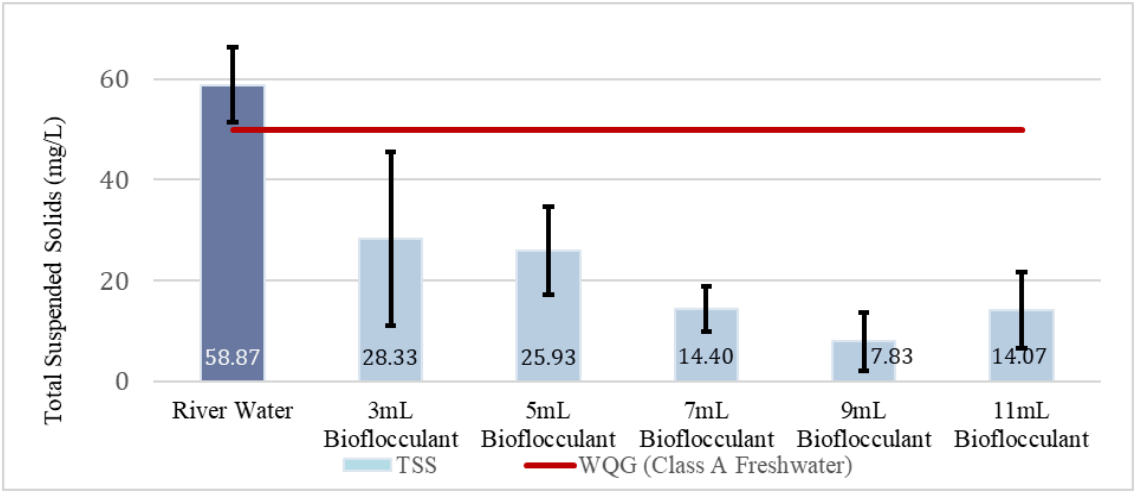

Figure 12. Bioflocculant effect on total suspended solids of river water. The dark bar gives the TSS of untreated river water. Light bars represent TSS of river water according to volume of bioflocculant. Red line represents TSS guidelines for Class A freshwater.

Total suspended solids of river water treated with the SNP bioflocculant have passed the 50 mg/L threshold for Ngalan River based on the water quality guidelines of Class A freshwater (Department of Environment and Natural Resources 2016). Lowest TSS of 7.83 was achieved at 9 mL bioflocculant dose.

Water quality measurements after flocculation using SNP bioflocculant were compared to the initial quality of untreated river water to determine the percent reduction of color, turbidity, and TSS. Percent removal was determined using the formula:

$$\%\ turbidity\ removal\ =\ \frac{NTU_{initial} - NTU_{final}}{NTU_{initial}}$$

$$\%\ color\ removal\ =\ \frac{Color_{initial} - Color_{final}}{Color_{initial}}$$

$$\%\ TSS\ removal\ =\ \frac{TSS_{initial} - TSS_{final}}{TSS_{initial}}$$

Percentage removal is shown in Figure 13.

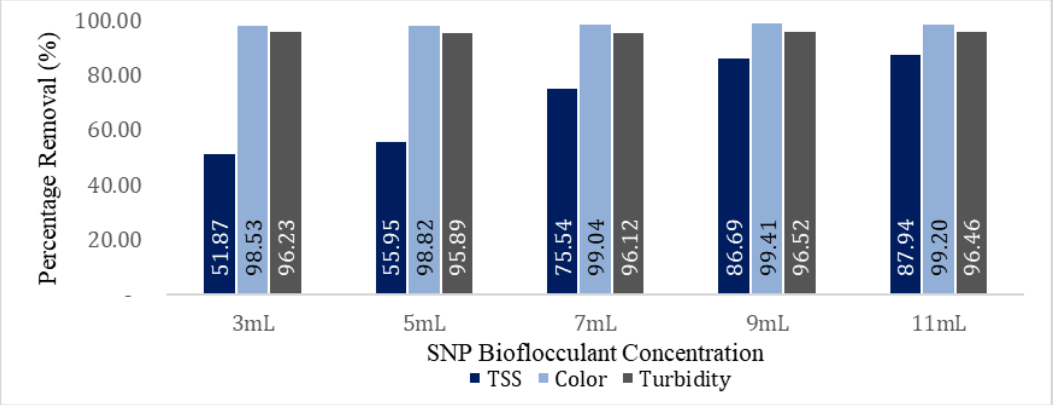

Figure 13. Percentage removal of TSS, color and turbidity using SNP bioflocculant.

The highest percentage of removal for color, turbidity, and TSS was achieved with a 9 mL bioflocculant dose.

Three set-ups were established using equal flocculant doses to compare the performance of the commercial flocculant and SNP bioflocculant. River water from the three set-ups were administered by the prescribed 25 wt. % Polyaluminum Chloride coagulant dose, following the standard procedures for the jar test. A control set-up was established as a reference to assess the preexisting effect of the coagulant, without the addition of any flocculant. A standard 3 mL dose of commercial flocculant was added in the next set-up, while the synthesized SNP bioflocculant was used in the other.

Figure 14 shows the flocculation set-ups using the multiple spindle stirrer equipment.

Figure 14. Flocculation tests conducted for this study.

Results of the experiment are shown in the succeeding figures.

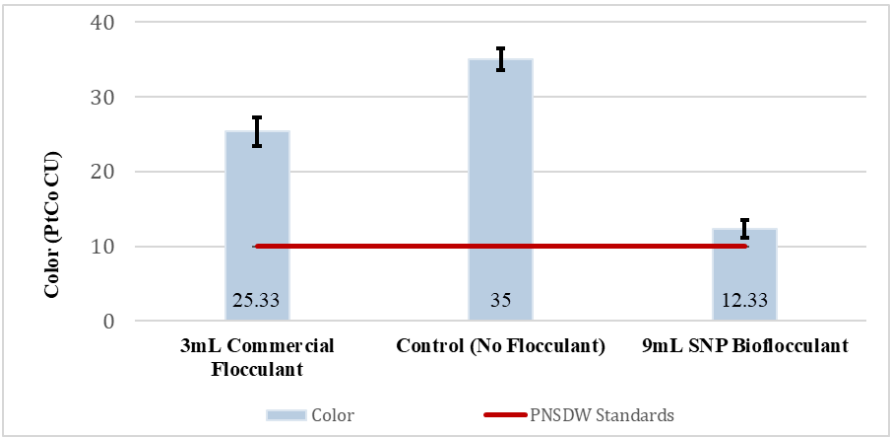

Figure 15. Efficiency of flocculant type in color removal. Bars represent color resulting from the type and optimum volume of flocculant used. Red line represents PNSDW standards for color.

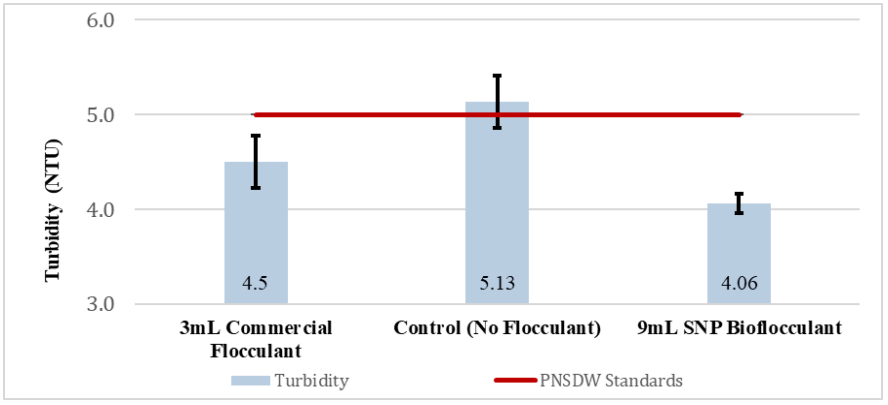

Figure 16. Efficiency of flocculant type in turbidity removal. Bars represent turbidity resulting from the type and optimum volume of flocculant used. Red line represents PNSDW standards for turbidity.

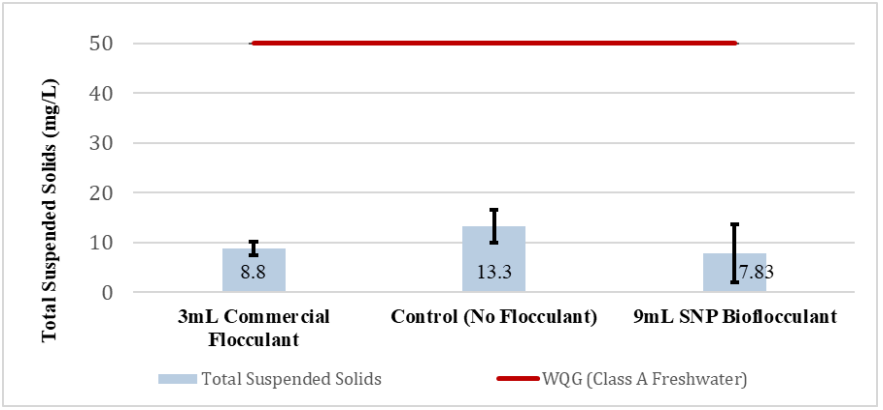

Figure 17. Efficiency of flocculant type in total suspended solids removal. Bars represent TSS resulting from the type and volume of flocculant used. Red line represents TSS guidelines for Class A freshwater.

It can be seen from the figures that water quality results from coagulation alone, in the control set-up, were not very far from the ones involving both coagulation and flocculation treatments. Flocculation contributes to the improvement of water quality based on the overall reduction in color, turbidity, and TSS as flocculant dose is administered.

Comparison of the acceptable values for drinking water, based on the Philippine National Standards, with jar test results for flocculation using the lowest effective or optimum concentrations of commercial flocculant and SNP bioflocculant is shown in Table 2 (Department of Health 2017).

Table 2. Turbidity, color and TSS of river water treated with optimum doses of commercial flocculant and SNP bioflocculant

	PNSDW/ DAO 2016-08	3mL commercial flocculant	9mL SNP bioflocculant
Turbidity (NTU)	5	4.50	4.06
Apparent Color (PtCo CU)	10	25.33	12.33
Total Suspended Solids (mg/L)	50	8.80	7.83

Statistical Treatment

Findings from the analysis variance for water quality parameters, resulting from the modification of SNP bioflocculant concentration using one-way ANOVA, are shown in the succeeding tables.

Table 3. ANOVA Table for Color

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-value	F-critical	p-value	η^2
Between Groups	605.067	4	151.267	18.7521	3.4780	<0.001	0.882
Within Groups	80.667	10	8.067				
TOTALS	**685.733**	**14**					

The F-critical from the distribution table at 0.05 level of significance is less than the calculated F-Ratio therefore; the null hypothesis is rejected. This is backed-up by the p-value, which is less than 0.05. Using one-way ANOVA. The results show a significant difference in the dosage of SNP bioflocculant for color reduction. In other words, the difference is big enough to be statistically significant. Effect size of 0.882 is considered large in magnitude and signifies a strong difference (Cohen 1992). Post-Hoc test was performed to determine the group pairs that were significantly different.

The descriptives table for color is shown in Table 4.

Table 4. Descriptives (Color)

Dosage	N	Mean	SD	SD	Coefficient of Variation
3mL	3	30.667	2.517	1.453	0.082
5mL	3	24.667	1.155	0.667	0.047
7mL	3	20.000	4.583	2.646	0.229
9mL	3	12.333	1.528	0.882	0.124
11mL	3	16.667	3.055	1.764	0.183

The lowest mean color of 12.333 PtCo CU is achieved using the 9mL dose. River water treated with 9 mL of SNP bioflocculant yields a color significantly lower than that from all other bioflocculant dosages in the table above. Using descriptive statistics, 9 mL is identified as the most effective dose for color reduction.

Table 5 displays the ANOVA results for turbidity.

Table 5. ANOVA Table for Turbidity

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-value	F-critical	p-value	η^2
Between Groups	1.093	4	0.273	15.324	3.478	<0.001	0.860
Within Groups	0.178	10	0.018				
TOTALS	**1.271**	**14**					

Null hypothesis is rejected since the F-critical is less than the calculated F-value and the p-value is less than 0.05, which suggests a significant difference between the groups. Following an ANOVA with a significant result, the post-Hoc test was performed.

The descriptives table for turbidity is shown in Table 6.

Table 6. Descriptives (Turbidity)

Dosage	N	Mean	SD	SD	Coefficient of Variation
3mL	3	4.400	0.120	0.069	0.027
5mL	3	4.797	0.146	0.084	0.030
7mL	3	4.527	0.195	0.113	0.043
9mL	3	4.057	0.120	0.069	0.030
11mL	3	4.127	0.032	0.019	0.008

Treatment using the 9 mL dose gives the lowest mean turbidity of 4.057 NTU. River water treated with 9 mL of SNP bioflocculant result to the lowest turbidity. This indicates that the 9 mL dosage is the most efficient for reducing turbidity.

The statistical table for TSS is provided in Table 7.

Table 7. ANOVA Table for Total Suspended Solids

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F-value	F-critical	p-value	η^2
Between Groups	904.331	4	226.083	1.560	3.478	0.259	0.384
Within Groups	1448.987	10	144.899				
TOTALS	**2353.318**	**14**					

The F-critical is greater than the calculated F-Ratio; therefore, the null hypothesis is accepted. Moreover, the p-value is greater than 0.05. The one-factor ANOVA for total suspended solids shows no significant difference, indicating that the result is independent of bioflocculant concentration. Consequently, varying the bioflocculant dosage produces similar results in TSS, signifying no significant effect.

The descriptives for Total Suspended Solids are presented in Table 8.

Table 8. Descriptives (Total Suspended Solids)

Dosage	N	Mean	SD	SD	Coefficient of Variation
3mL	3	28.333	21.057	12.157	0.743
5mL	3	25.933	10.772	6.219	0.415
7mL	3	14.400	5.453	3.148	0.379
9mL	3	7.833	7.067	4.080	0.902
11mL	3	14.067	9.240	5.335	0.657

Since the flocculant dosage produces similar results and has no notable influence on TSS, the dose that achieves the lowest TSS level should be considered optimal. The 9 mL dose of SNP bioflocculant results in the lowest mean TSS of 7.833 mg/L, indicating that this dosage is the most effective at reducing TSS.

The results of the hypothesis testing for the 3 mL commercial flocculant against the 9 mL SNP bioflocculant are shown in Table 9.

Table 9. ANOVA Table for Total Suspended Solids

Parameters	t	df	p	Cohen's d	SE Cohen's d
Color	-8.132	4	0.001	-6.640	2.831
Turbidity	-2.127	4	0.101	-1.737	1.081
Total Suspended Solids	-0.230	4	0.829	-0.188	0.820

The p-value obtained for color is less than 0.05, leading to the rejection of the null hypothesis and indicating that color removal is statistically significant. T-test for both turbidity and TSS yields p-values greater than 0.05, prompting the acceptance of the null hypothesis.

This indicates no significant difference in the percent removal for turbidity and TSS between the 3 mL commercial flocculant and the 9 mL SNP bioflocculant.

Table 10 summarizes the descriptives for T-test.

Table 10. Descriptives (T-test)

Parameters	Flocculant	N	Mean	SD	SD	Coefficient of Variation
Color	Commercial (3mL)	3	0.988	0.001	6.371×10^{-4}	0.001
	Bioflocculant (9mL)	3	0.994	7.299×10^{-4}	4.214×10^{-4}	7.343×10^{-4}
Turbidity	Commercial (3mL)	3	0.961	0.003	0.002	0.003
	Bioflocculant (9mL)	3	0.965	0.001	5.949×10^{-4}	0.001
Total Suspended Solids	Commercial (3mL)	3	0.851	0.030	0.017	0.035
	Bioflocculant (9mL)	3	0.867	0.120	0.069	0.138

A visual comparison of the percent color removals between the commercial flocculant and SNP bioflocculant is illustrated in Figure 18.

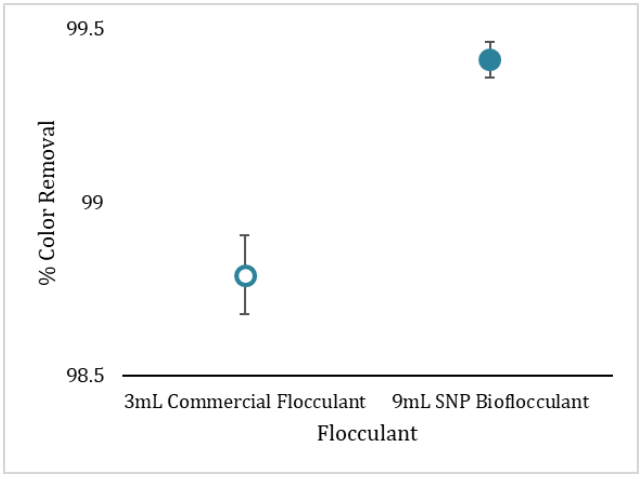

Figure 18. Percentage color removal resulting from optimum doses of commercial and SNP bioflocculant.

Figures 19 and 20 show that results from the 9mL SNP bioflocculant have a higher mean percent removal for turbidity and TSS than commercial flocculant.

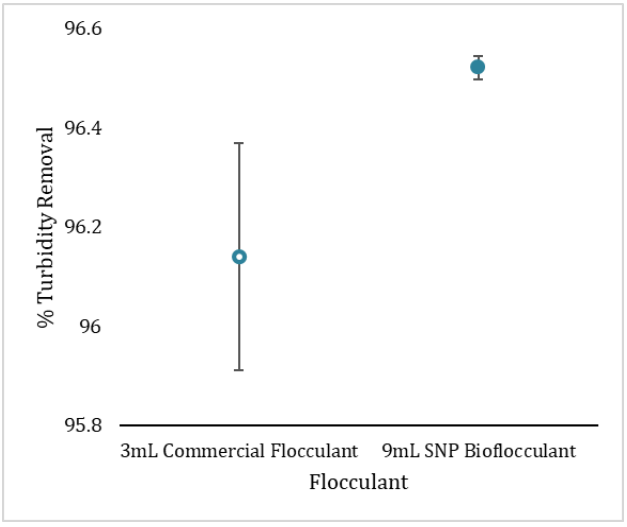

Figure 19. Percentage color removal resulting from optimum doses of commercial and SNP biofloccculantPercentage Turbidity Removal of Flocculants.

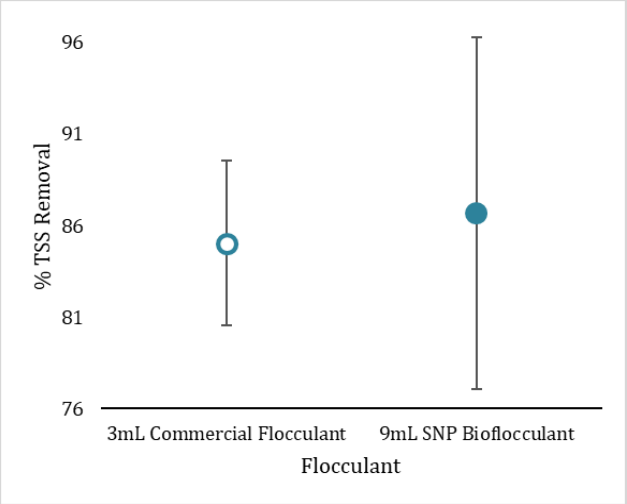

Figure 20. Percentage TSS removal resulting from optimum doses of commercial and SNP bioflocculant.

DISCUSSION

Extraction of Starch from Breadfruit

Isolation yield of breadfruit starch was relatively low compared to the 3.19% and 10% yields reported in the studies by Awokoya et al. (2018) and Ningsih Nst et al. (2024), respectively. Both studies also utilized the same extraction method described by Adebowale et al. (2005). Additionally, breadfruit starch isolation was carried out by Akanbi et al. (2009) using the same procedure, resulting in 14.26% yield.

Other than the maturity and nature of the fruit, several factors related to starch processing were suspected to have caused the low starch recovery. One factor is the product loss from the scraping of impurities formed during the starch isolation process such as yellow mucilage in the steeping process and the grey matter formed at the junction. Starch loss also occurs due to residues retained in the vessels and carryover with the impurities. Repetition of the sedimentation, decantation, and centrifugation process thrice increased the wastage probability and greatly contributed to a decline in the amount of recovered starch.

Acetylation of Isolated Starch

Among the various starch modification methods, acetylation has been extensively employed which involves the substitution of hydroxyl groups in starch with acetyl groups from acetic anhydride resulting to starch acetate (Subroto et al. 2023). The reaction is given by Figure 21.

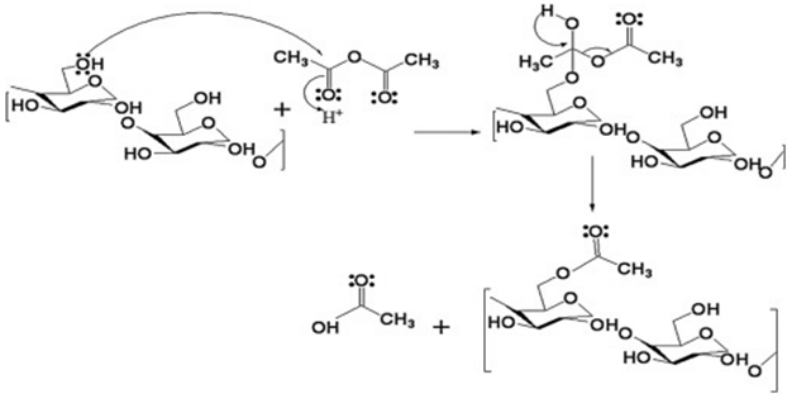

Figure 21. Starch Acetylation Reaction (Ho et al., 2024).

The purpose of acetylation is to prevent retrogradation and to enhance the flocculation performance of starch. By increasing the swelling power and solubility of starch, acetylation weakens the bonds between amylose and amylopectin molecules. The structural modification provides more active sites for impurity adsorption and improves the bridging interaction with flocculants, leading to more efficient flocculation. (Posada – Velez, et al. 2023; Ho et al. 2024) The flocculation mechanism by interparticle bridging is illustrated in Figure 22.

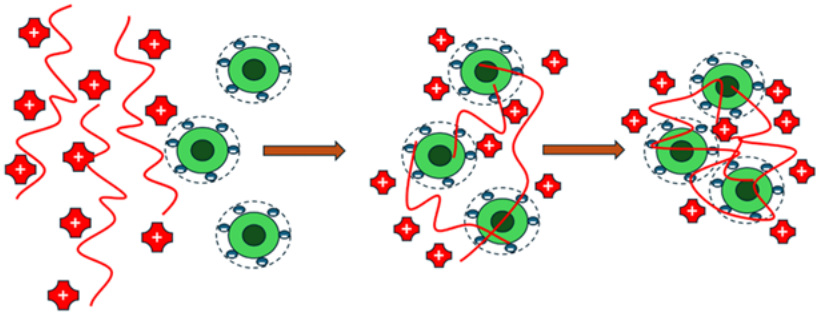

Figure 22. Interactive Mechanism of Interparticle Bridging (Ho et al., 2024).

Synthesis of Acetylated Starch Nanoparticles

Since acetylation is an aggregating process (Gilet et al. 2023), it was performed prior to ultrasonication to prevent particle clumping. The acoustic cavitation induced by ultrasonic treatment helps mitigate the agglomeration caused by acetylation. Although size reduction was achieved through the application of ultrasonic energy, the low yield of acetylated SNP is attributed to the limited amount of breadfruit starch that was isolated.

Characterization of Synthesized Starch Nanoparticle (SNP) Bioflocculant

(a) Particle Size

A size reduction of 93.51% was observed in breadfruit starch particles, after acetylation and ultrasonic treatment using a 50 Hz ultrasonic homogenizer set at 140 W for a total of 75 minutes. The applied power led to the total disintegration of all starch particles into nanosize, or less than 1000 nm.

(b) Structure and Surface Modification

Acetyl percent is directly proportional to the Degree of Substitution; an increase in acetyl percent leads to an increase in DS (Trela et al. 2020). Both values quantitatively determine the extent of the substitution reaction during acetylation (Ogundipe et al. 2019).

Compared to the maximum value of 3, the DS obtained from the experiment indicates that less than a quarter of the acetyl groups from the acetic anhydride successfully substituted the OH groups in breadfruit starch (De Freitas et al. 2017). A DS value in the range of 0.1-1.0, are classified as medium or intermediate degree starch acetate (Trela et al. 2020). Starches with a medium DS and those with a low DS (less than 0.1) are typically used in commercial food applications (Subroto et al. 2023). One of the effects of acetylation is the increase in starch's water absorption capacity, which is essential for baked goods and food systems, enhancing flavor, stability and texture (Ogundipe et al. 2019). Starches with a high DS are used as alternatives to cellulose acetate and in other non-food applications, such as adhesives and coatings. The calculated DS of acetylated breadfruit starch is classified as medium but falls within the range acceptable for food applications, as potable water is consumed and should be safe for drinking (Trela et al. 2020).

Quality of River Water

The quality of untreated river water greatly exceeded the accepted values of 10 PtCo CU for apparent color and 5 NTU for turbidity, as specified by the national standards for drinking water (Department of Health 2017). The value for total suspended solids also exceeds the 50 mg/L

standard for Class A freshwater, which is the classification of Ngalan River. The river is also classified as Public Water Supply Class II according to its beneficial use, indicating that it requires conventional treatment to meet potability standards. (Department of Environment and Natural Resources 2016). The considerable difference in values suggests that treatment is necessary for the river water to pass national standards for potable water.

Flocculation Studies

The lowest dips in the graph of bioflocculant dosage versus river water quality were observed at 9 mL, indicating the lowest levels of color, turbidity and TSS.

Sedimentation in a typical water treatment plant, as reported by Omar and Aziz (2020), yields an average turbidity removal efficiency of 92.25%. The process caused the initial turbidity of the river water, ranging from 75 to 100 NTU, to drop significantly to 10-12 NTU (Omar and Aziz 2020). In comparison, this study simulated the sedimentation process of a water treatment plant using a jar test, where coagulant and flocculant were added to river water. Resulting turbidity was over 4 NTU for both the commercial flocculant and the bioflocculant, with around 96% turbidity removal efficiency. At varying dosages of SNP bioflocculant, percentage removal for color ranges from 98% to over 99% while TSS removal increases with the dosage.

The typical operation of a surface water treatment plant involves conventional processes carried out in the following order: coagulation, flocculation, sedimentation, sand filtration and disinfection, in order to meet Philippine National Standards for Drinking Water (Masten and Davis 2020). It is important to note that this study represents only the coagulation – flocculation stage of treatment. Full compliance with water quality standards requires the subsequent stages of filtration and disinfection.

Nevertheless, turbidity results from water treatment using both commercial flocculant and SNP bioflocculant, have met the national standard of 5 NTU. While color levels from treatment using both flocculants exceeded the allowable standard of 10 PtCo CU; such colorant impurities would normally be addressed and reduced during the filtration and disinfection stages of a complete treatment process. Total suspended solids of river water treated with SNP bioflocculant and commercial flocculant passed the standard 50 mg/L with reference to the water quality guidelines for Ngalan River, which is Class A freshwater (Department of Environment and Natural Resources 2016). At its optimal dose of 9 mL, the bioflocculant demonstrates greater effectiveness in reducing color, turbidity, and TSS than the commercial flocculant, which is most effective at 3mL.

Statistical Treatment

Based on descriptive statistics, the 9 mL dose of SNP bioflocculant yields the best overall results, producing the lowest mean for color, turbidity and TSS. The 9 mL bioflocculant dose produces the best outcome among all other dosages in this study. It follows that 9 mL is the most effective minimum dosage of the synthesized SNP bioflocculant for river water treatment. This was used in comparison with the optimum dosage of commercial flocculant, which is 3 mL.

The performance of the SNP bioflocculant and the commercial flocculant was investigated in terms of percent reduction in color, turbidity, and TSS of river water. While there is a significant difference in color, the SNP bioflocculant yields a higher percent removal mean than the commercial flocculant, suggesting that it performs better than the latter. The T-test analysis for percent removal of both turbidity and TSS reveals no significant difference. For these parameters, the SNP bioflocculant at a 9 mL dosage performs similarly to the 3 mL commercial flocculant. Overall, the synthesized bioflocculant results in higher impurity removal, although it requires a larger volume than the commercial flocculant to optimally remove color, turbidity and TSS. However, it can surpass the performance of the commercial-grade flocculant in terms of color removal and replicate its effectiveness for turbidity and TSS removal. In conclusion, the SNP bioflocculant can serve as an environmentally accepted alternative to the commercial flocculant.

CONCLUSION AND RECOMMENDATIONS

This study successfully demonstrated the potential of breadfruit starch as a viable source for synthesizing a bioflocculant, providing a sustainable use for an underutilized local crop. Although breadfruit starch was successfully isolated, the yield of extracted starch was relatively low at 2.28%, resulting in a correspondingly low SNP yield of 1.27%.

Treatment of the acetylated starch using an ultrasonic homogenizer at 140 W for 75 minutes has reduced the particle size from 6,270.80 nm to 407.20 nm, achieving a 93.51% reduction. The applied power from the ultrasonic treatment led to the total disintegration of all starch particles into nanosize, resulting to 93.51% size reduction of the isolated breadfruit starch. The ultrasonic treatment completely disintegrated the starch granules into nanosized particles. Acetylation using acetic anhydride served as a key modification step to prevent retrogradation and enhance the starch's flocculation capability, as confirmed by the calculated acetyl percentage (9.78%) and degree of substitution (0.41).

Through these processes, the synthesized SNP bioflocculant demonstrated high efficiency in water treatment, achieving up to 99.41% color removal, 96.52% turbidity reduction, and 86.69% TSS removal at an optimal dose of 9 mL. At this concentration, the treated water recorded the lowest color (12.33 PtCo CU), turbidity (4.06 NTU), and TSS (7.83 mg/L). These findings establish the breadfruit-based SNP bioflocculant as a promising, eco-friendly alternative to conventional chemical flocculants, with comparable or superior performance in color removal and effective reduction of turbidity and suspended solids.

More than demonstrating performance, the study's key contribution lies in introducing a novel synthesis pathway combining acetylation with ultrasonic homogenization for bioflocculant production from breadfruit starch. This innovation paves the way for developing locally sourced, biodegradable materials for water treatment applications in the country and other regions with similar agricultural resources.

Future research should focus on enhancing starch extraction and recovery efficiency to improve overall yield, assessing the storage stability and shelf life of the synthesized bioflocculant, and evaluating the performance of unmodified breadfruit starch to establish comparative efficiency. Further optimization may include testing the commercial flocculant at equivalent dose ranges (3–11 mL) under identical conditions, as well as conducting scale-up and cost analyses to determine feasibility for potential industrial application.

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CONFLICT OF INTEREST

The author declares that there are no conflicts of interest regarding the publication of this thesis.

ETHICS STATEMENT

The study utilized the experimental method and no animal or human engagements were conducted in the research.

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