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Latifa Mirzatika AL-ROSYID[1*], Sawitri KOMARAYANTI[2]
Pramesya Ramadhana ARIFIN[1] and Wulan GURITNO[1]

BIOREMEDIATION OF FISHERY WASTE USING WATER LETTUCE (*Pistia stratiotes* L.)

Abstract: One of the relatively affordable and safe waste treatment is biological treatment by utilising certain plants as biofilters. The water lettuce (*Pistia stratiotes* L.) is an aquatic plant that is usually considered a weed by the community. These plants can be used to absorb toxic elements in wastewater. The purpose of this study was to analyse the relationship between *Pistia stratiotes* L. and the decrease in the organic matter content of fishery processing wastewater. The research method involved varying the effectiveness of the relationship between the bacteria and the water lettuce, the cover rate of the water slide plants and measuring water quality. Water quality measurements include: analysis of BOD_5, COD, TSS, pH and temperature. Data analysis with regression and correlation tests using SPSS 28 software. In this study, the density of *Pistia stratiotes* L. used was 0 %, 25 %, 50 %, 75 % and 100 %. Based on the results, it can be concluded that the effectiveness of the *Pistia stratiotes* L. in reducing levels of BOD_5, COD and TSS is highest at a density of 100 % with the effectiveness values of each 23.7 mg/L, 58.7 mg/L and 2.67 mg/L.

Keywords: bioremediation, fishery waste, water lettuce, *Pistia stratiotes* L.

Introduction

The fishing industry in its operations will produce the final product and unwanted material that will be discarded or can still be reprocessed, which is called waste. If waste containing certain hazardous and toxic chemical compounds is released into the environment, this will result in pollution in rivers, soil and air [1].

Jember District, which is located on the south coast, has fish-producing beaches that contribute to national fish stocks [2]. Puger sub-district is the biggest fish auction place (FAP) in Jember. FAP waste can be in the form of liquid waste or solid waste. Liquid waste in the form of washing water from fish processing. The effect caused by this liquid waste is different on the environment because it is influenced by the concentration of organic materials contained in it, the quantity of liquid waste discharged into nature and the mixing capacity of the waters that receive it. Countermeasures need to be taken so that it does not cause pollution to the environment [3]. The BOD/COD ratio itself

[1] Department of Environmental Engineering, Faculty of Engineering, University of Muhammadiyah Jember, Karimata Street No. 49, Jember 68122, Indonesia, phone +62 0331 336728, fax +62 0331 337957, email: pramesyaramadhana29@gmail.com, wguritno112@gmail.com, ORCID: LMA 0000-0003-2308-5580
[2] Department of Biology Education, Faculty of Teacher Training and Education, University of Muhammadiyah Jember, Karimata Street No. 49, Jember 68122, Indonesia, phone +62 0331 336728, fax +62 0331 337957, email: sawitrikomarayanti@unmuhjember.ac.id
[*] Corresponding author: latifa@unmuhjember.ac.id

indicates the biodegradability of wastewater, where the higher the ratio, the higher the biodegradability [4].

Fishery product waste that is not managed properly will cause environmental pollution. The pollution caused is: discoloration, odour and colloidal deposits in the aquatic environment around FAP, unpleasant odours, and causes coughing and itching [5]. Therefore, it is important and necessary to research the processing of fishery waste which is relatively inexpensive but efficient in terms of results.

One of the relatively affordable and safe waste treatment is biological treatment by utilising certain plants as biofilters. This is because plants have the ability to bind elements from the surrounding environment and are sensitive to all changes in environmental habitats, both physical and chemical [6]. The water lettuce (*Pistia stratiotes* L.) is an aquatic plant that is usually considered a weed by the community [7]. However, these plants can provide benefits for polluted waters [8]. These plant is a type of water weed that grows very fast and has the ability to adapt to new environments. These disturbing plants can be used to absorb toxic elements in wastewater [9, 10].

In the current study, variations in the effectiveness of the relationship between bacteria and watercress were analysed using regression and correlation tests using SPSS 28 software, analysis of cover rates of watercress plants, measurement of water quality (analysis of BOD_5, COD, TSS, pH and temperature). This will make the analysis results better.

Materials and methods

Preparation of test plant

Before being used in research, the water lettuce were first acclimatised. This acclimatisation aims to neutralise the water lettuce from the previous media. The way is to clean the water lettuce from the mud and dirt that is still attached, then prepare a reservoir filled with water. Choose healthy and fresh water lettuce then put it in the reservoir. Acclimatisation is carried out for a week with a natural lighting system (sunlight). The next step is to select plants that have a uniform size and then weigh the biomass.

Acclimatisation stage

Plants were acclimatised by placing them in an acclimatisation tank filled with water. Before acclimatisation, the plants are cleaned of dirt attached to the roots and leaves. Then the plants were acclimatised for ± 7 days (Table 1). If during this time the plant does not change, it can be continued in the process of exposure to fishery waste. The condition of the plants at the time of acclimatisation can be seen in Table 1.

Measurement of biological oxygen demand (BOD)

BOD_5 analysis uses the Winkler method [11]. The principle uses iodometric titration. The wastewater sample was first added with a solution of $MnCl_2$ and NaOH-KI (Merck, Germany), so that MnO_2 precipitate would occur. By adding H_2SO_4 (Merck, Germany) the precipitate that occurs will dissolve again and will also liberate iodine (I_2) molecules which are equivalent to dissolved oxygen. The liberated iodine was then titrated with a standard solution of sodium thiosulphate ($Na_2S_2O_3$) (Merck, Germany) and using a starch solution indicator (starch).

Table 1

Acclimatisation stage

Day	Stage
1	Acclimatisation of fresh water lettuce and the leaves are light green. The roots are long, white-brown and expand freely
2	Acclimatisation leaves and plant roots still look fresh and green
3	Acclimatisation, the plants are still green, fresh with floating roots
4	Acclimatisation, seen from the morphology of the plant leaves, did not experience any significant changes. Plant leaves still look green and fresh
5	There is no significant change in plant acclimatisation where the leaves are still green with floating roots
6	Acclimatisation of the outer leaves of the plant begin to turn yellow at the tips because they are old and the other leaves are still fresh and green. Fresh floating plant roots
7	Acclimatisation of old plants where the tips of the leaves start to turn yellow, while the other leaves are still green and fresh with floating roots

Measurement of chemical oxygen demand (COD)

The next research stage is COD analysis with closed reflux spectrophotometry [12]. Organic compounds are oxidised by $Cr_2O_7^{2-}$ in closed reflux to produce Cr^{3+}. The amount of oxidant required is expressed in oxygen equivalents (O_2 [mg/L]) measured by visible spectrophotometry. $Cr_2O_7^{2-}$ strongly absorbs at a wavelength of 420 nm and Cr^{3+} absorbs strongly at a wavelength of 600 nm. For COD values of 100 mg/L to 900 mg/L the increase in Cr^{3+} is determined at a wavelength of 600 nm. In the test sample with a higher COD value, dilution was carried out prior to testing. For COD values less than or equal to 90 mg/L the decrease in $Cr_2O_7^{2}$ concentration was determined at a wavelength of 420 nm. Making a calibration curve is done by adjusting the wavelength of the spectrophotometer test equipment at 600 nm or 420 nm, measuring the absorbance of each working solution, then recording it and plotting it against COD levels.

Measurement of total suspended solid (TSS)

Then a gravimetric TSS analysis was carried out in accordance with SNI 6989.3-2019 concerning Methods for Testing Total Suspended Solids. This standard uses Standard Methods for the Examination of Water and Wastewater 23rd Edition (2017) Method 2510 as the main reference. In this method, the homogeneous sample is filtered with a weighed filter media. The residue retained on the filter media is then dried at a temperature of 103 °C - 105 °C until it reaches a constant weight. The increase in filter weight represents Total Suspended Solids (TSS).

Research variations

In this study, laboratory scale experimental methods were used. The variation of the experimental design used was a completely randomised design (CRD) with 4 different treatments (Table 2), namely different closures with 3 replications [13]. Replications are placed randomly. This is done so that the data analysis becomes valid besides that all samples get the same treatment.

Table 2

Research variations

Variation	Treatment of covering aquatic plants
A	Fishery product processing waste with 100 % plant cover, *Pistia stratiotes* L. 250 g
B	Fishery product processing waste with 75 % plant cover, *Pistia stratiotes* L. 200 g
C	Fishery product processing waste with 50 % plant cover, *Pistia stratiotes* L. 150 g
D	Fishery product processing waste with 25 % plant cover, *Pistia stratiotes* L. 100 g
E	Fishery product processing waste without closure (control)

Results and discussion

Measurement of pH and temperature

The data obtained from the analysis shows that the pH value of the initial waste before being processed meets the wastewater quality standards. After undergoing processing, the pH value fluctuated but still met the wastewater quality standards.

The decrease in pH value is due to organic (protein and fat) and inorganic (mineral) materials that have been filtered or bound by the roots of the water lettuce, making it easier for microbial decomposers in the degradation process [1]. Organic materials that have been filtered or bound by the roots of the Water lettuce are degraded into simple compounds, namely, amino acids and fatty acids (organic acids) to obtain ammonia, nitrate, nitrite and nitrogen. With the formation of organic acids resulting from the breakdown of proteins and fats, the pH will continue to decrease to near neutral pH. The inorganic materials in the leather tanning liquid waste are filtered or bound by the roots of the plant so that the levels of organic matter in the waste can be reduced [9].

The average temperature in this study of 26 °C - 27 °C fulfilled the optimum temperature limit, namely the optimum temperature for phytoremediation ranged from 25 °C - 30 °C and was at the optimum temperature limit for growing water lettuce, namely 22 °C - 30 °C [2]. Temperature is related to metabolic processes and photosynthesis, so that an increase in temperature will indirectly affect the level of absorption. According to [3], the higher the plant's ambient temperature, the higher the absorption rate by plants. However, an increase in temperature can also cause toxicity to increase so that plant growth is hampered, which in watercress plants is characterised by shortening of the roots [6].

Biological oxygen demand (BOD) analysis

The characteristic test on wastewater is used to determine the initial content of waste referring to the parameters of wastewater for industrial businesses or activities in PermenLH No. 06 of 2007 concerning Wastewater Quality Standards for Fishery Product Processing Businesses and/or Activities. The BOD_5 value obtained exceeds the quality standard, which is 121.9 mg/L.

The results of this study showed that the ability of the water lettuce to reduce BOD_5 levels in the treatment group with a density of 0 %, 25 %, 50 %, 75 % and 100 %. The highest decrease in BOD_5 levels was found at a density of 100 % from day 0 to day 28, which was 23.7 mg/L (Fig. 1).

There was a decrease in the concentration of BOD_5, due to the presence of aquatic plants which caused an increase in the concentration of dissolved oxygen in wastewater. This oxygen was obtained from the results of plant photosynthesis and the addition of aeration in the reactor [14]. The dissolved oxygen in the reactor is getting higher.

If dissolved oxygen is sufficient, symbiotic microorganisms in plant roots can decompose more organic contaminants [15].

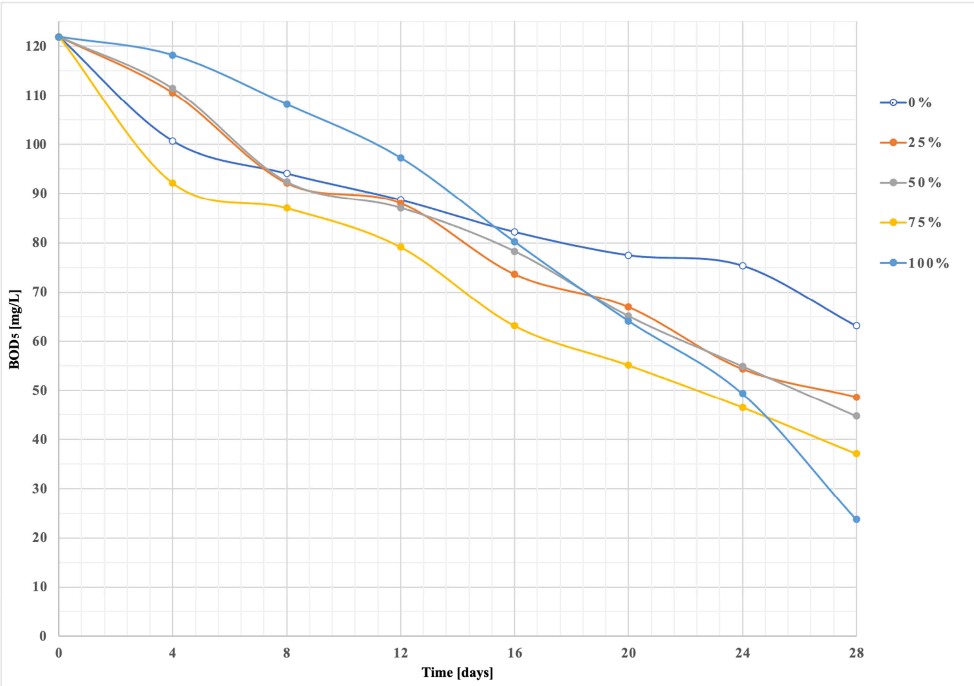

Fig. 1. The effectiveness of water lettuce in reducing BOD_5

The decrease in BOD_5 levels can also be caused by the process of phytodegradation. Organic contaminants are absorbed through the roots and decompose through metabolic processes in plants. The decrease in pollutant substances can be caused by the phytovolatisation process which is the absorption of pollutants by plants after the above process and is released in the form of water vapour into the atmosphere. This process is appropriate for use by contaminants of organic substances [16].

Chemical oxygen demand (COD) analysis

The COD value obtained exceeds the quality standard, which is 303.4 mg/L. The results of the study showed that the ability of the water lettuce to reduce COD levels in the treatment group with densities of 0 %, 25 %, 50 %, 75 % and 100 %. The highest decrease in COD levels was found at a density of 100 % from day 0 to day 28, which was 58.7 mg/L (Fig. 2).

COD analysis in this study was carried out with a variation of time, so that it can be seen that the longer the time, the decreasing level of COD in wastewater will decrease. The COD level before the phytoremediation process was 303.4 mg/L, which means that this value exceeds the quality standard. According to [17], the COD value is a value that indicates the thickness of the organic matter in the waters so that it can cause low levels of dissolved oxygen in the waters needed by organisms for respiration.

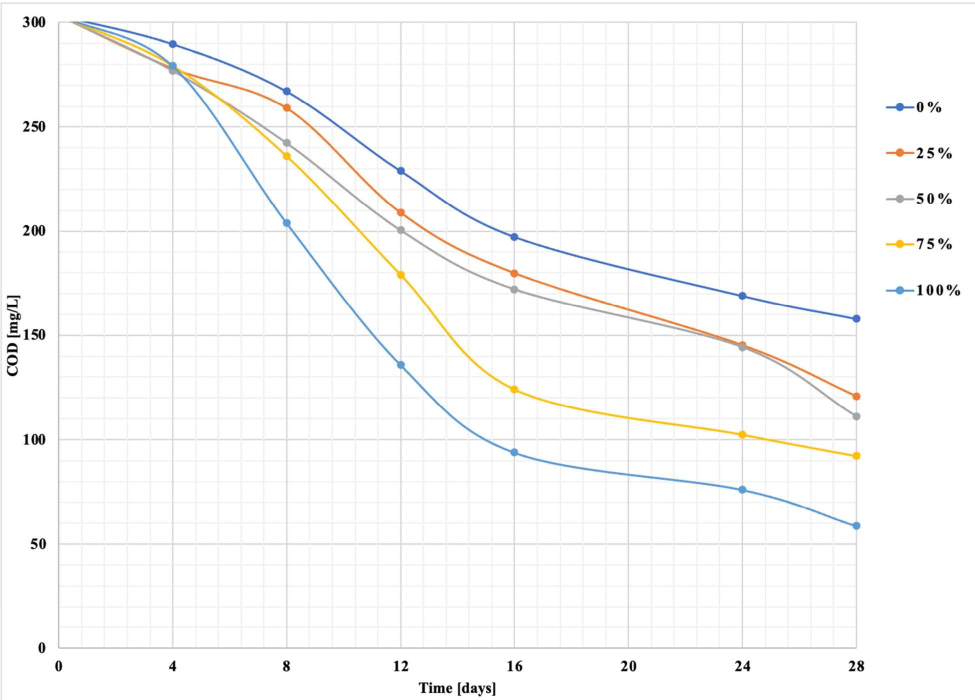

Fig. 2. The effectiveness of water lettuce in reducing COD

The increase in COD removal value is increasing day by day. This happens because the degradation process will begin to be effective when the microorganisms in the root zone have started to grow in large numbers. The superiority of water lettuce greatly affects the detention time in degrading organic matter (protein and fat) and inorganic (minerals) contained in liquid waste. The faster the process of absorption of organic and inorganic materials by the roots of the watercress plants, the faster the process of improving the quality of waste and the effectiveness of the detention time required to obtain standard waste water quality can be achieved [18].

Total suspended solid (TSS) analysis

TSS analysis in this study was carried out with variations in time, so that it can be seen that the longer the time, the lower the level of TSS reduction in wastewater will decrease. The TSS level before the phytoremediation process was 25 mg/L, which means that this value met the quality standards.

The results of the research showed that the ability of the water lettuce to reduce TSS levels in the treatment group with a density of 0 %, 25 %, 50 %, 75 % and 100 %. The highest decrease in TSS levels was found at a density of 100 % from day 0 to day 28, which was 2.67 mg/L. This shows that the longer the waste is in contact with the water lettuce and the more the number of plants, the lower the TSS level (Fig. 3).

The decrease in the TSS value was due to the fact that the hardwood plant has fibrous roots which can become a place for colloids floating in the water to attach. The more fibrous roots one has, the more colloid will stick to these roots [19]. Precipitates and colloids as well as dissolved materials originating from solid waste materials will settle to the bottom if they cannot dissolve and some will become colloidal if they can dissolve. Precipitates that cannot dissolve before reaching the bottom will float in the water with the colloids. Organic waste materials in the form of colloids will agglomerate on the surface of the water so that the water will appear cloudy. The colloidal clumping will inhibit the diffusion of oxygen into the wastewater so that the availability of oxygen becomes less and will inhibit the work of aerobic microorganisms in decomposing organic matter.

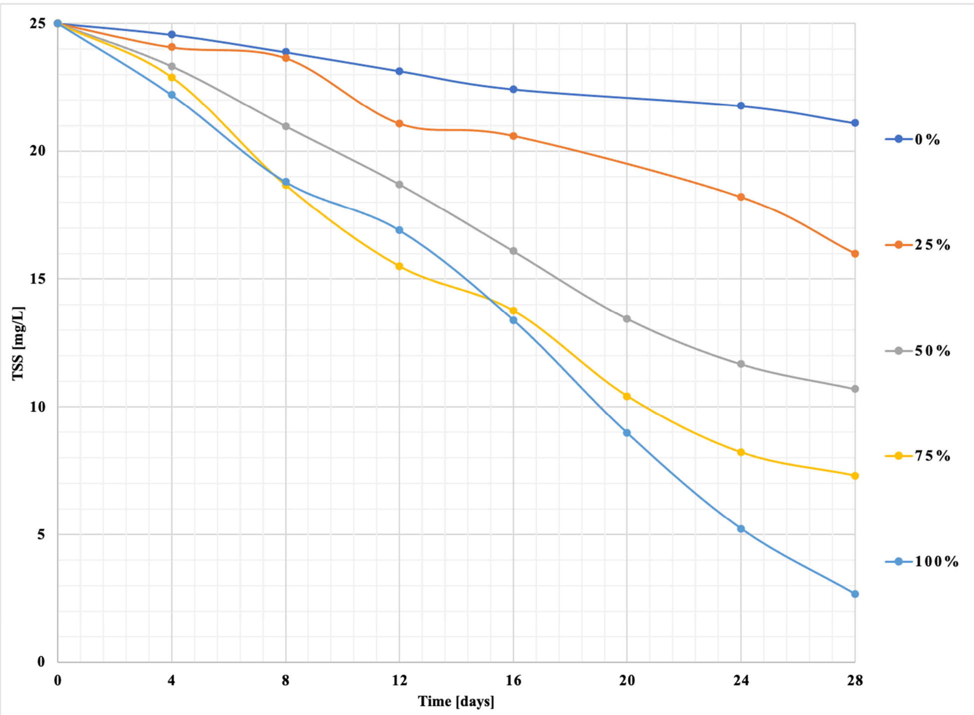

Fig. 3. The effectiveness of water lettuce in reducing TSS

TSS is closely related to water turbidity. The higher the TSS value, the more turbid the water will be. This can result in blocking sunlight from entering the water, so that photosynthesis will be disrupted and have an impact on decreasing dissolved oxygen levels. Dissolved oxygen levels that fall will greatly disrupt the activities of living things in the water [20].

Statistical analysis

The decrease in BOD_5, COD and TSS levels using the phytoremediation principle on the variable density of water lettuce was supported by statistical tests using SPSS 28. The results stated that $p < \alpha$ (0.05) so that the hypothesis was accepted, it could be seen that there were differences in the decrease in levels of BOD_5, COD and TSS in fishery waste using water lettuce (Table 3).

Table 3

SPSS ANOVA statistical test. Correlations

		BOD_5	COD	TSS
BOD_5	Pearson correlation	1	$.846^{**}$	1
	Sig.		.000	
	N	5	5	5
COD	Pearson correlation	$.846^{**}$	1	$.846^{**}$
	Sig.	.000		.000
	N	5	5	5
TSS	Pearson correlation	1	$.846^{**}$	1
	Sig.		.000	
	N	5	5	5

** Correlation is significant at the 0.01 level

To prove whether there is a significant effect of total bacteria on the decrease in organic matter content, a statistical test was carried out. The regression test results show a coefficient of determination of 0.846 with a significance of 0.000 < 0.05. This shows that at the 95 % significance level, the phytoremediation factor using water lettuce has a significant influence on reducing organic matter content.

Conclusion

One of the relatively affordable and safe waste treatment is biological treatment by utilising certain plants as biofilters. The water lettuce (*Pistia stratiotes* L.) is an aquatic plant that is usually considered a weed by the community. These plants can be used to absorb toxic elements in wastewater. In this study, involved varying the effectiveness of the relationship between the bacteria and the water lettuce, the cover rate of the water slide plants and measuring water quality. Water quality measurements include: analysis of BOD_5, COD, TSS, pH and temperature. The density of water lettuce (*Pistia stratiotes* L.) used was 0 %, 25 %, 50 %, 75 % and 100 %. Based on the results of the research that has been done, it can be concluded that the effectiveness of the water lettuce (*Pistia stratiotes* L.) in reducing levels of BOD_5, COD and TSS is highest at a density of 100 % with the effectiveness values of BOD_5, COD and TSS each of 23.7 mg/L, 58.7 mg/L and 2.67 mg/L. In addition, the regression test showed a coefficient of determination of 0.846 with a significance of 0.000 < 0.05, then H0 was rejected or in other words, at a significance level of 95 %, the phytoremediation factor used water lettuce gave a significant effect on the reduction of organic matter content.

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