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Phitoremidiation of Combination *Pistia Stratiotes* and *Eichhornia Crassipes* Towards Changes in Waste Liquid Waste Changes and Plant Morphological Structure

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Abstract

Tofu liquid waste tofu contains a low pH of 3 (acidic), because in the manufacturing process there is the addition of a prickly heat, it will cause pollution. One of the efforts to minimize pollution is by doing phytoremediation by combining *Pistia stratiotes* and *Eichornia crassipes*. The purpose of this study was to determine the effect of phytoremediation *Pistia stratiotes* and *Eichornia crassipes* on changes in pH and plant morphology. The method used was an experiment with four treatments and three replications by combining 0 g (control) biomass (P0), 75 g *Pistia stratiotes* and 25 g *Eichhornia crassipes* (P1), 50 g *Pistia stratiotes* and 50 g *Eichhornia crassipes* (P2), and 25 g of *Pistia stratiotes* and 75 g of *Eichhornia crassipes* (P3) which will be phytoremediated into four liters of waste with a concentration of 25%. The experimental data were processed by analysis of variance at the 5% level. The results showed that the four treatments were able to increase the pH value of tofu liquid waste for P1 with an average of 6.0 while P0, P2 and P3 had an average pH of 5.7. The morphological structure that showed the best results was P3 with green leaves and stems.

Keywords: Phytoremediation; Tofu Liquid Waste; pH; Morphological Structure.

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Introduction

The process of making tofu produces solid waste and liquid waste. Solid waste from the tofu industry is usually reprocessed into animal feed or tempe gembus. Meanwhile, tofu liquid waste has not been reused so it has the potential to pollute the environment. Based on the results of observations, the process of making tofu in the Tandang area of Semarang produces tofu liquid waste of ± 1,527 liters/day which can pollute the environment. Tofu liquid waste is produced from the process of making tofu. The process of making tofu goes through several stages ranging from soaking, grinding, boiling, filtering, clumping, tofu printing and pressing which will produce a large volume of waste. Most of the tofu industrial waste is in the form of liquid waste. The liquid waste is in the form of tofu milk waste that does not clot into tofu. The waste product contains high complex organic materials, especially protein and amino acids because the basic ingredient of tofu is soybeans. Other organic content is carbohydrates, protein and fat. Another characteristic of tofu liquid waste is that it is cloudy and thick white in color. The cloudiness is due to the presence of suspended substances in it which are insoluble in water.

Based on the preliminary test, the liquid waste of the tofu industry in the Tandang area of Semarang has a pH of 2.6. The content exceeds the industrial waste quality standard stipulated by the Regulation of the Minister of the Environment of the Republic of Indonesia Number 5 of 2014 concerning the pH quality standard for wastewater, which is 6-9. The results of the observation of low pH in the waste are due to the tofu clumping process there is an addition of a prickly heat. The culprit or stinging is the remaining liquid after the protein deposition stage or the remaining liquid from separating the tofu lumps that have been left for one night. The low degree of acidity (pH) can cause disruption of biotic in the aquatic environment.

Efforts to overcome these waste problems, so that they are safely disposed of into the environment, a liquid waste treatment is needed. One way to overcome these problems is with biological agents by utilizing aquatic plants or what is called phytoremediation. Phytoremediation is a natural way to remove contaminants in wastewater using plants. This process utilizes metabolic processes to remove nutrients and contaminants from wastewater and store them in biomass. The ideal plant for phytoremediation requires a large root system, with these roots the plant is able to interact with contaminated wastewater (Wirandani, 2016). Phytoremediation has advantages compared to other processes, namely cheap in terms of cost, easier operation and maintenance and has a fairly high efficiency, can remove pollutants and support ecological functions (Sungkowo *et al*, 2015).

Based on the results of research by Novita *et al* (2019), *Pistia stratiotes* was not optimal in increasing the pH, namely the initial pH was 4.5 and neutralization was carried out using NaOH so that the pH value was 7.0 and at the end of the treatment it was 4.5. Therefore, the combination of *Pistia stratiotes* and *Eichhornia crassipes* is expected to increase the pH of tofu wastewater. Both plants have potential as phytoremediators. *Pistia stratiotes* commonly found in water or ponds are used as aquarium protection plants. While *Eichhornia crassipes* is considered a weed because of its fast growing so that it covers the waters. Both plants have many and dense root systems. Many root systems can be used in the process of absorbing organic matter contained in wastewater.

This study aims to determine the morphological structure of plants and changes in the pH of tofu wastewater on phytoremediation with *Pistia stratiotes* and *Eichornia crassipes*. In addition, the research results obtained can be used for the management of liquid waste in the tofu industry, so that environmental pollution can be minimized.

Method

Sample or Participant

The research subjects were kiapu plant (*Pistia stratiotes*), water hyacinth plant (*Eichhornia crassipes*) and tofu liquid waste.

Instrument

pH measurement and observation of the morphological structure of plants are carried out during the phytoremidiation process , for 7 days. pH measurement is carried out using a pH meter, while the morphological structure is observed directly on the leaves, stems and roots. Data on pH measurement and observation of morphological structure are filled in the observation table.

Data collection

The research was conducted at the Biology Education Laboratory, Universitas PGRI Semarang in August- November 2021.

Procedure

The experimental design was prepared with variations in biomass of *Pistia stratiotes* and *Eichhornia crassipes*, carried out four treatments with three replications. This results in 12 experimental units. According to Fachrurozi *et al* (2010), the use of plant weight is 50 grams and a dilution of tofu liquid waste is 25% (Vidyawati *et al*, 2019). The dilution of tofu liquid waste is 1 liter of tofu liquid waste added 3 liters of water, so a total of 4 liters (Natalina *et al*, 2013). The study used four treatments, namely with a total biomass of 0 g (control) (P0), 75 g *Pistia stratiotes* and 25 g *Eichhornia crassipes* (P1), 50 g *Pistia stratiotes* and 50 g *Eichhornia crassipes* (P2), and 25 g *Pistia stratiotes* and 75 gr *Eichhornia crassipes* (P3).

Data Analysis

Data analysis used analysis of variance to see the effect of treatment on the pH of tofu liquid waste at the 5% level.

Result

Effect of Phytoremediation of *Pistia stratiotes* and *Eichhornia crassipes* on the pH of Tofu Liquid Waste

The effect of the combination of *Pistia stratiotes* and *Eichhornia crassipes* on changes in the pH of tofu wastewater (Figure 1).

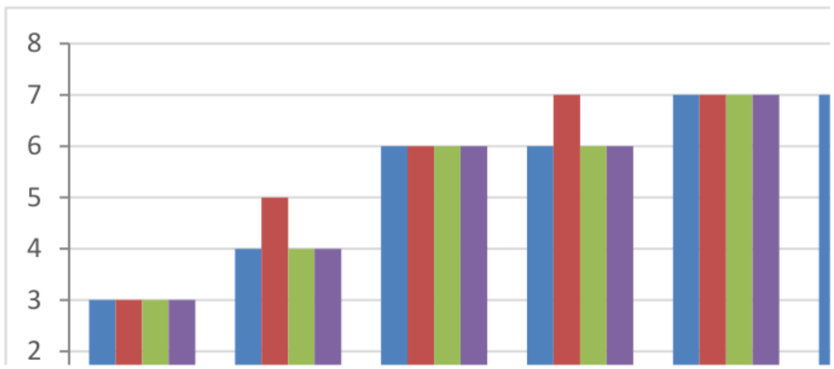

Figure 1. Measuring the pH of Tofu Liquid Waste

Table 1. Analysis of pH Measurement of Tofu Liquid Waste

Treatment	Mean	Std. Deviation	N	F	Sig
P0	5,714	1,6036	7		
P1	6,000	1,5275	7		
P2	5,714	1,6036	7	0,057	0,982
P3	5,714	1,6036	7		
Total	**5,786**	**1,4996**	**28**		

Effect of Phytoremediation of *Pistia stratiotes* and *Eichhornia crassipes* Plant Morphological Structure

Based on the results of plant morphology observations, it can be seen that there are morphological changes of *Pistia stratiotes* and *Eichornis crassipes* (Figure 3).

P0 Day-1 P0 Day-7 P1 Day-1 P1 Day-7

P2 Day-1 P2 Day-7 P3 Day-1 P3 Day-7

Figure 2. Morphological structure of *Pistia stratiotes* and *Eichhornia crassipes*

Table 2. Morphological structure of *Pistia stratiotes*

Morphological Structure	***Treatment***	**Day**						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Leaf	**P1**	*Green*	*Green, rolled edge*	*Yellow, central green*	*Yellow, dry, central green*	*Yellow, dry, central green*	*Yellow, dry, central green*	*Yellow, dry, central green*
	P2	*Green*	*Green , rolling edge*	*Yellow, central green*	*Yellow, dry, central green*	*Yellow, dry, central green*	*Yellow, dry, central green*	*Yellow, dry, central green*
	P3	*Green*	*Green, rolling edge*	*Yellow, central green*	*Yellow, dry, central green*	*Yellow, dry , central green*	*Yellow, dry, central green*	*Yellow, dry, central green*
Root	**P1**	*Fresh*	*white White fresh*	*Dirty*	*Dirty, falling off*	*Dirty, falling off*	*Dirty, falling off*	*Dirty, falling off*
	P2	*fresh*	*Fresh*	*Dirty*	*Dirty, falling off*	*Dirty, falling off*	*Dirty, falling off*	*Dirty , drop*
	P3	*Fresh*	*white Fresh white*	*Dirty*	*Dirty,*	*dirty, dirty,*	*dirty, dirty,*	*dirty*

Table 3. Morphological structure of *Eichhornia crassipes*

Morphological structure	*Treatment*	**Day**						
		1	**2**	**3**	**4**	**5**	**6**	**7**
Leaf	**P1**	*Green*	*Green, edges curled*	*Yellowed, edges curled*	*Yellowed, dry, curled*	*Green, dry edges*	*Green, edges dry*	*Dry edge green*
	P2	*Green*	*Green, edge curled*	*Yellowed, edge curled*	*Yellowed, dry, curled*	*Dry*	*Dry*	*Dry*
	P3	*Green*	*Green, edge curled*	*Yellowed, edge curled*	*Yellowed, dry, curled*	*Green, some dry*	*Green, dry edge*	*Green*
Bar	**P1**	*Green*	*Green*	*Green*	*Yellowing*	*Green Yellowing*	*Green Yellowing*	*Green*
	P2	*Green*	*Green*	*Green*	*Yellowing*	*Green Yellowing*	*Green Yellowing*	*Green*
	P3	*Green*	*Green*	*Green*	*Yellowing*	*Green Yellowing*	*Green Yellowing*	*Green Yellowing*
Roots	**P1**	*Fresh*	*white Fresh white*	*Dirty*	*Dirty, fall off*	*Dirty, fall out*	*Dirty, fall off*	*Dirty, fall*
	P2	*Fresh*	*white Fresh white*	*Dirty*	*Dirty, fall out*	*Dirty, fall out*	*Dirty, fall out*	*Dirty, fall out*

P3	*Fresh*	*white Fresh white*	*Dirty*	*Dirty, fall out*	*Dirty, fall out*	*Dirty, fall out*	*Dirty, fall out*

Discussion

Effect of Phytoremediation of Pistia stratiotes and Eichhornia crassipes on the pH of Tofu Liquid Waste

The degree of acidity (pH) is the level in expressing the degree of acidity in water (Novita *et al* 2019). The effect of the combination of *Pistia stratiotes* and *Eichhornia crassipes* on changes in the pH of tofu wastewater (Figure 1). The combination *Pistia stratiotes* and *Eichhornia crassipes* has the ability to increase the pH of tofu wastewater.

Based on the research results, the pH of the waste ranges from 3-7. During the observation that the pH increased every day, the initial pH of the effluent was 3 and on the last day of observation the pH of the effluent became 7. In the P1 treatment the pH value tended to rise faster than the P2 and P3 treatments. The average value of the highest pH measurement results was found in treatment P1 of 6.0 compared to the average results of P2 and P3 of 5.7. The calculated F value is 0.057 with sig = 0.982. Because the sig value is > 0.05, it can be interpreted that there is no difference in the average increase in pH with the combination of *Pistia stratiotes* and *Eichhornia crassipes* in tofu liquid waste (Table 1). The value of the waste category is in the safe category, because it is in accordance with the Regulation of the Minister of the Environment of the Republic of Indonesia Number 5 of 2014 concerning the quality standard of tofu industrial wastewater with a pH value of 6-9.

The increase in pH that occurred after phytoremediation treatment by combining *Pistia stratiotes* and *Eichhornia crassipes* for 7 days with a waste concentration of 2[illegible] the quality would be better and safer if discharged into the waters. In treatment P0, P1, P2, and P3 all experienced an increase in pH. The increase in pH is caused by the process of photosynthesis, and the breakdown of organic nitrogen (Fitriyah, 2011 in Haryati *et al*, 2012).

In the P0 treatment, it was seen that the pH increased the same as the P1, P2 and P3 treatments. This is due to the high content of organic matter in the waste which is degraded by microorganisms. Microorganisms obtain energy through the oxidation of organic compounds with the main product CO_2 being released back into nature, and a carbon source for the synthesis of new cells (Simanungkalit *et al* 2006). These organic materials include carbohydrates, proteins and fats contained in tofu liquid waste (Ratnani, 2012). Microorganisms cannot directly metabolize insoluble organic matter. Microorganisms will produce extracellular enzymes for depolymerization of large compounds into smaller and water-soluble (substrate for microbes) (Simanungkalit *et al*, 2006). Microbes transfer these substrates to cells through the cytoplasmic membrane for the decomposition of organic matter. The organic matter is decomposed by microorganisms aerobically, it can be seen in the following reaction equation (1) (Ratnani, 2012):

$$\text{CHONS (Organic material)} + O_2 \longrightarrow CO_2 + H_2O + NH_3 + C_5H_7NO_2 + H_2S$$
$$\text{CHONS (Organic material)} + H_2 \longrightarrow \text{fatty acids + protein + carbohydrates}$$

The results of the decomposition will be used by microorganisms in the body's metabolic processes. The decomposition process is also known as microbial respiration (Simanungkalit *et al* 2006). The results of the decomposition of organic matter glucose, fatty acids and glycerol are used by microorganisms for the process of cell reproduction and multiplication, the final products are CO_2 and H_2O. The results of the decomposition of amino acids that have an NH_3 group are converted into urea and excreted by the body. Urea will react with water to produce NH_3 and CO_2 (Figure 2). Ammonia which has alkaline properties can increase the pH of wastewater (Yuni *et al*., 2014). It can be seen that the reaction equation (2) is as follows:

CH_4N_2O (urea) + H_2O $\longrightarrow$ $2NH_3$ (ammonia) + CO_2 (2)

Therefore, P0 experienced an increase in pH due to microorganisms that help the decomposition process of organic matter in tofu liquid waste (Vidyawati *et al*, 2019). The degradation products are used by microorganisms, not for plant life processes (Rahadian *et al*, 2017). Treatment P1, P2 and P3 the average value of the pH value becomes large. This is due to the process of decomposition of organic matter by microorganisms, absorption of organic matter by roots, the process of photosynthesis, and the supply of O_2 resulting from photosynthesis to the roots and the O_2 is used by microorganisms to decompose organic matter. The decomposition products are CO_2, H_2O , and NH_3. Ammonia (NH_3) will bind to O_2 and undergo nitrification. It can be seen that the reaction equation (3) is as follows (Vidyawati *et al*, 2019):

$$2NH_3^+ + 3O_2 \longrightarrow 2NO_2 \text{ (nitrite)} + 4H + 2H_2O$$

$$2H_2O + O_2 \longrightarrow 2NO_3 \text{ (nitrate)} + \text{energy}$$

Plants that require nitrogen for their metabolic processes. Plants absorb nitrogen in the form of NH_3 and NO_3. Nitrogen supply that is able to accelerate the process of vegetative planting of plants both on branches, stems and leaves (Damayanti *et al*, 2018). Root absorption by simpler compounds occurs in several ways, including diffusion through the solution in the media, passively carried by water in mass flow to the roots, and roots that grow close to these elements (Salisbury *et al*., 1995). The absorption of NO_3 is too fast, causing the pH of the tofu liquid waste to be large, because the absorption of NO_3 accompanied by the absorption of H^+ or the release of OH^- to maintain the charge balance (Salisbury *et al*., 1995).

According to Effendi (2003) in (Rahadian *et al*, 2017) the increase in the pH of tofu liquid waste is caused by the photosynthesis process carried out by plants. The photosynthesis process will produce CO_2 at night during respiration (Salisbury *et al*, 1995) and release OH- ions$^-$ into the water Photosynthesis will take H^+ in wastewater. Photosynthesis converts CO_2 to $C_6H_{12}O_6$ in the chloroplast which requires H_2 and energy. Hydrogen is obtained from H^+ obtained from wastewater and air. So that taking H^+ and releasing OH^- will cause the pH of the tofu liquid waste to be neutral (Widya *et al al*, 2015). It can be seen that the reaction equation (4) is as follows:

$$OH^- + H^+ \longrightarrow H_2O$$

The process of photosynthesis is possible due to the release of O_2 rhizosphere in the root zone (*zone*). Zone *rhizosphere* which is rich in O_2, causes the development of aerobic microorganisms in decomposing organic compounds better. These organic compounds are decomposed into other simpler compounds which are then absorbed by plants for metabolic processes, especially photosynthesis and can increase the pH of liquid waste (Sungkowo *et al*, 2015).

The difference in biomass between P1, P2 and P3 affects the increase in pH. Treatment P1 with biomass of *Pistia stratiotes* and *Eichhornia crassipes* 75 gr and 25 gr. This happens because *Pistia stratiotes* absorbs H^+ to quickly, leaving OH ions$^-$ which causes the pH of the tofu wastewater to be neutral (Rohmani, 2014). In addition, in the study of Sari *et al* (2014) *Pistia stratiotes* has the ability to absorb organic materials that contain the greatest nutrients at the beginning of the study. According to Vidyawati *et al* (2019), aquatic biota likes a pH value of 7-8.5 and is sensitive to changes in pH. Aquatic plants will die if the pH value is <4 this is because the plants are not able to tolerate low pH.

Effect of Phytoremediation of Pistia stratiotes and Eichhornia crassipes Plant Morphological Structure

The morphological structure of plants is the shape and arrangement of the plant body (Tjitrosoepomo, 1985). Polluted aquatic plants will show adaptability and can be seen from their anatomical, morphological and physiological structures (Munawwaro *et al*, 2018). Based on the results of plant morphology observations, it can be seen that there are morphological changes of *Pistia stratiotes* and *Eichornis crassipes* (Figure 3).

Tofu liquid waste causes damage and changes in plant physiology which are expressed in crop disturbances. According to Fontes (1995) in (Hasyim, 2016) pollution causes changes at the biochemical level of cells, followed by physiological changes at the individual level to the plant community level.

condition *Pistia stratiotes* at the beginning The research is fresh green, the roots are long and white and free-floating, while the condition of *Eichornia crassipes* is early In the study, the plants were fresh green, oval-shaped leaves, erect stems, and brownish-white roots. Plants that get nitrogen usually have green leaves (Salisbury *et al*, 1995). Good roots (long and dense roots shaped like threads, lots of root hairs) will be able to absorb nutrients well (Ni'ma *et al,* 2014). After the research, there were changes in the morphological structure, namely the leaves turned yellow, and the roots lost. Other conditions, some plants are still alive and some are damaged and rotting at the bottom of the research bucket.

Day 1, treatments P1, P2 and P3 showed green leaves. After some time, the leaves of *Pistia stratiotes* began to turn pale white and turned grayish white, while at the tips of the leaves of *Eichornia crassipes* began to curl, this indicates that the plant is adapting to the new environment (Table 2 and Table 3).

On the 2nd day of the study, the treatments P1, P2 and P3 were almost the same, namely *Pistia stratiotes* and *Eichornnia crassipes* experienced changes in leaves that were getting wilted and leaf edges curled. This indicates that the two plants are adapting due to an acidic environmental stress. An acidic environment makes it difficult for plants to obtain other ions needed for their metabolic processes. In addition, on day 2, it is possible that the process of overhauling organic matter carried out by microorganisms has not been optimal, so that the product from the reshuffle is not yet available optimally (Table 2 and Table 3).

On the 3rd, 4th, 5th, 6th, and 7th days of the study, in treatments P1, P2 and P3 the leaves of *Pistia stratiotes* were yellow, dry and the central part of the plant was green, while the leaves of *Eichornia crassipes turned* yellow, dry and the edges of the leaves curled. Research days 3 and 4 liquid waste pH 6 (Table 2 and Table 3). This makes NH_3 still in small amounts. Another thing is that plants absorb NH_3 only to grow and will show symptoms of deficiency, experiencing chlorosis in old leaves. Chlorosis is a condition of plant tissue in leaves that are damaged or fail to form chlorophyll (Nurfitriana, 2019). This causes the photosynthesis process carried out by plants to be disrupted, due to the less chlorophyll content. Worse yet, the leaves are yellow and slightly brown when they die. Usually, the leaves fall in the yellow or brownish yellow phase. Yellow and dying leaves are unable to photosynthesize because of the destruction of chlorophyll and loss of chloroplast function (Salisbury *et al*., 1995). The central part of the plant remains green because the central leaf is a young leaf. Young leaves stay green longer because young leaves get soluble nitrogen from older leaves (Salisbury *et al*, 1995). Whereas on days 5, 6 and 7 the pH of the wastewater has reached 7, anion ions and cation ions are already available for the metabolic processes of the plant body, causing the leaves on both plants to appear springy and green again (Table 2 and Table 3).

The morphological structure of the roots of the two plants had dirty and fallen characteristics. These gross changes are due to suspended substances adhering to plant roots. The attachment of these suspended substances is due to the attractive force of water molecules present in plants. After the attractive force continues, the absorption process continues. According to Hardyanti (2007) in Ibrahim (2017) the process of absorption of substances contained in waste is carried out by the ends of the roots with meristem tissue. The substances that have been absorbed by the roots will enter the stem through transport vessels (xylem), which will then be passed on to the leaves. This is supported by Yusuf (2001) in Ibrahim (2017) that the process of moving ions towards the xylem vessels when transporting through the cell wall from the epidermis to the endodermis, some of the ions will be absorbed by the cells in its path.

According to Fitter and Hay (1991) in Paramitasari (2014) there are two mechanisms carried out by plants in dealing with toxic concentrations, namely amelioration and tolerance. Amelioration is done by localization approach in the root, excretion actively through the crown gland or passively through accumulation in old leaves and then leaf abscission occurs. Tolerance is carried out by plants by developing metabolic systems that function at certain concentrations. The mechanism of changes in root loss is carried out by plants due to the toxicity response of acidic pH. The results that showed the best morphological structural conditions were found in the P3 treatment, namely the green leaves. This is because the most dominant biomass is *Eichornia crassipes*. *Eichornia crassipes* has a body structure that supports the absorption of organic matter by having large stomata holes, which are twice as large as most other plants (Indah *et al* ., 2014).namely releasing O2$_{CO2}$ and absorbing., chloroplast smooth absorption of CO2 will be used in the process of photosynthesis in the chloroplasts have chlorophyll that keeps the leaves of *Eichornia crassipes* green.

Conclusions

Based on the research it can be concluded:

1. All treatments were able to increase the pH value of tofu liquid waste. The highest average increase was in treatment P1 with a pH value of 6.0, while for treatment P0, P2 and P3 had the same average pH value of 5.7.
2. Treatments P1, P2 and P3 experienced changes in morphological structure. Treatment P3 remained fresh green leaves and stems compared to treatments P1 and P2. Treatments P1 and P2 experienced a change in the color of the leaves and stems to yellow and withered.

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