Phytoremediation of Combination Pistia Stratiotes and Eichhornia crassipestowards Changes in Waste Liquid Waste Changes and Plant Morphological Structure

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Phytoremediation of Combination *Pistia Stratiotes* and *Eichhornia crassipes* towards Changes in Waste Liquid Waste Changes and Plant Morphological Structure

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Abstract

Background: Tofu liquid waste tofu contains a low pH of 3 (acidic) because, in manufacturing, adding prickly heat will cause pollution. One of the efforts to minimize pollution is phytoremediation by combining Pistia stratiotes and *Eichornia crassipes*. This study aimed to determine the effect of phytoremediation of Pistia stratiotes and *Eichornia crassipes* on changes in pH and plant morphology. **Methods:** 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 phytoremediation into four liters of waste with a concentration of 25%. The experimental data were processed by analysis of variance at the 5% level. **Results:** The results showed that the four treatments increased 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. **Conclusions:** 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. Making tofu goes through several stages, from soaking, grinding, boiling, filtering, clumping, pressing, and pressing, producing 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 highly complex organic materials, especially protein and amino acids because soybeans are the essential ingredient of tofu. Other organic content is carbohydrates, protein, and fat. Another characteristic of tofu liquid waste is its cloudy and thick white color. The cloudiness is due to suspended substances that 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; prickly heat is added. The culprit or stinging is the remaining liquid after the protein deposition stage or the remaining liquid from separating the tofu lumps left for one night. The low degree of acidity (pH) can disrupt biotics in the aquatic environment.

Liquid waste treatment is needed to overcome these waste problems and safely dispose of them in the environment. One way to overcome these problems is with biological agents by utilizing aquatic plants or phytoremediation. Phytoremediation is a natural way to remove contaminants in wastewater using plants. This process utilizes metabolic processes to remove nutrients and pollutants from sewage and store them in biomass. The ideal plant for phytoremediation requires an extensive root system; with these roots, the plant can interact with contaminated wastewater (Wirandani, 2016). Phytoremediation has advantages compared to other processes. Namely cheap, more straightforward operation and maintenance, reasonably high efficiency, and 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. At the same time, Eichhornia crassipes is considered a weed because of its fast-growing so that it covers the waters. Both plants have many dense root systems. Many root systems can be used to absorb 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 to manage liquid waste in the tofu industry to minimize environmental pollution.

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 phytoremediation process for seven 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. (2014), the use of plant weight is 50 grams, and the dilution of tofu liquid waste is 25% (Vidyawati & Fitrihidajati, 2019). The dilution of liquid tofu waste is 1 liter of tofu liquid waste added to 3 liters of water, so a total of 4 liters (Natalina & Hardoyo, 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), 50g *Pistia stratiotes* and 50g *Eichhornia crassipes* (P2), and 25g *Pistia stratiotes* and 75gr *Eichhornia crassipes* (P3).

Data Analysis

Data analysis used variance analysis to see the treatment's effect 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 is shown in Figure 1.

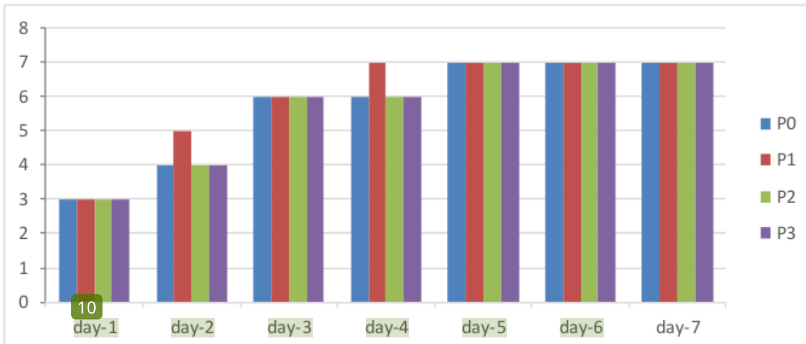

Figure 1. Measuring the pH of Tofu Liquid Waste

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 in *Pistia stratiotes* and *Eichornis crassipes* (Figure 2.).

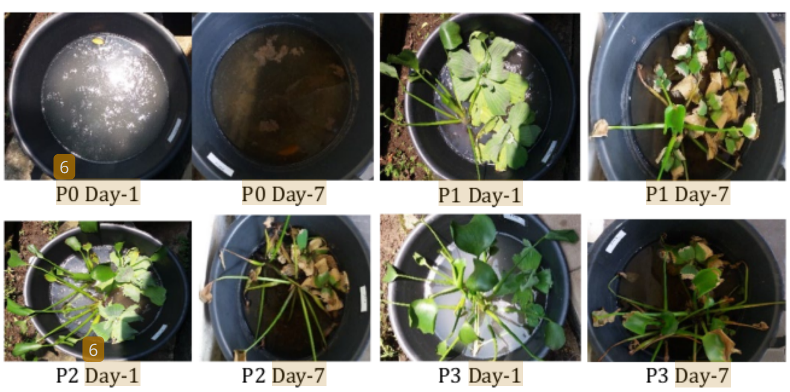

Figure 2. Morphological structure of *Pistia stratiotes* and *Eichhornia crassipes*

Table 2. Morphological structure of *Pistia stratiotes*

Morphological Structure	Treatment	Day						
		1	2	3	4	5	6	7
Leaf	**P1**	Green	Green, rolled edge	A yellow, central green	A yellow, dry, central green	A yellow, dry, central green	A yellow, dry, central green	A yellow, dry, central green
	P2	Green	Green, rolling edge	A yellow, central green	A yellow, dry, central green	A yellow, dry, central green	A yellow, dry, central green	A yellow, dry, central green
	P3	Green	Green, rolling edge	A yellow, central green	A yellow, dry, central green	A yellow, dry, central green	A yellow, dry, central green	A 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) expresses the edge 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 is shown in Figure 1. The variety of *Pistia stratiotes* and *Eichhornia crassipes* can 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 observance, the pH of the effluent became 7. In the P1 treatment, the pH value tended to rise faster than in 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 intermediate 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 by 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 after phytoremediation treatment by combining *Pistia stratiotes* and *Eichhornia crassipes* for seven days with a waste concentration of 25%, 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. Photosynthesis causes a rise in pH and the breakdown of organic nitrogen (Fitriyah, 2011; Haryati et al., 2012).

In the P0 treatment, it was seen that the pH increased the same as in the P1, P2, and P3 treatments. This is due to the high organic matter content in the waste degraded by microorganisms. Microorganisms obtain energy through the oxidation of organic compounds, with the main product, CO_2, being released back into nature as a carbon source for synthesizing new cells (Simanungkalit et al., 2006). These organic materials include carbohydrates, proteins, and fats in tofu liquid waste (Ratnani, 2012). Microorganisms cannot directly metabolize insoluble organic matter. Microorganisms will produce extracellular enzymes for depolymerizing large compounds into smaller, water-soluble substrate for microbes) (Simanungkalit *et al.*, 2006). Microbes transfer these substrates to cells through the cytoplasmic membrane to decompose organic matter. The organic matter is decomposed by microorganisms aerobically, and 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 decomposition results 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 cell reproduction and multiplication, and the final products are CO_2 and H_2O. The results of the decomposition of amino acids with 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:

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

Therefore, P0 experienced an increase in pH due to microorganisms that help decompose organic matter in tofu liquid waste (Vidyawati & Fitrihidajati, 2019). The degradation products are used by microorganisms, not for plant life processes (Rahadian et al., 2017). The average pH value becomes significant in treatments P1, P2, and P3. This is due to the operation of decomposition of organic matter by microorganisms, absorption of organic matter by roots, the process of Photosynthesis, and the supply of O_2 from Photosynthesis to the germs, and microorganisms use the O_2 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 need nitrogen for metabolic processes. Plants absorb nitrogen in the form of NH_3 and NO_3. Nitrogen supply can accelerate the vegetative planting process of plants 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 & Ross, 1995). The absorption of NO_3 is too fast, causing the pH of the tofu liquid waste to be significant because the absorption of NO_3 is accompanied by the absorption of H^+ or the release of OH^- to maintain the charge balance (Salisbury *et al.*, 1995).

According to Effendi (2003); Rahadian et al. (2017), the increase in the pH of tofu liquid waste is caused by the photosynthesis process carried out by plants. Photosynthesis 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, taking H^+ and releasing OH^- will cause the pH of the tofu liquid waste to be neutral (Widya et al., 2015). It can be seen that the reaction equation (4) is as follows:

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

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* absorb H^+ too quickly, leaving OH ions$^-$ which causes the pH of the tofu wastewater to be neutral (Rohmani, 2014). In addition, in the study of Indah et al. (2014), *Pistia stratiotes* can absorb organic materials that contain the essential 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 because the plants cannot 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 are adaptable and can be seen from their anatomical, morphological, and physiological structures (Munawwaroh & Pangestuti, 2018). Based on the results of plant morphology observations, it can be seen that there are morphological changes in *Pistia stratiotes* and *Eichornis crassipes* (Figure 2.).

Tofu liquid waste causes damage and changes in plant physiology, expressed in crop disturbances. According to Fontes & Cox (1995); 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. The study showed new 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 and many root hairs) can 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 were lost. Some plants are still alive in other conditions, and some are damaged and rotting at the bottom of the research bucket.

On day 1, treatments P1, P2, and P3 showed green leaves. After some time, the leaves of *Pistia stratiotes* began to turn pale White and grayish White, while the tips 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 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, there is a possibility that the process of overhauling organic matter carried out by microorganisms has not been optimal, so the reshuffled products are not yet optimally available (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 were 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 is 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 cannot 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 main leaf is young. 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. These broken substances are attached due to the attractive force of water molecules present in plants. After the attractive force continues, the absorption process continues. According to Hardyanti & Rahayu (2007); Ibrahim (2017), the process of absorption of substances contained in waste is carried out by the ends of the roots with meristem tissue. The importance the seeds have absorbed will enter the stem through transport vessels (xylem), which will then be passed on to the leaves. This is supported by Yusuf (2001); Ibrahim (2017), that some ions will be absorbed by moving ions toward the xylem vessels when transported through the cell wall from the epidermis to the endodermis by the cells in their path.

According to Fitter & Hay (1991); Paramitasari (2014), there are two mechanisms carried out by plants in dealing with toxic concentrations: 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. Plants carry out tolerance by developing metabolic systems that function at specific concentrations. Plants carry out the mechanism of changes in root loss due to the toxicity response of acidic pH. The results showed the best morphological and structural conditions in the P3 treatment, namely the green leaves. This is because the most dominant biomass is *Eichornia crassipes*. *Eichornia crassipes* have a body structure that supports organic matter absorption by having large stomata holes twice as large as most other plants (Indah et al., 2014). namely releasing O_2 and absorbing CO_2, chloroplast smooth absorption of CO_2 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 that all treatments increased 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. 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 difference in the leaves' color, containing yellow and withered.

Declaration statement

The authors reported no potential conflict of interest.

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