Jurnal Presipitasi

Media Komunikasi dan Pengembangan Teknik Lingkungan e-ISSN: 2550-0023

Regional Case Study

Spirulina Platensis as Biocoagulant to Reduce Turbidity and Total Suspended Solids in Domestic Wastewater

Fathiya Nur Afifah^{1*}, Fayza Rachmalia¹, Frista Ananda Rizky¹, Hasna Khairunnisa¹, Nida Ulhaq Filardhiani¹, Lia Kusumaningrum^{1*}

¹Department of Environmental Science, Faculty of Mathematics and Natural Sciences, Universitas Sebelas Maret. Jalan Ir. Sutami 36A Surakarta 57126, Central Java, Indonesia * Corresponding Author, email: <u>fathiyanurafifah20@uns.ac.id</u>



Abstract

Wastewater must be treated before it is discharged into water bodies. The most widely used wastewater treatment method is coagulation using the synthetic coagulant PAC, whose continuous use can harm human health and reduce the pH value of the water. Therefore, innovation in wastewater treatment is required to overcome this problem. This study examined the potential of Spirulina platensis as a biocoagulant for reducing turbidity and TSS. This research was conducted in three stages: extraction, protein test, and optimum dose test. The results showed that Spirulina platensis has 0.014375% protein in 20 g and can reduce the lowest turbidity at doses of 10-5 ml / L and 10-10 ml / L (in two injections) with an effectiveness value of reducing turbidity levels by 63.02% and reducing the lowest TSS levels at doses of 10-10 ml / L (in two injections) with an effectiveness value of reducing levels by 85%. Based on the Wilcoxon test, it was found that the P-value < 0.05, which means that there is a significant difference in TSS values and turbidity between the results before and after Spirulina platensis biocoagulant treatment

Keywords: Biocoagulant; dosage; extraction; potency; Spirulina platensis.

1. Introduction

Domestic wastewater is a type of waste produced by anthropogenic activities that has the potential to harm living things if not treated. Domestic wastewater consists of 70% organic matter and 30% inorganic matter (Kholif 2020). A high organic content can cause environmental pollution. Solids in the form of microscopic particles cause total suspended Solid) levels and water turbidity levels to be high. The average TSS content in the wastewater reached 77.15 mg / l (Sattuang et al., 2020). Domestic wastewater has an average acidity (pH) value of 6.9 (Sattuang et al., 2020). However, it can damage the environment because harmful substances or microorganisms pollute the environment. Dealing domestic wastewater with untreated water bodies causes pollution (Tariq and Mushtaq, 2023). According to PP No.22 of 2021, water pollution is defined as the entry or inclusion of living things, substances, energy, and other components into water by human activities to exceed the established Water Quality Standards. Water pollution in a water body impacts non-optimal functions, reduces aesthetic value, and can affect and increase the number of health risks (Hu et al., 2019). Therefore, treatment is needed before water is discharged into the river body (Nurhidayanti et al., 2021). Wastewater can be treated using appropriate domestic wastewater management systems (Dirgawati et al., 2023).

Coagulants are used to lower colloidal charges and combine them into clots. Generally, there are two types of coagulants: synthetic and natural (Owodunni and Ismail 2021). Synthetic coagulants (PAC) and orthokinetic flocculants are widely used in domestic wastewater treatment. This is because PAC can remove color, neutralize electric charges, precipitate and reduce heavy metals (Islam and Mostafa 2020), reduce the Chemical Oxygen Demand (COD), and form large flocs. However, its use continuously and in large quantities can reduce the pH value of water and harm health because it is neurotoxic and triggers Alzheimer's disease owing to the alum compounds contained therein.

Thus, synthetic coagulants can be replaced by biocoagulants. Biocoagulants are made from natural ingredients obtained from nature. Biocoagulants derived from natural ingredients can be alternatives to synthetic coagulants. In previous studies, tamarind seeds, papaya seeds, aloe vera, moringa seeds (Sharma 2023), and bean seeds have been used as biocoagulants in the wastewater bioremediation process. This type is similar in protein content and can act as a polyelectrolyte. In addition, *Spirulina platensis* contains 37.5% protein (Muyassaroh et al. 2020). Another study by Konur (2020) stated that *Spirulina platensis* has protein levels ranging from 65-75%. The high levels of protein make *Spirulina platensis* has the potential to be a biocoagulant, and this research was conducted by considering the protein content of Spirulina, which has benefits not only in the field of food and beauty, but also in the field of environment. The biocoagulant dose was determined using the optimum dose test with a jar test with two injections. This study is the first and new step in developing the potential of *Spirulina platensis* to manage liquid waste in an environmentally friendly and sustainable manner. Therefore, this study aims to determine the potential of *Spirulina platensis* to reduce water turbidity, pH, and Total Suspended Solid (TSS).

2. Methods

2.1. Location and Research Time

This research was conducted at IPAL Pialam Yogyakarta, located in Jl. Selarong No.4a, RT.9, Rice Field Area, Pendowoharjo, Sewon District, Bantul Regency, Special Region of Yogyakarta, Indonesia. Sample testing was conducted at the Toya Wening Drinking Water Perumda Laboratory Lab in Surakarta City (Jar Test) and the UPT UNS Laboratory (Protein Extraction and Test). The study was conducted from July to September 2023.

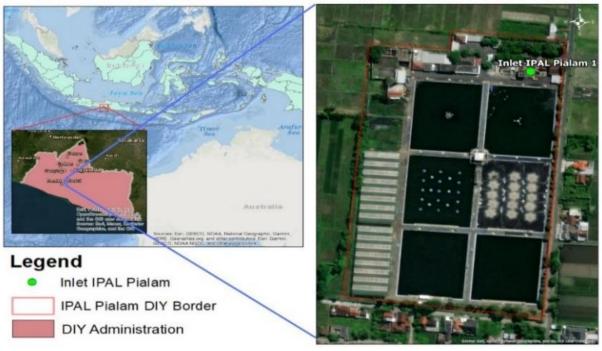


Figure 1. Sampling area

2.2. Materials

Tools used in this study include a set of glassware, stirring rods, porcelain cups, ultrasonic homogenizers model 150 VT (Biologics Inc.), centrifuge (Hettich Micro 22), spray bottles, gloves,

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analytical balance, 1.5 mL cuvette, quantitative UV-Vis spectrophotometer (Shimadzu UV-1800), bucket, dipper, multi-parameter lutron YK-2005WA, horiba multi-parameter water quality U-50, jerry cans, thermos, rope mine, markers, floccucator (VELP Scientifica), suction pump model 1132GL (Thomas), Eutech TN-100 turbidimeter (Thermo Scientific), petri dish, oven (Binder ED 53), pH meter (WTW Inolab series), rotary evaporator (Bibby Sterilin). The materials used included *Spirulina platensis* powder purchased from the distributor company Fuqing King Dnarmsa Spirulina Co., Ltd., aquades, aluminum foil, filter paper, tissues, ice cubes, Na₂CO₃, NaOH, CuSO₄.5H₂O 1%, Na/K Tartarat solution 1%, Cioceleau folin reagent, BSA, biuret solution, and domestic wastewater WWTP Pialam Yogyakarta.

2.3. Extraction of Spirulina platensis

Spirulina platensis powder weighing as much as 20 g was dissolved in 100 mL of the aquades. The cells were sonicated with ultrasonic homogenizers for 15 min (Agustina et al., 2018) to destroy the cell wall of Spirulina platensis and attract bioactive compounds. However, the timing was based on the consideration of the sonication effect on decreasing the active compound content in Spirulina platensis. Research conducted by Lestari et al. (2021) proved that an extraction time of 30 min was able to reduce the concentration of reduced sugar in Spirulina platensis, which is suspected to be due to the longer sonication time that can reduce the active compound content due to excess energy to the system, and then allowed to stand for 24 h (Fayyad et al., 2019). According to previous research, the maceration time is too short, which causes the acquisition of suboptimal extract results (Purba et al., 2019). Thus, if the extraction was carried out for 12 h, it was likely that the active compounds in Spirulina platensis were not extracted optimally. Subsequently, the maserat was inserted into a 1.5 mL cuvette and centrifuged for 30 min at 10,000 rpm (Fachri et al., 2022). The extracted liquid was filtered and evaporated using a rotary evaporator until a thick extract of 20 mL was obtained at a temperature of 50°C (Fayyad et al., 2019). The condensed extract obtained was then weighed and calculated so that the percentage yield value was known. The equation used to calculate the percentage yield in equation (1) is:

 $\% Yield = \frac{Actual Yield}{Theoretical Yield} x 100\%$

(1)

2.4. Protein Test of Spirulina platensis Lowry Method

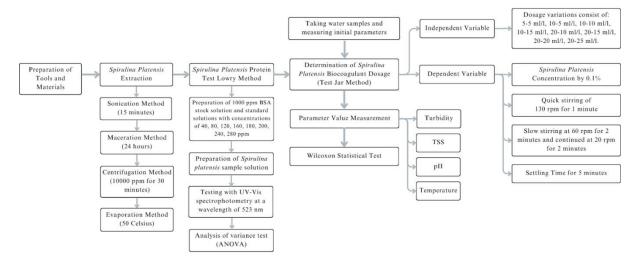
The Lowry method protein test was carried out by measuring the absorption using a UV-Vis spectrophotometer. Sample solutions were prepared with Reagent A solutions including Reagent A (2 g of Na₂CO₃ dissolved in 100 ml of NaOH), Reagent B (5 mL CuSO₄.5H₂O 1% was added to 3 mL of 1% tartarite Na/K solution), Reagent C (2 mL of reagent B + 100 mL of reagent A), and Reagent D (Cioceleau folin reagent diluted with aquades (1:1)).

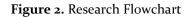
The result of 1 ml filtrate extract is added aquades up to a volume of 4 ml. After that, 5 mL of reagent C is mixed and stirred well and allowed to stand for 15 minutes at room temperature. Then, o.5 ml of reagent D was quickly added, stirred perfectly, and left for 30 minutes at room temperature (Muyassaroh et al., 2020). A total of 1 ml of sample solution is diluted to 10 ml (dilution 10 times). Then a stock solution of BSA (Bovine Serum Albumin) is made at 1000 ppm (40 mg BSA + 40 ml aquades). BSA standard curve standard solution concentration: 40; 80; 120; 160; 180; 200; 240; 280 ppm in a 10 ml measuring flask with 1 ml of standard solution; 2 ml; 3 ml; 4 ml; 4.5 ml; 5 ml; 6 ml; 7 ml, then each added with 0.4 ml biuret solution and aquades until the limit mark is then homogenized (Ghanny et al., 2022). The maximum wavelength is determined by measuring one of the BSA standard solutions in the 400-800 nm range and obtaining a maximum wavelength of 523 nm. Then, absorption measurements were made in the sample and BSA standard solutions using a UV-Vis spectrophotometer at the maximum wavelength.

2.5. Optimum Dose Test of Biocoagulant Spirulina platensis (Jar Test)

The dose of biocoagulant was set at 0.1% (0.1mg+100ml aquades). The optimum dose test uses jar test / Velp JLT 6 Flocculator F105A / 109 concerning SNI 19-6449-2005. 1 L of homogeneous raw water is

put into eight beaker glasses. Initial parameters are first measured: turbidity, temperature, pH, and TSS. The concentration variations used consist of 5-5 ml/l, 10-5 ml/l, 10-10 ml/l, 10-15 ml/l, 20-10 ml/l, 20-15 ml/l, 20-20 ml/l, 20-25 ml/l. The injection process or concentration is done gradually to avoid discoloration factors of *Spirulina platensis*. In the first concentration injection, a rapid stirring of 130 rpm is carried out for 1 minute. Followed by a slow stirring of 60 rpm for 2 minutes and continued at 20 rpm for 2 minutes. Further precipitation for 5 minutes. Then, proceed with injecting the second concentration with the same stages. After that, measurements of the final parameters, temperature, pH, turbidity, and TSS were carried out to compare the changes that occurred so that the effectiveness of each optimum dose of *Spirulina platensis* biocoagulant was known.





2.6. Data Analysis

The dose of biocoagulant was set at 0.1% (0.1mg+100ml aquades). Determination of protein concentration in *Spirulina platensis* was performed using a BSA standard curve incorporated into variance analysis (ANOVA) with a confidence level of 95% (p<0.05). Furthermore, to determine the effectiveness of using the optimum dose of biocoagulant against reducing turbidity levels, TSS and pH hypotheses were determined: Ho "There is no significant difference between before and after biocoagulant administration" and H1 "There is a significant difference between before and after biocoagulant administration" analyzed using Wilcoxon's non-parametric test with Ho rejected if p-value (sig.) < 0.05.

3. Result and Discussion

3.1. Extraction of Spirulina platensis

The extraction process is carried out with a ratio of 1: 5 (20 gr of Spirulina platensis powder + 100 mL aquades), which will attract polar compounds contained in Spirulina platensis, one of which is protein. The sonication method for 15 minutes with the help of ultrasonic vibrations can generate significant energy to destroy the cell walls of Spirulina platensis, attracting bioactive compounds (Vernes et al., 2019) and shortening the extraction time. Next, maceration for 24 hours so that it is well extracted. The extracted results are fed into a cuvette and centrifuged at 10,000 rpm for 30 minutes to separate the mixture. Centrifugation with a speed of 10,000 rpm is can obtain maximum extraction results. If centrifugation is carried out at low speed, the results of the extracted liquid is filtered and then evaporated with a rotary evaporator at a temperature of 50° C to obtain a viscous extract of 20 mL This is done because $60-70^{\circ}$ C heating can denature protein. Other studies also mention that proteins will experience denaturation when heated at temperatures around 80° C (Wang et al., 2020). The results of the viscous extract amounted to 2.95 gr (yield of 14.75% from Spirulina platensis powder). The yield results



show the amount of extract produced from the extraction process with a good category if it is more than 10% (Subaryanti et al., 2022).

3.2. Spirulina platensis Protein Test Results

	0
Wavelength (nm)	Absorbance
463.00	0.169
493.00	0.181
523.00	0.192
553.00	0.171
583.00	0.132
613.00	0.087

Table 1. Determination of maximum wavelength (nm)

The BSA standard curve determines the concentration of protein levels that connect the concentration and absorbance in the solution. Based on Table 1, the maximum wavelength in the 400-800 nm range is determined to be 523 nm. Absorption measurement using a UV-Vis spectrophotometer at a wavelength of 523 nm yields the BSA standard curve as follows.

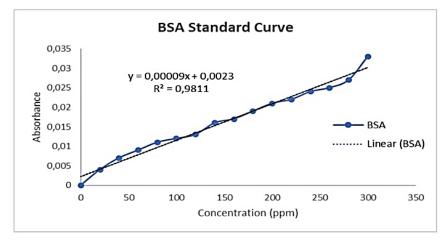


Figure 3. BSA standard curve

The results of the analysis are entered into a variance analysis (ANOVA) with a confidence level of 95% (p<0.05) to find the relationship between the two variables to produce a linear regression equation. Based on the figure above, a linear regression equation is obtained from the BSA standard curve: y = 0.00008x + 0.0037, where x is the concentration and y is the absorbance. The correlation coefficient is 0.9959, close to 1, showing a good relationship between concentration and absorbance. The regression equation is used to determine the value of the protein concentration of *Spirulina platensis*. The absorbance for the sample of *Spirulina platensis* was 0.022, and the sample concentration was 228.75 µg/mL. The protein content in *Spirulina platensis* is 0.014375% in 20 grams.

Spirulina platensis is considered a source of protein because it contains high protein, around 55-70%, with essential amino acids, such as lysine (2.6-4.63%), methionine (1.3-2.75%), tryptophan (1-1.95%), and cystine (0.5-0.7%). The protein content of microalgae is influenced by environmental conditions such as light intensity, nutrient limits (especially nitrogen), salinity, temperature, pH (Afifah et al., 2021), and culture age. The ability of *Spirulina platensis* as a biocoagulant to reduce turbidity values and Total Suspended Solid (TSS) is influenced by the protein content with cationic amino acids in its constituent chains. Among the 20 types of amino acids that make up proteins, there are three types of cationic amino acids: lysine, histidine, and arginine (Silfia et al., 2023). The content of positively charged proteins that act as polyelectrolytes results in attractive forces with colloidal particles in negatively charged wastewater, and biocoagulants can bind colloidal particles and form a precipitated floc (Lisa et al., 2022). Increasing the Spirulina platensis concentration can increase protein levels (Koli et al., 2022). Therefore, Spirulina *platensis* has the potential to be used as a biocoagulant because it contains protein.

Test Result of the Optimal Biocoagulant Dose of Spirulina platensis (Jar Test) 3.3.

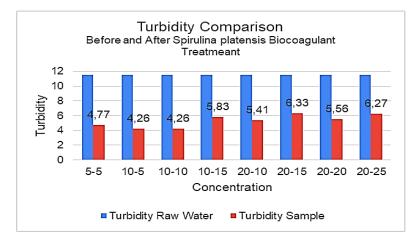
The jar test method aims to determine the optimum dose of the biocoagulant *Spirulina platensis*. Improper doses cause colloids not to be bound optimally, and excessive doses can result in the restabilization of colloidal particles (Sibiya et al., 2021). Yogyakarta urban domestic wastewater serves as a test medium for the biocoagulant potential of Spirulina platensis against reducing TSS levels and turbidity. Based on initial parameter measurements, the domestic wastewater of the Yogyakarta WWTP has a slight turbid tendency in terms of physical parameters. Based on Table 2. found wastewater characteristics with high turbidity and TSS levels. The monthly report on wastewater measurement also supports exceeding wastewater quality standards at the Pialam WWTP Yogyakarta.

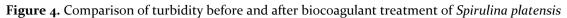
Table 2.	. Characteristics of the initial parameters of domestic waster	vater

Parameter	Early Raw Water
Temperature (°C)	27°C
pН	7.2
Turbidity (NTU)	11.52 NTU
TSS (mg/L)	20 g/L

Turbidity Parameter Jar Test Results

The highest decrease in turbidity levels was at doses of 10-5 ml/L and 10-10 ml / L (Figure 4). The correct dose will cause destabilization of fine particles to produce attractive forces and form flocs. Hussain et al., (2019) state that maximum coagulant efficiency can occur when the optimum initial coagulant and turbidity dose is used. Excessive biocoagulant doses will produce dispersion from the surface charge of colloidal particles that turn so that biocoagulants are not optimal (Priyatharishini et al., 2019). Generally, a decrease in turbidity levels will affect the TSS value, but the two do not have a directly proportional relationship. This is influenced by the materials that cause water turbidity, which can consist of various materials with different properties and weights, so they are not really reflected in comparable TSS residue weights (Samudro and Rulian, 2011).





TSS Parameter Jar Test Results

Based on Figure 5. the optimum dose is 10-10 ml / L injection with a TSS value of 3 mg/L. Giving biocoagulants into raw water causes attractive forces to attract bonds between colloidal harmful particles and cation particles from biocoagulants and produces flocs. One of the factors in the floc formation process is the fast mixing speed. Proper stirring causes the coagulant to be dispersed quickly and evenly in the liquid waste (Hadiwidodo et al., 2019). So, the potential for collisions between particles with the coagulant runs better. The specific gravity of suspended solids heavier than water's specific gravity causes gravitational precipitation. Adding a dose of biocoagulant Spirulina platensis will give a green color. In addition, giving an excessive dose will cause restabilization of the colloid particles, namely, the negative charge will change to positive due to the absorption of the excess dose, resulting in a repulsive force. In biocoagulant research with a mixture composition of 85 mg/l tamarind and 1 mg/l aluminum sulfate using river water samples with an initial turbidity content of 55 NTU, it was found that the optimum biocoagulant reduced to 3.18 NTU at a dose of 33 mg/l. In contrast, the addition of The dose experienced restabilization, marked by an increase in the turbidity value again (Afiatun et al., 2018). Then, in another study, optimal alum coagulant reduced turbidity from 40.2 NTU to 3.08 NTU with a dose of 40 ppm and increased again when the dose was increased (Sisnayati et al., 2021). This is because, at the optimum dose of 40 ppm, the alum content as a dispersion colloid, which has a positive value, will bind fine waste particles with a negative nature and then be neutralized until small flocs form settle. However, increasing the dose will cause the adsorption of excess cations on the surface of negatively charged colloidal particles with the opposite charge of Al₃₊ from aluminum sulfate.

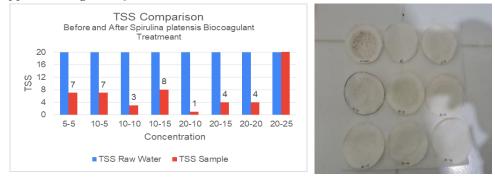


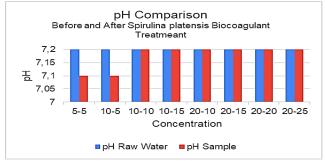
Figure 5. TSS comparison before and after biocoagulant treatment of *Spirulina platensis* (a); TSS result (b)

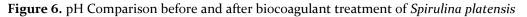
(b)

(a)

pH Parameter Jar Test Results

Based on Figure 6. the use of biocoagulants was found not to affect changes in the pH condition of the sample water. The characteristics of wastewater at a neutral pH can cause the coagulation process to be non-optimal. Based on research by Silva et al., (2021), wastewater with a pH between 5-7 has a lower turbidity reduction efficiency than alkaline pH due to the pH-sensitive nature of *Spirulina platensis*.





Effectiveness of the Biocoagulant Spirulina platensis Against TSS and Turbidity 3.4.

The effectiveness of biocoagulant Spirulina platensis on all three parameters, the optimum dose is a dose of 10-10 ml/L with turbidity effectiveness of 63.02% and TSS of 85% (Figure 7). This is proven based on the Wilcoxon test (Table 3), resulting in significant changes between before and after administration of Spirulina platensis biocoagulant to turbidity parameters and TSS with p values (Asymp. Sig.) < 0.05. The pH parameter p-value (Asymp. Sig.) > 0.05 or no significant change between before and after administration of biocoagulant Spirulina platensis. There is a correlation between the decrease in TSS parameters and turbidity; the lower the content of suspended particles in a solution, the lower the turbidity level (Rahmawan et al., 2019). This is because suspended particles that do not dissolve and cannot immediately settle cause turbidity (Suhernomo et al., 2022)). In the pH parameter, there is a relationship between the type of biocoagulant and the pH of the wastewater used. pH parameter is one of the parameters that influences the coagulation-flocculation process. This is due to using protein, the primary active substance, as a coagulant. Protein characteristics have amphoteric molecular properties, so they depend on the pH of the solution (Fitriagustiani et al., 2022).

Table 3. Wilcoxon Test Results of the Effectiveness of Biocoagulant Dosing Against Parameter Reduction

Test Statistics				
	PostTest_pH -	PostTest_Turbidity-	PostTest_TSS-	
	PreTest_pH	PreTest_Turbidity	PreTest_TSS	
Z	.000 ^b	-2. 521 ^c	-2. 383 ^c	
Asymp. Sig. (2-	1.000	.012	.017	

tailed)

Wilcoxon Signed Ranks Test a.

The sum of negative ranks equals the sum of positive ranks b.

Based om positive ranks c.

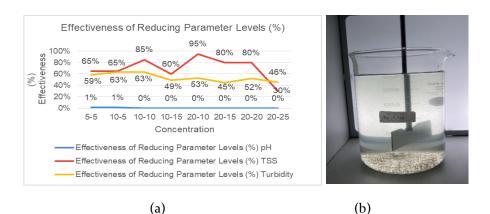


Figure 7. effectiveness of parameter reduction (%) (a); raw water condition from dosing 10-10 ml/L (b)

So, the optimum dose of Spirulina platensis biocoagulant based on pH, Turbidity, and TSS parameters is 10-10 ml / L. Excessive doses will cause colloidal particles to restabilize (Ejimofor et al., 2021). Restabilization will impact the reversal of colloidal particles from negative to positive due to the absorption of excess doses so that larger floc does not form.

SWOT Analysis of Biocoagulant Potential of Spirulina platensis to the Environment 3.5.

In general, SWOT analysis can be interpreted as a technique that can be used to evaluate Strengths, Weaknesses, Opportunities, and Threats. SWOT analysis is considered a primary analysis method that assesses a topic or problem from 4 different sides, which can then ultimately help in



formulating recommendations to maintain strength to increase the advantage of existing opportunities and reduce shortcomings to avoid threats (Anissa et al., 2019). In this section, SWOT analysis is carried out to determine the strengths, weaknesses, opportunities, and threats to the use of *Spirulina platensis* as a biocoagulant in reducing turbidity and TSS levels in domestic wastewater to provide optimal strategies by maximizing existing strengths and opportunities and minimizing existing weaknesses and threats simultaneously. In this section, SWOT analysis is carried out by identifying internal and external factors based on test results and interviews with several experts. The alternative development strategy for using *Spirulina platensis* as a biocoagulant (Table 4).

	Strength	Weakness
Internal Factor	1. The use of <i>Spirulina</i>	1. The costs incurred are more
	<i>platensis</i> as a Biocoagulant	significant than synthetic
	does not damage or pollute	coagulants
	the environment	2. Inappropriate doses have the
	2. Spirulina platensis grows and	potential to cause
	multiplies very quickly	discoloration of green
External Factor	3. No effect on water salinity	3. Inappropriate doses leads to
	levels	a non-optimal process of
	<u></u>	coagulation-flocculation
Opportunities	S – O Strategy	W - O Strategy
1. Increasing industry	1. The use of biocoagulants by	1. Implement cultivation of
awareness in environmentally	industry to reduce the	Spirulina platensis in
friendly waste management	potential impact of waste	wastewater systems to reduce
2. Spirulina platensis, is	and the application of	production costs in large
relatively faster to reproduce	environmentally friendly	quantities (W1, O1, O2)
than other types of	waste management (S1, O1)	2. Conduct research and testing
microalgae, so its cultivation	2. Utilization of technology as	related to the optimum dose
is relatively easier	a basis for effective	of Spirulina platensis as a
3. The development of	cultivation of Spirulina	biocoagulant (W2, W3, O3)
technology and information	platensis and to avoid the	
of Spirulina platensis as an	presence of invasive plants	
environmentally friendly	that interfere (S2, O2, O3)	
biocoagulant		
Threats	S – T Strategy	W – T Strategy
1. The use of synthetic	1. Making Spirulina platensis	1. Optimizing the use of
coagulants is cheaper and	cultivation itself in larger	Spirulina platensis
more practical	quantities to reduce costs	biocoagulant with optimal
2. The emergence of secondary	incurred (S2, T1)	doses to reduce large
pollution due to the use of	2. Trials were conducted to	amounts of costs (W1, W2,
biocoagulant <i>Spirulina</i>	find the correct dose of	W3, T1, T2)
platensis in inappropriate	Spirulina platensis	2. Build zoning of cultivation
doses	biocoagulant (S1, T2)	suitability areas (W1, T3)
3. Biodiversity threats due to	3. Expansion of Spirulina	
the expansion of Spirulina	platensis cultivation land in	
platensis cultivation land if	freshwater and seawater	
the demand for biocoagulant	types because the content in	
production is higher	Spirulina platensis does not	
	affect water salinity (S3, T3)	

Table 4. SWOT Analysis of biocoagulant potential of Spirulina Platensis to the environment

4. Conclusions

Spirulina platensis has the potential to be a biocoagulant with a protein content of 0.014375% in 20 gr. With this protein content, as much as 0.1% biocoagulant Spirulina platensis can reduce turbidity and TSS levels of domestic wastewater with an optimum dose of 10-10 ml (in 2 injections) with an effectiveness value of reducing turbidity levels by 63.02% and an effectiveness value of reducing TSS by 85%. Based on the Wilcoxon test, it was also found that the P-value < 0.05, which means there is a significant difference in TSS value and turbidity between the results before and after the Spirulina platensis biocoagulant treatment. In contrast, the pH parameter found that the P-value > 0.05, meaning there is no significant change before or after the administration of Spirulina platensis biocoagulant.

Acknowledgement

Thanks to the Directorate General of Higher Education, Research, and Technology for funding Student Creativity Program Activities so that this research can occur. We would also like to thank the IPAL Pialam Yogyakarta for cooperating in providing domestic wastewater samples. We want to thank all WWTP officers of Balai Pialam Yogyakarta, all officers of the Toya Wening Drinking Water Perumda Laboratory of Surakarta City, and the UPT UNS Laboratory who have provided support and facilitated all the the author needs to run the research.

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