Potential of three aquatic plants as phytoremediator for liquid waste of tofu

Wardiah, Supriatno, A Amalia and D Andayani
Department of Biology Education, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
E-mail: supriatno@unsyiah.ac.id

Abstract. Phytoremediation is a method of using plants to remove pollutants from contaminated soil or waters. Several species of aquatic plants have been used in reducing pollutants in the waters. The purpose of this study was to determine the effectiveness of three species of aquatic plants as phytoremediators to reduce levels of COD and BOD of liquid waste, the effect of tofu liquid waste on the fresh weight of the species, and the chlorophyll of the plants content after treatment. Data obtained through experimental methods through a quantitative approach with non-factorial Complete Randomized Design. Data analysis used Analysis of Variance. The results showed that three types of water plants were effectively used as phytoremediator in tofu waste water with the highest percentage of BOD and COD reduction obtained in a combination of Water lettuce (Pistia stratiotes L.) and Yellow velvetleaf (Limnocharis flava L.) which was 99.42%. Besides, liquid tofu waste is effective in an increase in the species chlorophyll content. In brief, the aquatic plant species are effective to reduce COD and BOD levels and to increase of chlorophyll content and fresh weight of the species.

1. Introduction
Tofu industry is an important food industry in Indonesia as the demand for the products increase as the population rise. However, the industry, small and large scales, produce liquid waste that contaminates waters and lands, leading to a decrease in quality of the ecosystems. Tofu waste contains organic matters such as proteins, fats, carbohydrates and solid-form waste products. These organic compounds increase BOD and COD levels, and total suspended solid [1].

Various parameters can determine the quality of the water ecosystem, and Water quality can be determined by the levels of biological oxygen demand (BOD) and chemical oxygen demand (COD). Biological Oxygen Demand (BOD) is a parameter to determine the amount of dissolved organic matter and indicates the amount of oxygen needed by organisms in decomposing the organic waste in waters [2]. The higher BOD concentration indicates the higher amount of oxygen required to oxidise organic matters [3]. Chemical Oxygen Demand (COD) is the amount of oxygen needed to oxidize organic waste through chemical reactions [4]. However, COD values are higher than that BOD’s as the biodegradation is slower than that chemical-degraded processes.

Initial measurement indicated that BOD and COD concentrations were greater than that of national standard (BOD (150 ml/L and COD (800 ml/L), accounting for 273, 97 ml/L and 800 ml/L respectively. Therefore, treatment before realising to waters is crucial to increase the quality of waste. Some aquatic plants have been utilized to the decomposition of water waste which is called
phytoremediation. This technique has been known as an economical and eco-friendly method. In this study, we combined and used three aquatic species (Yellow velvetleaf, Water lettuce, and Water hyacinth) to reduce the COD and BOD levels of tofu liquid waste. The study was aimed to determine the reduction of BOD and COD levels using the three aquatic species and the effect of the waste on growth and chlorophyll content of those plants.

2. Method

The research was conducted at the Biology Laboratory of FKIP UNSYIAH, while the analysis of COD and BOD levels of tofu liquid waste was carried out at the MIPA Laboratory of the University of Syiah Kuala. The research was an experimental study with a non-factorsial Complete Randomized Design (RAL) consisting of 8 treatments and three replicates. The treatments were P0 (control = without plants), P1 (AAA), P2 (BBB), P3 (CCC), P4 (ABB), P5 (ACC), P6 (BCC), P7 (ABC), with A = Yellow velvetleaf (Limnocharis species L.), B = Water lettuce (Pistia stratiotes L.), and C = Water hyacinth (Eichornia crassipes Solms).

The equipment used were Winkler bottles, 50 ml beakers, UV spectrophotometer, digital balances, mortals, cuvettes, filter paper, and label paper. The materials were fresh tofu liquid waste, Yellow velvetleaf plants, Water lettuce, Water hyacinth, and 96% alcohol.

The three aquatic plants were weighed, and the initial weight obtained was ± 72 g (Yellow velvetleaf), ± 23 g (Water hyacinth) and, ± 13 g (Water lettuce). Furthermore, the roots of the plants are washed using distilled water before acclimatisation. The wastewater used was collected from the Tofu Solo production house in Gampong Punge Blang Cut, Jaya Baru District, Aceh Besar Regency.

Acclimatisation was done by putting the three aquatic plants into a chamber containing water. Water was replaced every two days. Acclimatisation was carried out for ten days with a natural lighting system (sunlight), and after that, the plants are washed with distilled water before they are treated [5].

Tofu wastewater has flowed into each treatment chamber, then the initial BOD and COD levels were measured using the Winkler and COD mn methods, respectively. After 14 days of treatment, the BOD and COD levels of tofu waste were measured again with the same methods. The percentage reduction in BOD and COD is obtained through the formula [6]:

\[
\%\text{COD reduction} = \frac{(\text{initial COD concentration} - \text{final COD concentration})}{\text{Initial COD concentration}} \times 100\%
\]

\[
\%\text{BOD reduction} = \frac{(\text{initial BOD concentration} - \text{final BOD concentration})}{\text{Initial BOD concentration}} \times 100\%
\]

After 14 days of treatment, as much as 0.3 grams of leaves of the plants were weighed and crushed, and then 30 ml of 96% alcohol were added to dissolve the crush. The extract was filtered and transferred to a 10 ml cuvette. Chlorophyll levels were measured using a UV spectrophotometer at wavelengths of 649 and 665 nm. Total chlorophyll levels were analysed using the following formula:

\[
\text{Total Chlorophyll (mg/L)} = 20.2 \ OD_{649} + 6.1 \ OD_{665}
\]

Fresh weight was obtained by weighing the plants after 14 days of treatment. The difference between initial and final weight was obtained through the formula:

\[
\text{Fresh weight increase (gram)} = \text{final weight (gram)} - \text{initial weight (gram)}
\]

Data of COD and BOD levels and fresh weight were analysed by Analysis of Variance, whereas the graph showed the chlorophyll content among the treatments.
3. Results and Discussion

3.1. COD and BOD levels

Organic materials from tofu industry releasing to waters without pre-treatment will decrease in water quality. The activity of microorganisms in breaking down the organic matters increase producing inorganic molecules. The oxygen consumption also rises as the decomposition increases leading to the decrease in dissolved oxygen in waters. The oxygen deficit is immediately replaced by oxygen from photosynthesis and aeration. However, a large amount of organic molecules in waters generates anaerobic conditions that produce ammonia, carbon dioxide, acetic acid, hydrogen sulfide, and methane. These compounds are very toxic to most aquatic animals and produce unpleasant odour [7].

Chemical oxygen demand (COD) and biological oxygen demand (BOD) has been used as a parameter of water quality. COD expresses the oxidation of organic and inorganic matter in waters, wastewater, and natural water. BOD has been utilised to determine the biodegradation of the waste [8]. Biological oxidation is slower than that of a chemical one. Within five days (BOD5), organic carbon oxidation is 60% - 70% and within 20 days (95%). Therefore, COD values are higher than that of BOD’s [9].

There are several techniques of tofu liquid waste treatment, namely anaerobic-aerobic reactor, aerobic biofilter, and phytoremediation. However, phytoremediation is an environmentally friendly and cost-effective way as it uses aquatic plants to decompose contaminations [10].

Figure 1 shows the percentage of reduction COD and BOD levels after treatment. It has been revealed that the three aquatic species and plant combinations (treatments) have decreased of COD and BOD levels of tofu liquid waste. However, the treatment using combination one Water lettuce and two Water hyacinths has reduced the level of BOD and COD nearly 100% (97.50%), followed by combination of Yellow velvetleaf and Water hyacinth (less than 97%). The combination of Water hyacinth and Water lettuce was the best combination of phytoremediator in reducing BOD and COD levels. Water Hyacinth has been used for wastewater treatment and is effective in reducing both parameters. Tofu wastewater treatment using biofiltration using Water hyacinth can reduce BOD and BOD values by 68.06% and 72.76% respectively in six days of retention [11]. The plant root tips absorbed the organic substances and transported to the plant organs. Through the process, organic substances will react to other molecules and then accumulate in plant organs [12]. Water lettuce has been known for its roles in wastewater treatment. The water plant can absorb organic compounds from the water and accumulate them in its organs. It is assumed that the
association between the plant root and microorganisms may accelerate the decomposition of organic matters that serve as the plant nutrient [13].

3.2. Increase in fresh weight
The rise in fresh weight of the plants has been revealed in Figure 2. The graph indicates that the liquid waste has increased the fresh biomass of the plants with the highest one was obtained from the combination of three Water hyacinth plants (1.4 grams). Water hyacinth has well-developed fibrous roots that take more than 50% of plant biomass. The root system has increased the surface area for nutrient absorption leading to increase in the plant biomass [14].

![Figure 2](image)

**Figure 2.** The increase in fresh weight after 14 days of treatment. A= Yellow velvetleaf (*L. flava* L.), B= Water lettuce (*P. stratiotes* L.), and C= Water hyacinth (*E. crassipes* Solms), P0=control. The values were obtained by log 2 conversion.

The organic matters in tofu liquid waste have served as the plant nutrient. In 100 mL of tofu liquid waste contains Nitrogen (1.64%), Phosphorus (0.15%), and Potassium (6.25%) that are crucial for physiological and metabolic processes [15] [16]. Nitrogen is an important element for plant growth and development regarding protein production, vegetative organ growth and development, and metabolisms. Nitrogen absorbed from the waste is converted nucleic acids and amino acids used for protein biosynthesis and vegetative and generative growth [17]. Moreover, Phosphate and Potassium are used for the formation of adenosine triphosphate (ATP) and an enzyme activator in photosynthesis and photosynthates, respectively. ATP is a high energy molecule required for various cell activities including cell division and enlargement increasing plant weight [15].

3.3. Concentration of total chlorophyll
We develop a questionnaire based on Park [8]. It consists of six scales, namely perceived ease of use (EU), perceived usefulness (PU), attitude (AT), behavioral intention (BI), online learning self-efficacy (SE), external variables (EV), dan accessibility (AC). To validate the questionnaire, we used convergent and divergent. Moreover, in terms of reliability, we utilized composite reliability and variance extracted value. Moreover, the instrument consists of 19 liker scale items ranging from strongly agree to strongly disagree.

The highest chlorophyll concentration was obtained from Yellow velvetleaf plants and Water lettuce. Not only have many fibrous roots that increase the surface area for nutrient absorption but also, they are broadleaf plants. It increases photosynthesis rate as surface area rises subsequently promotes the accumulation of organic compounds (photosynthates) in plant tissues. These organic
compounds, namely carbohydrates, fats and proteins, are required in the form of other compounds such as chlorophyll [15].

![Figure 3. Total chlorophyll content. A= Yellow velvetleaf (L. flava L.), B= Water lettuce (P. stratiotes L.), and C= Water hyacinth (E. crassipes Solms), P0=control. The values were obtained by log 2 conversion.](image)

The photosynthetic rate has been linked with several factors in different plants consisting of stomatal and mesophyll resistance, RuBP carboxylase activity, mesophyll cell size, chloroplast number, and chlorophyll content [18]. The decomposition of the liquid waste releases Nitrogen, one of chemical substance required for the formation of chlorophyll. It is assumed that the increase in chlorophyll content of the plants is due to the abundant of Nitrogen released from the decomposition of protein from the waste. The pigment molecule acts as the absorbent of sunlight in photosynthesis to produce photosynthates subsequently promotes the plant growth.

4. Conclusions
The three of aquatic plants (Yellow velvetleaf (Limnocharis flava L.), Water lettuce (Pistia stratiotes L.), and Water hyacinth (Eichornia crassipes Solms) have effectively reduced the levels of BOD and COD of tofu wastewater. The most effective combination of phytoremediator plants in reducing BOD and COD levels is the combination of Water lettuce and Water hyacinth. The fresh weight and chlorophyll content of the aquatic plants have increased indicating that the waste may serve as an organic liquid fertilizer for agricultural purposes.

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