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Effectiveness of Aerobic Microbes in the Treatment of Shrimp Pond Wastewater

Eli Hendrik Sanjaya[1,2,3]*, Aulia Amara Asha[1], Suharti[1], Muntholib[1] and Hong Chen[4]
[1]Department of Chemistry, State University of Malang (Universitas Negeri Malang), Jl. Semarang No. 5, Malang, East Java, 65145, Indonesia
[2]Department of Biotechnology, State University of Malang (Universitas Negeri Malang), Jl. Semarang No. 5, Malang, East Java, 65145, Indonesia
[3]Center for Excellence in Science and Technology-Higher Education Center of Advanced Materials for Renewable Energy (PUI-PT Camry) State University of Malang (Universitas Negeri Malang)
[4]Key Laboratory of Water-Sediment Sciences and Water Disaster Prevention of Hunan Province, School of Hydraulic Engineering, Changsha University of Science & Technology, Changsha 410004, China
*Corresponding author (e-mail: eli.hendrik.fmipa@um.ac.id)

Shrimp pond wastewater (SPW) can harm the environment since it contains high organic matter and ammonia. Biological treatment using aerobic microbes is needed to break down waste into CO_2, H_2O, sludge, and energy. This study aims to determine the characteristics of shrimp pond wastewater and investigate the effectiveness of aerobic microbes in enhancing the water quality parameters such as Chemical Oxygen Demand (COD), carbohydrates, protein, ammonia, Total Dissolved Solid (TDS), and Total Suspended Solid (TSS) as well as aerobic bacteria as biosorption of heavy metals Fe and Pb. The stages included sampling, characterization, and preparation of aerobic microbial starter in a semi-batch system for 268 days with various concentrations for 12 phases. The results indicate that the initial concentration of COD in shrimp pond wastewater was 1084.33 mg/L. The removal of several parameters by aerobic microbes was optimum in phase 8 with the shrimp pond wastewater dilution of 8 times. The removal of COD, carbohydrates, protein, ammonia, TDS, and TSS are 96.24%, 98.20%, 94.22%, 99, 15%, 99.4%, and 94.24%, respectively, while the heavy metals Fe and Pb can be adsorbed respectively by 44.68% and 100%. The results show that aerobic digestion is potential for shrimp pond wastewater treatment.

Keywords: Shrimp pond wastewater; biological wastewater treatment; aerobic microbes; heavy metals Fe and Pb

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Shrimp farming in Indonesia is one of the fisheries sectors which is a priority to improve the national economy. According to the Ministry of Maritime Affairs and Fisheries (KKP) in 2024 shrimp production is targeted to increase by 250% [1]. The production of shrimp caused an increase in the amount of waste produced, including shrimp pond wastewater (SPW).

Shrimp pond wastewater consists of feces, leftover feed, and urine. The leftover feed and feces waste is almost entirely made of organic matter. It is characterized by high amounts of COD, BOD, TSS, dissolved particulate matter, VSS, nitrogen, phosphorus, and heavy metals [2]. Nitrogen and phosphorus in shrimp pond waste amounted to 77.73% and 90.21%, respectively, wasted into the aquatic environment around the pond [3]. Nitrogen and phosphorus are the main elements that drive eutrophication in aquaculture wastewater resulting in ecosystem damage. In addition, social problems arise such as bad odors and can cause food safety problems and poor health quality [4]. Therefore, it needs a method of processing shrimp pond waste that is appropriate and environmentally friendly to overcome this problem.

Many conventional methods can be used in the treatment of this waste. Some of them are coagulation, advanced oxidation processes, membrane filtration, adsorption, dialysis, photocatalytic degradation, and biological methods [2]. Biological processing methods can be the right choice in processing SPW because of low operating costs, simple processes, and energy saving as well as environmental friendliness. However, the study of aerobic digestion of shrimp pond wastewater is still veery limited. Biological treatment utilizes aerobic microbial activity in the presence of oxygen to convert large organic complexes into CO_2, H_2O, sludge, and energy as shown in equation 1 [5, 6]. In addition, treatment with aerobic microbes also acts as a biosorbent in separating heavy metals from various wastes including liquid waste even at low concentrations [7].

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$$C_xH_yO_Z + O_2\,(g) + \text{microbes} \rightarrow CO_2\,(g) + H_2O\,(l) + \text{sludge} + \text{energy} \quad (1)$$

There is a shrimp pond in Prigi Trenggalek, East Java, that lacks waste treatment. Therefore, this study aims to determine the characteristics of shrimp pond wastewater and investigate the effectiveness of aerobic microbes in reducing water quality parameters such as Chemical Oxygen Demand (COD), carbohydrates, protein, ammonia, Total Dissolved Solid (TDS), and Total Suspended Solid (TSS) as well as aerobic bacteria as biosorption of heavy metals *Ferrum* (Fe) and *Plumbum* (Pb).

EXPERIMENTAL

Chemicals and Materials

The tools used in this study were a water bath shaker (Faithful), a UV-Vis spectrophotometer (Thermo Fischer Scientific), a Thermo Ice 3000 Atomic Absorption Spectrophotometer, a pH meter, 5-20 mL pipettes, 1000 μm micropipettes, vortexes, balances. analytical, centrifuge, hot plate, oven, magnetic stirrer, thermoreactor, 10-1000 mL volumetric flask (Pyrex), 10 mL measuring cup (Pyrex), 50-500 mL beaker glass (Pyrex), test tube (Iwaki), glass funnel (Pyrex), reagent bottle, spatula, watch glass, stirring rod, 1 mL tip, knife, scissors, dry ice, serum bottle, sample bottle, gauze, wrap, evaporating cup, tools vacuum filtration, Erlenmeyer glass 100 and 250 mL (Pyrex), 5 ml measuring pipette (Pyrex), suction ball, aluminum foil, Whatman filter paper 0.45μm pore size.

The basic material used in this research is shrimp pond wastewater taken from Prigi Trenggalek. The material used is an aerobic microbial starter from PT. Intuisi Tata Bestari, Bovine Serum Albumin (Sigma Aldrich), $CuSO_4.5H_2O$ (Merck), sodium tartrate (Merck), Na_2CO_3 (Merck), NaOH (Merck), Follin (Merck), anthrone (Merck), H_2SO_4 95-97% (Merck), glucose pro analysis (Merck), $K_2Cr_2O_7$ (Merck), $HgSO_4$ (Merck), $AgSO_4$ (Merck), Potassium Hydrogen Phthalate (Merck), distilled water, Whatman filter paper, Phenol 89% (Merck), Ethyl alcohol 95% (Merck), Standard solutions of Pb and Fe, Sodium nitroprusside (Merck), Trisodium citrate (Merck), NaOH (Merck), HNO_3 (Merck), ethylene gas, $CaCO_3$ (Merck), HCl (Merck), Sodium hypochlorite (Merck).

Characterization Methods

Sampling

Shrimp pond wastewater was taken from Prigi Trenggalek. Samples of shrimp pond wastewater were collected from the outlet at the coordinates (-8.2859297, 111.7207227). Sample bottles were prepared in advance before the sampling process. Subsequently, the samples were collected using these bottles. After collection, the sample bottles were placed into a cooling box.

Formatting Processing of Shrimp Pond Liquid Waste Samples with Aerobic Microbes

The sample was shaken first, and then 300 mL was put into an Erlenmeyer containing consortium microbial culture (*Enterococcus faecalis*; *Clostridium beijerickii*; *Clostridium sacharopertylacetonicum*) from PT. Intuisi Tata Bestari (0.6 g) diluted in 60 mL water which had previously been incubated for 24 hours. Then, the Erlenmeyer's mouth was closed with gauze. Samples and microbial cultures in Erlenmeyer were incubated in a water bath shaker at 37ºC and 120 rpm. The treated samples were taken 40 mL 2 times a week for testing water quality parameters. Then, the initial sample was added to 40 mL of the Erlenmeyer. Samples were diluted if the aerobic microbial performance was not good.

The samples were diluted with water to improve the performance of aerobic microbes in degrading organic matter in the waste. Samples were processed in 12 phases with variations in the dilution of each phase. Phase 1 conditions began with sample processing without dilution, then continued with phase 2 where the sample was diluted 4 times. Furthermore, dilution with other variations was carried out for the next phase while observing the performance of aerobic microbes in the previous phase.

pH Measurement

Samples were measured with a calibrated pH meter using pH 4, 7, and 10 buffers.

Testing of Proteins Content of Shrimp Pond Effluent Samples

A total of 0.2 mL of the sample was pipetted and transferred into a test tube. Then, 1 mL of Biuret solution was added and vortexed. Next, the mixture was incubated at room temperature for 10 minutes. After that, 0.1 mL of Follin's reagent was added. The mixture was incubated for 30 minutes. The absorbance of the sample was measured at a wavelength of 750 nm using a UV-vis spectrophotometer [8].

Testing of Carbohydrate Content of Shrimp Pond Wastewater

The sample of 0.5 mL was put into a test tube. Anthrone solution was added to 2 mL, and then shaken until homogeneous. Next, the mixture was put into the bath for 8 minutes at 100ºC. The mixture was cooled in a beaker filled with tap water. The absorbance of the sample was measured at a wavelength of 630 nm using a UV-vis spectrophotometer [9].

Testing of COD Content of Shrimp Pond Wastewater

A sample of 1.25 mL was put into a test tube. A high concentration solution of the digestion solution of 0.75

mL and sulfate reagent solution of 1.75 mL was added. The test tube was closed and shaken slowly until homogeneous. The test tube was inserted into the thermoreactor at 150ºC for 2 hours. The absorbance of the sample was measured at a wavelength of 600 nm using a UV-Vis spectrophotometer [10].

Testing of Ammonia Content of Shrimp Pond Wastewater

A sample of 5 mL was put into the Erlenmeyer. Phenol and sodium nitroprusside solutions were each added as much as 1 mL and 2.5 mL of oxidizing solution was added, then homogenized. Erlenmeyer glass covered with aluminum foil and left for 1 hour until the color formed. The absorbance of the sample was measured at a wavelength of 640 nm using a UV-Vis spectrophotometer [11].

Testing of TSS Content of Shrimp Pond Wastewater

The filter paper was prepared by rinsing 20 mL of distilled water, and sucking it dry using vacuum filtration. The filter paper was transferred to a porcelain cup and then baked in the oven for 1 hour at 103ºC. The filter paper was cooled in a desiccator and weighed, and the weight was recorded as W0. The sample was shaken as much as 30 ml and then filtered using vacuum filtration. Samples were given the same treatment as the preparation and their weight was recorded as W1. The volume of the sample tested is recorded as V[12]. The formula for calculating TSS levels was listed in Equation 1.

$$TSS\ (mg/L) = \frac{(W1-W0)}{V} \times 1000 \qquad \text{(Eq. 1)}$$

Testing of TDS Content of Shrimp Pond Wastewater

The cup was prepared by baking for 1 hour at 180 ºC. The cup was cooled in a desiccator and weighed, and the weight was recorded as W0. The sample was shaken as much as 30 ml and then filtered using vacuum filtration. The filtrate was put into the prepared cup, then given the same treatment as the preparation, and recorded the weight as W1. The volume of the sample tested was recorded as V [13]. The formula for calculating TDS levels was listed in Equation 2.

$$TDS(mg/L) = \frac{(W1-W0)}{V} \times 1000 \qquad \text{(Eq. 2)}$$

Testing of Fe Heavy Metal Levels

Sample of 50 mL was put into Erlenmeyer. The sample was added 5 mL of concentrated HNO_3 and closed with a funnel. Then, the mixture was heated slowly until the remaining volume was 15 mL - 20 mL. If the digestion was not complete (not clear), then the mixture needs to be added 5 mL concentrated HNO_3 again. Then, the mixture was covered and heated but not brought to a boil. This process was repeated until all the metal dissolved. The funnel was rinsed and the rinse water was added to the Erlenmeyer. The mixture was transferred to a volumetric flask (filtered if necessary) and mineral-free water was added until the markings were right, then homogenized. The absorbance of the sample was measured at a wavelength of 248.3 nm using an Atomic Absorption Spectrophotometer (AAS) [14].

Testing of Pb Heavy Metal Levels

A sample of 100 mL was put into a beaker glass. Then, 5 mL of nitric acid was added to the sample. The mixture is heated on a hot plate until it is almost dry. Then, 50 mL of distilled water was added to the mixture and put into a volumetric flask through filter paper. The mixture was added with distilled water up to the boundary mark. The absorbance of the sample was measured at a wavelength of 217 nm using AAS [15].

RESULTS AND DISCUSSION

Characterization of Shrimp Pond Wastewater

In this study, samples were taken from Shrimp Pond Outlets in Prigi Trenggalek. The first thing to do in this research is to characterize some of the water quality parameters from samples that will be treated with aerobic microbes to reduce the levels of some of the parameters. Based on the test results it is known that the pH of the sample is 6.47. In addition, the results of the characterization of shrimp pond wastewater samples on several parameters are presented in Table 1.

Table 1. Characterization of the original shrimp pond wastewater.

Parameter	Concentration (mg/L)	Parameter	Concentration (mg/L)
COD	1084.33	TSS	1723.33
Proteins	539.89	TDS	22374.07
Carbohydrate	232.45	Heavy metal Fe	0.0994
Ammonia	1.33	Heavy metal Pb	0.0583

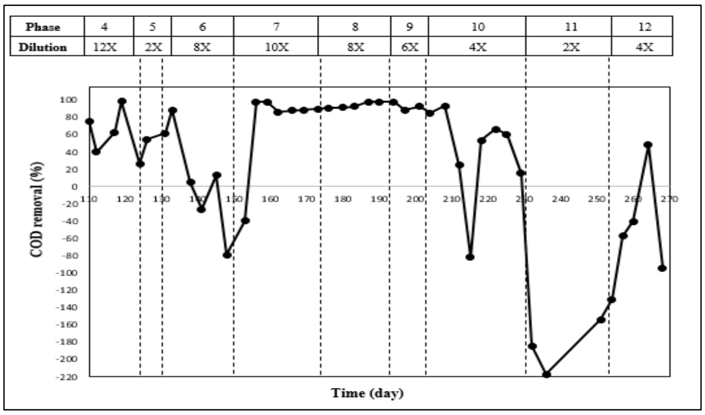

Figure 1. COD removal by Aerobic Microbes.

Shrimp pond wastewater treatment was carried out using aerobic microbes with a DO of 23.92 mg/L. Waste is processed using a water bath shaker which aims to shake and mix the sample with aerobic microbes and maintain a constant temperature so that the microbes can work optimally [16]. The sample before processing looks greenish and slightly cloudy. However, after processing the sample brighter with a slightly yellowish color and not cloudy. This indicates the presence of aerobic microbial activity in degrading organic matter-rich waste and reducing the levels of water quality parameters in the waste.

Performance of Aerobic Microbes in Treating Shrimp Pond Wastewater

Samples of shrimp pond wastewater taken from the outlet of shrimp ponds showed a COD level of 1084.33 mg/L as shown in Table 1. In phase 1 or samples without dilution after the waste was treated with aerobic microbes the level decreased to 404.33 mg/L or 62.71% on day 5 as shown in Fig. 1. The reduced COD level in this phase experienced sharp fluctuations and was unstable due to the adaptation phase of the bacteria to the new environment. At the end of this phase, there was a reduction in COD levels of 63.63% on the 61st day. The presence of high levels of COD waste samples may indicate low levels of O_2 in the sample. While the growth of aerobic bacteria is influenced by the availability of O_2, these bacteria are capable of adapting to low oxygen conditions. For instance, some bacteria can adjust their metabolism to use alternative electron acceptors when O_2 is limited, a process referred to as microaerobic growth. Additionally, the diffusion of O_2 into the cytoplasm of bacteria occurs at high rates, even at very low O_2 tensions, which is sufficient for aerobic growth [17]. Moreover, in this study, the experiment was designed with aeration by shaking the Beaker glass.

Therefore, the next phase, or phase 2 is carried out by diluting the sample in the hope of improving the performance of aerobic microbes in reducing COD levels. In phase 2 or diluting the sample to 4× the initial sample, the % reduction in concentration increased better than the previous phase, namely 76.24% on the 82nd day as shown in Figure 1. However, in phase 3 or dilution 8× the COD content in the effluent was only decreased by 4.92%. The COD levels reduced by the microbes in the next phase were much more unstable and even experienced a greater % decrease, reaching -217.30% on day 236.

The optimum conditions for % reduction in COD levels were obtained in phase 8 with 8× dilution where the average % reduction in COD levels in this phase was 96.24%. Some studies show that the dilution of substrate can enhance the performance of organic wastewater digestion [18-19]. If the concentration of wastewater is too high, it can inhibit the performance of microbes in digesting the wastewater. Additionally, the oxygen supply in dilute samples is not limited, which allows the bacteria to carry out their metabolic processes more effectively.

COD is a measure of the amount of oxygen required to oxidize organic matter in a sample which includes various complex organic compounds that are more difficult to break down than proteins and carbohydrates. Organic compounds that are difficult to break down in shrimp pond waste are fat, chitin, and lignin [20-22]. Therefore, aerobic microbes need a long time to reduce the COD levels contained in shrimp pond wastewater.

Based on the data listed in Table 1, samples of shrimp pond wastewater contained a protein content of 539.89 mg/L. During the initial processing phase (phase 1), aerobic microbes were able to reduce the protein content in the sample by 88.90%, reaching 59.89 mg/L on day 19, as shown in Fig. 2. However, the reduction decreased to 84.71% by day 54 before increasing again to 92.61% on day 56. Aerobic microbial activity in treating this waste was unstable in phase 1 due to the process of microbial adaptation to the new environment. However, at 4× dilution or two% phase, the decrease in protein levels began to appear stable. Optimum conditions for processing protein content were obtained in phase 8 or 8× dilution with an average percentage of 94.22%.

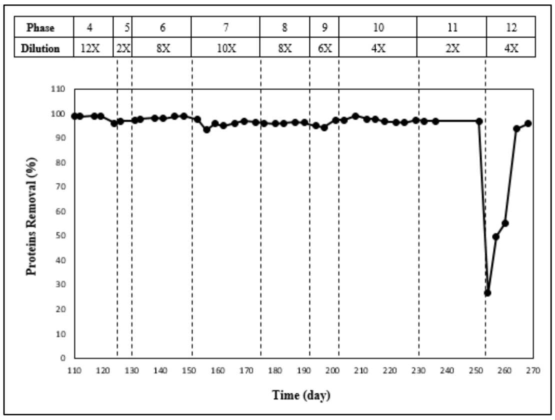

Figure. 2. Percentage Curve for Reducing Proteins Levels by Aerobic Microbes.

Characterization data for samples of shrimp pond wastewater showed a carbohydrate content of 232.45 mg/L which is shown in Table 1. In the adaptation phase or phase 1, the % removal in carbohydrate content experienced unstable fluctuations which was marked by a decrease in levels of 81.35%. on day 5 then increased to 93.47% on day 26 and decreased sharply to 33.24% on day 49 as shown in Fig. 3. Microbial activity in reducing carbohydrate levels was seen to be unstable from phases 1 to 4. However, its activity begins to increase and looks more stable starting from phase 5 which begins with a % decrease in levels of 96.98%. In phase 7 there was a sudden decrease in activity with a decrease in levels of only 71.31%. This could have been caused by changes in environmental conditions due to the addition of samples with different concentrations. Optimum conditions for reducing carbohydrate content were obtained in phase 8 or 8x dilution where the concentration can decrease to 99.99%. Carbohydrates take a long time to be reduced when compared to protein. This is because the structure of carbohydrates is more complex and consists of long chains of sugar molecules, making it more difficult to break down than protein-forming amino acids [23-24].

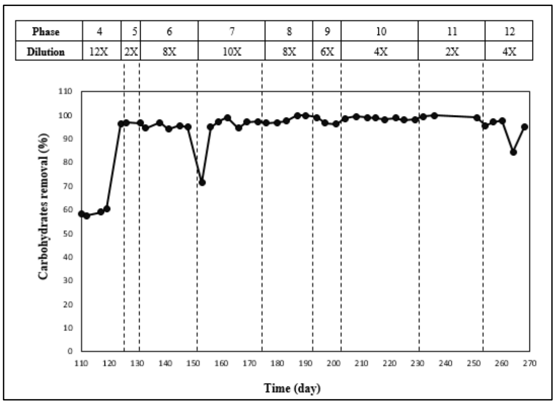

Figure. 3. Percentage Curve for Reducing Carbohydrates Levels by Aerobic Microbes.

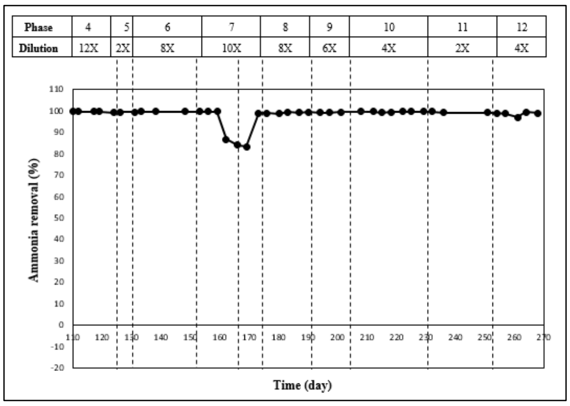

Figure. 4. % Curve for Reducing Ammonia Levels by Aerobic Microbes.

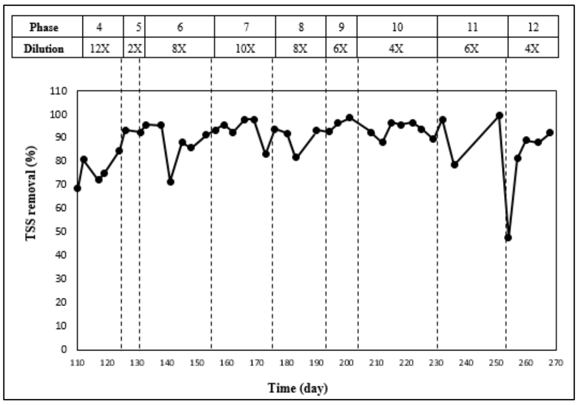

Figure. 5. % Curve for Reducing TSS Levels by Aerobic Microbes.

In Table 1, shrimp pond wastewater had an initial ammonia level of 1.33 mg/L. After 21 days, it decreased to 0.002 mg/L or 99.78% reduction which is shown in Fig. 4. On day 35, a drop in microbial activity caused a decrease in reducing protein and carbohydrate levels, and the COD reduction rate was only 50.10%. However, reduction rate increased to 99.78% on the 40th day. Shrimp pond wastewater is reduced effectively and stably by aerobic microbes.

Shrimp pond wastewater samples taken from shrimp pond outlets showed TSS levels of 1723.33 mg/L as shown in Table 1. In the initial phase of shrimp pond wastewater treatment using aerobic microbes can reduce ammonia levels by 42.94% on days 2-5 which is shown in Fig. 5. In this phase, microbial performance is unstable within a few days of processing due to the adaptation phase to the environment.

The performance of aerobic microbes in reducing TSS levels is very good during the processing of shrimp pond wastewater from phase 1 to the next phase with a significant increase in % reduction levels until the end of processing. However, there was a sharp decrease of 47.59% on day 254 with 4× dilution or phase 12. On day 254, the % decrease in COD and protein levels was very low with values of -130.62% and 26.52% respectively, as shown in Fig. 1 and Fig. 2. Thus, the decreased % concentration can be caused by the high concentration of protein and COD in the waste followed by an increase in the results of microbial degradation of organic matter in the form of sludge [18]. The presence of sludge resulting from the degradation of organic matter by aerobic microbes can affect the TSS levels in the waste. However, the % reduction in TSS levels increased again up to 92.15% at the end of processing.

Samples of shrimp pond wastewater taken from the outlet of the shrimp pond showed a TDS level of 22374.07 mg/L as shown in Table 1. In phase 1 or the microbial adaptation phase, there was an increase in TDS levels beyond the initial sample level, so the % decrease in levels indicated a value of - 7.91% on the 5th day as shown in Fig. 6. In the initial phase there was an unstable performance with a lower reduction value than the % reduction in TSS levels. After being processed for several days, the optimum TDS level decreased to 1296.77 mg/L on day 183 with the % reduction reaching 94.28% in phase 8

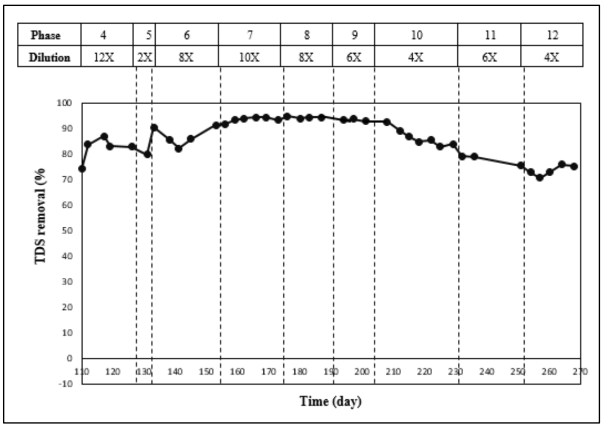

Figure. 6. % Curve for Reducing TDS Levels by Aerobic Microbes.

Table 2. Data on levels of heavy metals Fe and Pb at SPW.

Metal Type	**Phase**				
	Sample	2	4	6	8
Fe (mg/L)	0.0994	0.0015	0.0005	0	0.0052
Pb (mg/L)	0.0583	0.0607	0	0	0

Unlike the case with TSS, TDS can include various substances, such as minerals, metals, chlorides, calcium, magnesium, potassium, sodium, bicarbonate, sulfates, salts, heavy metals, and some organic compounds that dissolve in water [25]. The presence of various kinds of solutes can make it difficult for aerobic bacteria to degrade the TDS content in the waste [26]. The TDS content in waste can interact with other substances in water, such as electrolyte ions, which can slow down the rate of TDS reduction by aerobic bacteria [22]. Thus, TDS can be reduced longer than the TSS content in the sample.

Biosorption of Fe and Pb Heavy Metals

Based on the data in Table 2, samples of shrimp pond wastewater contained the heavy metals Fe and Pb, respectively 0.0994 and 0.0583 mg/L. In Phase 2, the Fe metal content decreased to 0.0015 mg/L, a 84.04% reduction, while the concentration of Pb increased to 0.0607 mg/L shown in Table 2. This condition is quite complex, however, in phase 4, the concentrations of the heavy metals Fe and Pb decreased respectively to 0.0005 mg/L and 0 mg/L with a % reduction of 94.68% and 100%. The levels of heavy metals Fe and Pb are no longer contained in the waste in phase 6. This proves the activity of aerobic bacteria as metal biosorption.

Fe metal decreased by 44.68% in phase 8 as shown in Table 2. This proves that the heavy metal Fe content which was not present in the sample was re-contained at a level of 0.0052 mg/L. Microbes can release metals they have absorbed back into the environment through processes such as cell lysis, excretion, and desorption [27, 28]. This desorption process can occur when the bacteria have reached the maximum absorption of metals so that the excess Fe and Pb absorbed can be released back into the environment. In addition, environmental factors such as pH, temperature, and the presence of other metals can also affect the adsorption and release of heavy metals by bacteria [29].

Although the exact function of Pb and Fe as cofactors of specific enzymes in bacteria is not yet understood in this study, it is evident that bacteria have the ability to absorb these metals from shrimp pond wastewater. The presence of these metals can have an impact on the growth and functions of bacteria, the microorgnism needs a little bit of Fe dan Pb. Heavy metals can compete with essential nutrients required by bacteria for growth, bind to essential nutrients like iron, which is required for various critical functions in bacteria, and be toxic to bacteria, which can inhibit their growth or even lead to cell death [2].

CONCLUSION

The aerobic microorganism is potential to be bioremediation agent of shrimp pond wastewater. The results of this study indicate that the best performance of the aerobic method is in phase 8 with substrate dilution to 8 times. In this phase, the average performance of the aerobic method in reducing the levels of COD, carbohydrates, protein, ammonia, TDS, and TSS respectively was 96.24%, 98.20%, 94.22%, 99.15%, 99.40%, and 94.24%. The heavy metal Fe can be adsorbed from a concentration of 0.0094 mg/L to 0.0052 mg/L with a performance of 44.68%, while the heavy metal Pb is adsorbed from a concentration of 0.0583 mg/L to 0 mg/L with a performance of 100%.

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