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Phytoremediation of Tofu Liquid Waste Using Water Hyacinth and Water Lettuce to Improve Waste Quality

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Abstract
Tofu businesses in Indonesia are primarily small-scale businesses that do not have independent wastewater treatment units. Most tofu liquid waste businesses still dispose of tofu liquid waste into nearby bodies of water without processing it first, damaging environmental quality and water pollution. This research aims to describe the influence of various combinations of water hyacinth *(Eichhornia crassipes (Mart.) Solms*) and water lettuce (*Pistia stratiotes Linnaeus*) plants in improving the quality of tofu liquid waste in terms of pH and Nitrate parameters. The research was carried out experimentally using a Randomized Block Design (RBD) and one of the treatment factors, namely the combination of plant biomass consisting of 4 treatments (control, 75:25, 50:50, and 25:75). There were 6 six repetitions in the research so there were 24 treatment units. The research was carried out in the Greenhouse Biology Study Program at Surabaya State University, with the parameters measured covered pH and Nitrate on days 0 and 7. pH and Nitrate parameter data were analyzed using one-way ANOVA, then continued with Duncan's test and compared with standards quality according to Minister of Environment Regulation Number 5 of 2014. The results of the research showed that combinations of biomass improved the quality of tofu liquid waste. The most effective combination was 25 water hyacinths : 75 water lettuces that could increase the pH value to 6.9 (6–9) and reduce Nitrate to 0.21 (30) mg/L.

Keywords: *Eichhornia crassipes (Mart.) Solms; Pistia stratiotes Linnaeus;* phytoremediation; waste water treatment
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INTRODUCTION

Some tofu businesses in Indonesia are small-scale businesses with minimum cost and simple technology. Most tofu industries don't have an independent wastewater treatment unit, so tofu liquid waste is thrown into the water without being treated first. High investment and operational costs constrain micro-scale tofu craftsmen from building wastewater treatment plants (WWTP) (Qatrunada *et al.,* 2023). The tofu industry produces liquid waste from washing, boiling, pressing, and molding. The content of organic compounds in tofu liquid waste is relatively high, which can cause the rapid proliferation of microorganisms in the water. The high organic compounds and suspended material of tofu liquid waste decrease oxygen levels in the water, making the water dirty and cloudy. The characteristics of tofu liquid waste are high levels of TSS (Total Suspended Solids), oil, ammonia, nitrite, and nitrate (Pranama *et al.,* 2023).

The phytoremediation technique is an alternative for processing tofu liquid waste. Compared to other waste processing techniques, phytoremediation techniques are relatively cheap, easy to implement, and do not damage the environment (Dewi *et al.,* 2021). Plants can remove, absorb, or convert many dangerous contaminants in the environment. Plant mechanisms for phytoremediation also vary; some plants carry out phytoextraction, hemofiltration, phytotransformation, phytodegradation, phytostabilization, and phytovolatilization (Juhriah & Alam, 2016). Some aquatic plants that can be used as phytoremediation agents are Eichhornia crassipes, Typha latifolia, Pistia stratiotes L, Salvinia molesta, and other aquatic plants. Water hyacinth (*Eichhornia crassipes (Mart.) Solms*) and water lettuce (*Pistia stratiotes* Linnaeus) can be used as phytoremediation agents for tofu liquid waste. In this research, the two aquatic plants were combined with the hope of synergizing and working together to improve the quality of tofu liquid waste by a more significant percentage. Water

hyacinths can absorb organic water substances through the tips of their roots. They have broad leaves with hollow stems and dense fibrous roots, which allows them to absorb toxic substances in liquid tofu waste (Ahmad & Adiningsih, 2019). Meanwhile, water lettuce has fibrous roots that extend downwards, making it easier for the plant to absorb pollutants (Nizam *et al.,* 2020).

This research uses journal references from a previous study conducted by Vidyawati and Fitrihdajati (2019) regarding phytoremediation using water hyacinth for tofu liquid waste. The results showed that phytoremediation with water hyacinth on tofu liquid waste using 25% diluted can improve the quality of the waste on the pH, NO_2, NH_3, and NO_3 parameters. Therefore, researchers are interested in studying by modifying the independent variable to provide differentiating variables from previous research, where the independent variable used was a combination of water hyacinth and waterc lettuce plant biomass. Based on the background description, tofu liquid waste can be processed using phytoremediation techniques using a combination of water hyacinth and watercress plant biomass to improve its quality in terms of pH and Nitrate parameters. Therefore, this research aims to determine the effect of the combination of water hyacinth and water lettuce biomass on the phytoremediation of tofu liquid waste in terms of pH and Nitrate (NO_{3-}) parameters and to find out which biomass combination is the most effective in improving the quality of tofu liquid waste.

MATERIALS AND METHODS

This research was conducted for four months, from September – December 2023. Location Phytoremediation research was Green House C10 Biology Department, Universitas Negeri Surabaya. Measurement Nitrate was carried out at the UPTD Chemistry Laboratory, Environmental and Hygiene Service Laboratory Regency Sidoarjo.

The manipulated variable in this study was the combination of water hyacinth and watercress biomass consisting of control, 75:25, 50:50 and 25:75. The control variables in this study were a concentration of tofu liquid waste of 25%, acclimatization time and contact time of seven days or one week, plant biomass weighing 300 grams, a gallon container with a volume of 15 liters, planting media with a volume of 5 liters, and a serving location. The response variables in this study were the pH and Nitrate (NO_{3-}) values in tofu liquid waste.

The initial research stage was to acclimatize water hyacinth and water lettuce plants for seven days. The 25% dilution technique is to dilute 1.25 liters of liquid tofu waste plus distilled water until the volume reaches 5 liters of water and then pour it into a planting media container. Then, combine the two plants with a total biomass of 300 grams. The control combination was no plants; combination 75:25 using 225 grams of water hyacinth and 75 grams of water lettuce; 50:50 combination using 150 grams of water hyacinth and 150 grams of water lettuce, 25:75 combination using 75 grams of water hyacinth and 225 grams of watercress (Sata, 2018). Then, place the combined plants in a gallon containing liquid tofu waste for exposure for seven days.

The parameters of tofu liquid waste measured were pH and Nitrate values. pH is measured using a pH meter that has previously been calibrated using a buffer solution. Meanwhile, nitrate levels were measured using the Hanna Instrument method at the Sidoarjo Regency Environment and Hygiene Service Laboratory. Measurements for each parameter were carried out for all treatment, at the beginning (day 0) and end of treatment (day 7).

Data regarding the effect of the biomass combination of water hyacinth and water lettuce on the phytoremediation of tofu liquid waste will be analyzed statistically using one-way ANOVA. The data will be presented in graphical form to show the effect of the combination on increasing or decreasing the quality of liquid tofu waste on the pH and Nitrate parameters, then compare the average data with the quality standards for processed soybean waste, which have been regulated by the Minister of the Environment of the Republic of Indonesia Number 5 of 2014. Meanwhile, data regarding the effectiveness of the biomass combination between water hyacinth and water lettuce as a phytoremediator for tofu liquid waste will be analyzed statistically using one-way ANOVA, followed by the Duncan test to determine the effectiveness of the reduction percentage. Data will be presented in table form with letter notation to show the effect of fundamental differences at the 0.05 level.

RESULTS

Phytoremediation research using a combination plants of water hyacinth and water lettuce to improve the quality of tofu liquid waste was carried out in the Greenhouse of the Biology Study Program, Surabaya State University with a contact time of 7 days. Improvements in the quality of tofu

liquid waste are measured from the pH and Nitrate (NO_{3-}) parameters. This research aims to find out the effect of a combination of biomass between water hyacinth plants and water lettuce with phytoremediation techniques in an effort to improve the quality of tofu liquid waste.

The influence of phytoremediation is the effect of using a combination of water hyacinth and water lettuce as a phytoremediator for tofu liquid waste. The results of phytoremediation research using various biomass combinations between water hyacinth and water lettuce on the quality of tofu liquid waste obtained average data on pH and nitrate (NO_{3-}) analyzed Statistically using the Onesample Kolmogorov-Smirnov Test to find out whether the data is usually distributed and obtained a significance value of more than 0.05. This value shows that the data is normally distributed, so further tests can be carried out using Analysis of Variance (one-way ANOVA). The following is a graphic image for each plant combination treatment's pH and Nitrate (NO_{3-}) values.

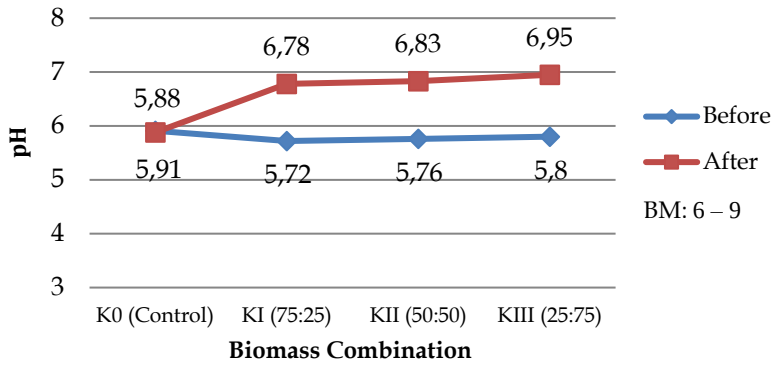

Figure 1. pH values before and after phytoremediation using a combination biomass of water hyacinth (*Eichhornia crassipes (Mart.) Solms*) and water lettuce (*Pistia stratiotes Linnaeus*). Note: K0=control, KI=combination of 75 water hyacinth : 25 water lettuce, KII= combination of 50 water hyacinth : 50 water lettuce, KIII=combination of 25 water hyacinth : 75 water lettuce.

The initial and final pH values in tofu liquid waste have increased significantly (Figure 1). Before diluting, tofu liquid waste has a very acidic initial pH in the range of 3 – 4, so before being given phytoremediation treatment, tofu liquid waste needs to be diluted using distilled water so that the phytoremediation plants can remain alive. The dilution concentration used is 25%. After dilution, the pH of the tofu liquid waste increased but still tended to be acidic, namely around 5.7 – 5.8. Then, phytoremediation will be carried out using a combination of water hyacinth and water lettuce for seven days. After phytoremediation, the pH value of tofu liquid waste increased significantly, even approaching normal pH, namely 6.7 – 6.9. The results of the one-way ANOVA test on the pH parameter show a significance value of 0.000, which means the significance value is less than 0.05, thus providing a significant influence. Therefore, there is an influence of the combination of biomass between water hyacinth and water lettuce improves the quality of tofu liquid waste on the pH parameter. The final pH value after phytoremediation follows the quality standard limits regulated in the Minister of Environment Regulation Number 5 of 2014, namely in the range of 6 – 9.

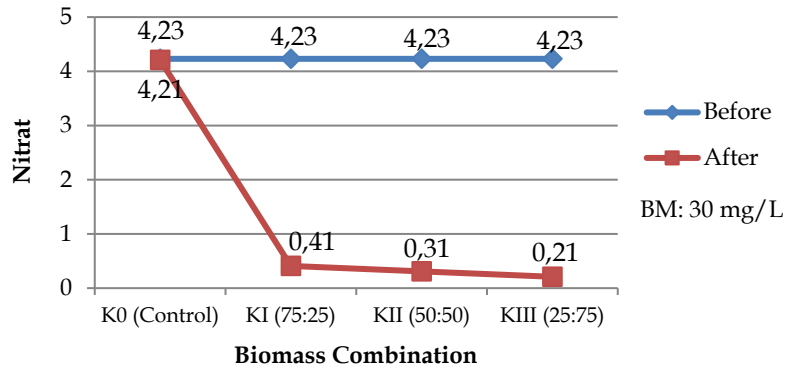

Figure 2. Nitrate value before and after phytoremediation using a combination biomass of water hyacinth (*Eichhornia crassipes (Mart.) Solms*) and water lettuce (*Pistia stratiotes Linnaeus*). Note: K0=control, KI=combination

of 75 water hyacinth : 25 water lettuce, KII= combination of 50 water hyacinth : 50 water lettuce, KIII=combination of 25 water hyacinth : 75 water lettuce.

The Initial and final nitrate levels in tofu liquid waste experienced a significant decrease, whereas tofu liquid waste before phytoremediation had an initial nitrate value of 4.23 mg/L (Figure 2). Meanwhile, the Nitrate value of tofu liquid waste after phytoremediation decreased, namely around 0.4 – 0.2 mg/L. Based on the results of the one-way ANOVA test on the Nitrate parameter, it shows a significance value of 0.000, which means the significance value is less than 0.05, thus providing a significant influence. Therefore, the biomass combination between water hyacinth and water lettuce influences the quality of tofu liquid waste in the Nitrate parameter. After phytoremediation, the final nitrate value follows the quality standard limit regulated in the Minister of Environment Regulation Number 5 of 2014, namely 30 mg/L.

The effectiveness of the combination of water hyacinth plant biomass and water lettuce in improving the quality of tofu liquid waste is a situation that shows the level of success of the combination of plants in improving the quality of tofu liquid waste. In this research, water hyacinth and water lettuce were combined with various ratios of different biomass combinations, namely the 75:25 combination obtained from 225 grams of water hyacinth and 75 grams of water lettuce, the 50:50 combination obtained from 150 grams of water hyacinth and 150 grams of water lettuce, a combination of 25:75 is obtained from 75 grams of water hyacinth and 225 grams of water lettuce. After previously carrying out the one-way ANOVA Test, the Duncan test was carried out to determine the effectiveness or best biomass combination treatment in improving the quality of tofu liquid waste. The following are the results of the Duncan Test for each plant combination treatment.

Table 1. pH and nitrate values (NO_{3-}) on tofu liquid waste with phytoremediation using a combination biomass of water hyacinth (*Eichhornia crassipes (Mart.) Solms*) and water lettuce (*Pistia stratiotes Linnaeus*).

Treatment	pH	Nirate (NO_{3-})
K0 (Control)	5.8 ± 0.029^{d}	4.21 ± 0.01^{d}
KI (75:25)	6.7 ± 0.033^{c}	0.41 ± 0.01^{c}
KII (50:50)	6.8 ± 0.028^{b}	0.31 ± 0.01^{b}
KIII (25:75)	6.9 ± 0.015^{a}	0.21 ± 0.01^{a}

Note: According to the Duncan Test, the numbers given notation different (a, b, c, d) shows different influences real between mark One with mark others at the 0.05 level.

The results of the Duncan Test are in Table 1 shows that the most effective treatment for improving the quality of tofu liquid waste in terms of pH and Nitrate parameters from the lowest value to the highest value, respectively, is KI (25:75), KII (50:50), KI (75:25) and K0 (Control). The most effective combination for improving the quality of tofu liquid waste is a combination of 25:75 with a weight of 75 grams of water hyacinth plants and 225 grams of water lettuce. The KIII combination has a pH value closest to neutral, namely 6.9, and has the lowest nitrate value namely 0.21 mg/L.

DISCUSSION

Based on the results of the data that has been obtained, it shows that the combined treatment of biomass between Water Hyacinth and water lettuce influences improving the quality of tofu liquid waste on the parameters pH, BOD and Nitrate (NO_{3-}). However, the temperature parameters did not show any influence of the biomass combination on the temperature of the tofu liquid waste. The most effective biomass combination in improving the quality of tofu liquid waste from the highest to the lowest value, respectively, is KI (25:75), KII (50:50), KI (75:25), and K0 (Control). The most effective combination for improving the quality of tofu liquid waste is a combination of 25:75 with a weight of 75 grams of water hyacinth and 225 grams of water lettuce. Improving the quality of tofu liquid waste by water hyacinth and water lettuce can occur because the two plants synergize with each other in improving the quality of tofu liquid waste by absorbing toxic substances in tofu liquid waste so that maximizing the improvement in the quality of tofu liquid waste. Two types of plants combined to carry out phytoremediation have their respective roles and synergize with each other in absorbing and reducing pollutants (Alivia *et al.*, 2024).

Water hyacinth and water lettuce are known as hyperaccumulator plants that can accumulate contaminants in liquid waste. These two types of aquatic plants were combined to have an advantage in improving the quality of tofu liquid waste through phytoremediation techniques. Of all the

combinations, namely KI (75:25), KII (50:50), and KIII (25:75), all three were able to improve the quality of tofu liquid waste to the maximum. However, the best combination is the KIII combination (25:75), obtained from 75 grams of water hyacinth and 225 grams of water lettuce. This combination has much more water lettuce biomass than water hyacinth biomass, so it can influence the effectiveness of improving the quality of tofu liquid waste. This is because the two plants have different fibrous root bundle morphology and phytoremediation mechanisms. Water hyacinth plants have fibrous roots that retain particles and organic materials in waste (Widiyanti et al., 2020). Meanwhile, water lettuce has fibrous roots with dense root hairs, making it easier for this plant to accommodate and absorb contaminants that pollute waters (Nizam *et al.,* 2020).

The tofu liquid waste in this study was placed in a 5-liter clear container so the waste only pooled and did not flow. As a result, organic contaminants were deposited at the bottom of the container. Water lettuce has bundles of roots that are long and branched so they can reach the bottom of the container, making it more effective in absorbing contaminants that settle at the bottom of the container. The water lettuce can grip sediment with its root bundles and absorb excess nutrients that cause pollution (Riyanto, 2023). Water lettuce directly absorbs toxic substances in tofu liquid waste through its roots using the rhizofiltration working mechanism through absorption, concentration, and deposition of contaminants in plant roots (Muhafaroh, 2023). Meanwhile, the water hyacinth plant has short root bundles, unbranched and dense fibrous roots so that the water hyacinth roots can only reach the top surface. Water hyacinth directly absorbs toxic substances in tofu liquid waste through its roots using a phytoextraction mechanism where the roots of the water hyacinth absorb and accumulate pollutants in other plant parts such as roots, stems, and leaves (Chou & Wipranata, 2021).

The degree of acidity is one of the parameters influencing the quality of tofu liquid waste. In this test, pH measurements were carried out using a pH meter at the beginning (day 0) and end (day 7). Before being given phytoremediation treatment, tofu liquid waste will be diluted with a concentration of 25% to neutralize the pH of tofu liquid waste so that aquatic plants can live in it. Processing by diluting liquid tofu waste using water can help to neutralize the pH (Vidyawati & Fitrihidajati, 2019). According to the research results, after the tofu liquid waste was diluted, the pH value remained acidic, namely 5.8. This is because the process of making tofu uses a coagulating agent in the form of vinegar, which then dissolves in the tofu liquid waste (Murdianto et al., 2023). Some living creatures in water are sensitive to changes in pH, and they tend to like neutral pH conditions ranging from 7.0 – 8.5 (Setiawan & Simon, 2021). Graph 1 shows that the average degree of acidity of the water after being given phytoremediation treatment with a combination of water hyacinth and water lettuce biomass has a pH value of 6.7 – 6.9, which tends towards neutral. The highest pH level on day 7 of treatment occurred in the combination of KIII plant biomass (25:75) with a pH of 6.9. All averages in the phytoremediation treatment show that the pH value does not exceed the quality standard limits regulated in the Minister of Environment Regulation Number 5 of 2014, ranging from 6 – 9. pH 6–9 is most suitable for the performance of aquatic plants in wastewater phytoremediation (Mustafa & Hayder, 2021). Changes in pH are caused by biochemical processes carried out by microorganisms in tofu liquid waste and the roots of water hyacinth and water lettuce plants. Plants need carbon dioxide in photosynthesis, so the carbon dioxide content in waste will decrease and cause the acidity value to increase (Fahmi *et al.,* 2020). pH is influenced by the amount of carbon dioxide (CO_2) in the water. pH conditions are related to carbon dioxide (CO_2) and alkalinity. Alkalinity is the concentration of bases or substances that can neutralize acidity. The higher the pH value, the higher the alkalinity value and the lower the carbon dioxide content (Mariah, 2021).

Nitrate (NO_{3-}) is a form of nitrogen dissolved in water that is easily absorbed directly by plants and is an essential nutrient needed for plant growth. In this study, nitrate (NO_{3-}) testing was carried out at the beginning (day 0) and end (day 7) at the Sidoarjo Environmental Service Laboratory UPTD using the Hanna Instrument method. Graph 2 shows that the phytoremediation process using a biomass combination of water hyacinth and water lettuce can reduce Nitrate (NO_{3-}) levels in tofu liquid waste. The average nitrate (NO_{3-}) after being treated with phytoremediation with a biomass combination of water hyacinth and water lettuce was 0.2 – 0.4 mg/L. The lowest Nitrate (NO3-) levels occurred on the seventh day of treatment in the combination of plant biomass KIII (25:75), namely 0.21 mg/L. The highest nitrate levels occurred in the control treatment on the seventh day of treatment, namely without plants. All average Nitrate (NO_{3-}) levels in the phytoremediation and control treatments were far below the quality standard limit regulated by Minister of the Environment Regulation Number 5 of 2014, namely 20 mg/L. This is because the tofu liquid waste was previously diluted in the control and combination treatments with a dilution concentration of 25%. Tofu liquid

waste processed using dilution techniques can help reduce the concentration of pollutants in the waste (Vidyawati & Fitrihidajati, 2019). Dilution with a concentration of 25% can also reduce Nitrate (NO_{3-}) levels in tofu wastewater. However, treatment with phytoremediators shows that phytoremediation can reduce Nitrate (NO_{3-}) levels better than without phytoremediators. Tofu liquid waste usually contains organic N compounds such as ammonia, nitrite, and nitrate (Piu *et al.,* 2023). In the nitrification process, ammonia and nitrite compounds are converted by microorganisms into nitrate compounds, which can only be absorbed directly by aquatic plants as nutrients to meet their growth needs (Fitrihidajati *et al.,* 2018).

Based on this explanation, the phytoremediation process using water hyacinth and water lettuce plants has been proven to improve the quality of tofu liquid waste because there is a symbiosis between the plants and the microbes in the roots. Each aquatic plant has a population of microbes of various types and each acts individually or forms a biofilm that attaches to the substrata or plant (Chatterjee *et al.,* 2019). Plant roots become a living medium for microbes to attach. The microbes will utilize oxygen from plant photosynthesis to break down organic materials in waste so that these simple compounds can be absorbed by plants as growth nutrients (Wijayanti & Purnomo, 2021). Tofu liquid waste initially has a very acidic pH value, so it has a high CO_2 content. That will benefit phytoremediator plants because water hyacinth and water lettuce plants will absorb CO_2 levels in the water during the photosynthesis process, so it can decrease carbon dioxide content in the water, causing the pH value of the water to increase. The degradation of organic substances in tofu liquid waste will produce CO_2 and H_2O. Some of the CO_2 will be released into the air while the other part will be retained in the water and dissolved into H_2CO_3 (carbonic acid). Carbonic acid dissociates into bicarbonate (HCO_{3-}) and then is absorbed by plants so that it can increase the degree of acidity (H^+) in the water (Santosa *et al.,* 2023). From the photosynthesis process, plants will produce oxygen which is used by microorganisms to break down organic substances in liquid tofu waste. The favorable pH conditions between 6.5 and 7.7 are suitable for microbial activity in degrading organic matter and nitrification (Kodituwakku & Yatawara, 2020). Microorganisms will conduct a nitrification process where ammonia and nitrite compounds will be converted into nitrate compounds and absorbed directly by water hyacinth and water lettuce plants as nutrients to meet their growth needs. Nitrification consists of two stages: the oxidation of Ammonium compounds to Nitrite by Nitromonas bacteria and the oxidation of Nitrite compounds to Nitrate by Nitrobacter bacteria (Islam *et al.,* 2021).

CONCLUSION

The effect of the combination of water hyacinth and water lettuce on phytoremediation techniques to improve the quality of tofu liquid waste in terms of pH and nitrate parameters shows that the three types of combination can enhance the quality of tofu liquid waste with a pH parameter of 6. 7 – 6.9 and Nitrate 0.2 – 0.4 ppm. All parameters have met the quality standards stipulated in the Minister of Environment Regulation Number 5 of 2014. The most practical combination of water hyacinth and water lettuce for improving the quality of tofu liquid waste in terms of pH and Nitrate parameters was a combination of 25:75 obtained with a ratio of 75 grams of water hyacinth plants and 225 grams of water lettuce plants.

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CONFLICT OF INTEREST

There is no conflict of interest

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