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Effects of Biofilms on Ammonium Removal Efficiency in Fish Pond Effluents

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Abstract. Treatment of wastewater in fish ponds effluent is an effort to reduce the impact of pollutants contained in the wastewater so that it can be disposed into the aquatic environment safely. The purpose of this study is to understand and analyze the effect of inoculant sources on removing of total ammonium in vanamae shrimp and marguensis shrimp pond wastewater, and to pre-design the technology for treating high-salinity organic waste with biological wastewater treatment using natural microorganisms. The study was conducted using a laboratory-scale reactor. At the beginning of the study, adherent biofilm media was planted at the bottom of the sea with a depth of 1.5 m for three weeks to grow nitrifying bacteria, then applied to treat fish pond wastewater. The results showed that the efficiency of ammonium reduction with adherent media from Telukawur is 74% while adherent media from Panjang Island is 66% with declining and fluctuating trends every day. The extensive -scale application for treating wastewater from brackishwater aquaculture is designed with 4 processing units, a 15.5 m x 7.7 m x 1.5 m sedimentation basin, a 10.5 m x 3.5 m x 1.5 m anaerobic basin, a 13 m x 13 m x 1.5 m aerobic basin, and a 15.5 m x 7.7 m x 1.5 m effluent basin. It concludes that biofilm adhesion technology from Panjang Island waters and Telukawur waters can work efficiently in removing ammonium concentration.

1. Introduction

Jepara Regency, the development of shrimp farming pond production has been quite rapid since 1980. However, shrimp pond production has declined since 1998 its caused by the many failures of shrimp ponds caused by many factors including shrimp disease and degradation of the aquatic environment. Degradation of the aquatic environment comes from organic waste produced by the remaining shrimp feed, shrimp dung, and dead microorganisms. The residual feed and organic material from the shrimp farming industry reach 60% of the total expenditure on shrimp farming that goes to water. The organic wastewater can pollute the pond waters and the coastal environment. High organic matter content will increase the ammonia content, which is toxic to shrimp and other biotas. On the other hand, the organic load content is also high, so it needs to be processed so that it can be discharged through an outlet to seawater safely.



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Biological processes in treatment plants are carried out by a diverse community of organisms (Henze, 2000). A bioremediation system that can use microorganisms to exclude, oxidize, or kill pollutants by not adding harmful chemicals. Bioremediation is very suitable to be applied in large areas where the concentration of contaminants is relatively low. In recent years, researchers have directed bioremediation mechanisms for a variety of priority pollutants, including chlorinated hydrocarbons, polyaromatic hydrocarbons, and heavy metals. However, most of the research published and related to planktonic culture that monitored at a laboratory scale. The bacterial community on biofilms can reliably alter organic matter through the process of nitrification and ammonification. This biofilm application will reduce nitrate levels in it. Organisms in biological processes are divided into the following groups, namely bacteria, fungi, algae, protozoa, and metazoa. Two types of biological treatment are biofilter and activated sludge. Both have different living conditions. The most varied bacterial community life is in the biofilter. This biofilter is due to the design and operation of these filters, so they have the right environment where different bacteria can flourish. On the other hand, in the process of activated sludge, animals are distributed evenly, and the species do not vary (Henze, 2000). So this research needs to be done to know, understand and analyze the influence of sticky biofilm media to eliminate toxic levels in wastewater in ponds after brackish water cultivation and provide planning recommendations for post-brackish water treatment plants in coastal areas to give a picture of large-scale treatment.

2. Methodology

This research conducted with experimental research methods, namely research used to find the effect of a treatment on others in controlled conditions (Sugiyono, 2009). In testing the effect of biofilm media, the reactor used was a biofilm reactor. In biofilm reactors, there are media in the form of plastic for the attachment of biofilms and aerator blowers for continuous aeration. The media needed is 1 type, namely synthetic grass fiber with the plastic base material to make it lightweight and inexpensive. Then each medium is used to treat waste from 2 different types of ponds. Then repeated two times and added the whole test without using biofilms so only use continuous blower aerators. So that the number of reactors was found to be ten variations plus 2 for reactors without biofilms containing only two different wastes. So, 12 total chambers will use. Placement of the reactor design using a random method, so not grouped according to treatment, reactor design layout. The experiment was carried out for 14 days, and laboratory-scale testing was carried out by sampling every day. At each test, measurements of ammonium, nitrate and total organic were measured.

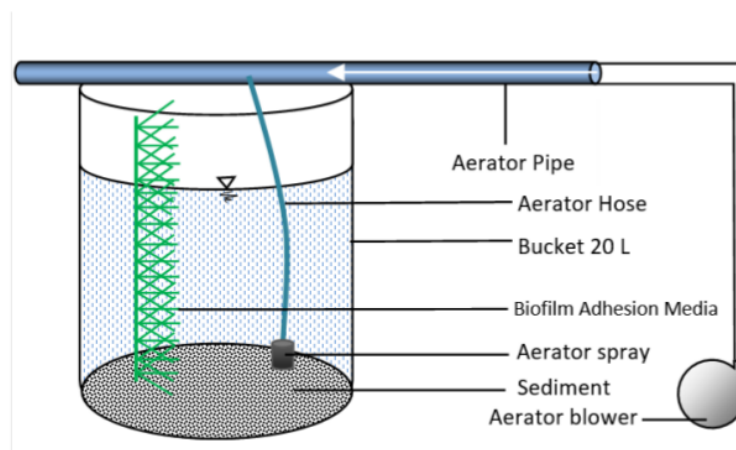


Figure 1. Reactor Design

In this study there are three types of variables used, namely is independent variable the sticky media grown in 2 marine waters, namely in the Teluk Awur MSTP Jepara Pier and on Pulau Panjang and the source variations from different pond waters wastes, namely prospective marguensis shrimp ponds and vanamae shrimp ponds. Then, dependent variable dependent variables are variables that are affected or which are the result due to the existence of independent variables. In this study, the dependent variable is the efficiency of changes in the concentration of ammonium. The control variables in this study are as follows pH, temperature, and salinity. The operating pH value is maintained around 6.0-8.5. The operating temperature keeps at about 20-45°C. The salinity value of the reactor is maintained at 23-25 PPT or g/l.

The sticky media is soaked and linked to the pier on two sea waters, namely Panjang Island and Telukawur waters. The sticky media is immersed as deep as 1.5 m in seawater. The sticky media was immersed as deep as 1.5 m because based on previous research that 50 species of trophic bacteria predominate at depths of 1-3.5 m in marine waters (Abdullah et al., 2018). The form of media used is fiber made from plastic in the form of synthetic grass. In shape, this fiber has a full surface and expected that many bacterial biofilms are attached to the fiber. The reactor volume used in this study was made with plastic material with a volume of 20 litres. The reactor is equipped with a hose that flows air into the waterbody which serves to maintain the condition of aerobes and gases do not add pressure to the reactor.

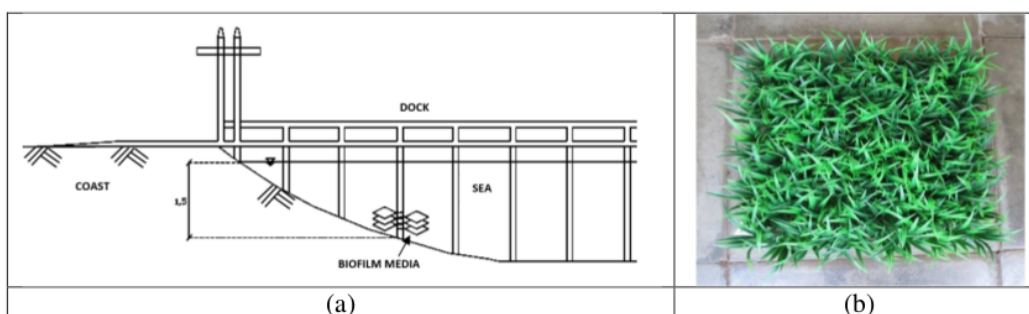


Figure 2. Design of Seeding, (a) Design of Seeding Media on Fork of the Dork, (b) Attached Media Form

The potential test of ammonium removal was carried out to determine the effect of biofilm media forms on treatment organic pond waste. So that the most effective form of media found for changes in ammonium, nitrate, and total organic concentration. Ammonium (NH_4) measurements are carried out by taking 25 ml of clear samples if it is cloudy then filtered first. Then given two drops of seignette salt and 0.5 ml of Nessler reagent. Beat and leave for 10 minutes then measure with a spectrophotometer with a wavelength of 420 nm (SNI 06-6989.30-2005).

3. Result and Discussion

The development of biofilms, at a minimum, is shared four stages, as summarized by Stoodley et al. (2002). Reversible attachment, which is the attachment of a single cell and free movement, initiates the formation of biofilm in the surface. Irreversible attachment, i.e. after non-permanent attachment to the surface turned into permanent adhesion. Bacteria must maintain contact with the substratum. Maturation - i.e. maturation during maturation, biofilms produce pores and resettlement of the bacteria that could be separated from the material. Detachment commonly described as cell release whether it's a single cell or a group of biofilms. The loose cell is believed to be the cover for the biofilm growth cycle (Stoodley et al., 2002). The process of biofilm formation can be seen in Figure 1.

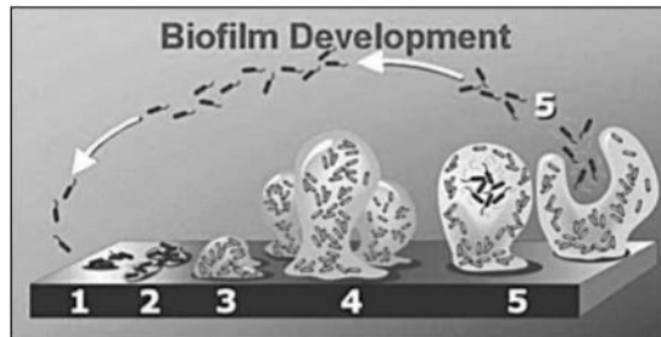


Figure 3. Biofilm Development, (1) Non-permanent, (2) Permanent, (3) Maturation, (4) Detachment, (5) Closure of Cycle

Source: Stoodley et al., 2002







Sources of seeding bacteria select from two different sources, namely biofilms placed at Pulau Panjang pier and Teluk Awur. From the sources of attachment media, biofilms attached to plastic media in the form of grass are obtained. The plastic media is kept in batches for three weeks with a depth of 1.5 m above sea level. Seeding biofilms was carried out for three weeks, and once a week, a sample characteristic was tested for the seawater:

Table 1. Sea Water Characteristics of Seeding Sticky Media

Sample	pH	Salinity (g/L)	Temperature (°C)
Pulau Panjang	01/12/2018	8,19	31,9
	08/12/2018	8,21	36,9
	17/12/2018	8,31	39,4
Telukawur	01/12/2018	8	32,0
	08/12/2018	8,21	33,3
	17/12/2018	7,98	39,0

Based on the data in table 4.1 pH in Pulau Panjang waters around 8.1-8.3, the highest salinity is 394 ppm with a temperature range of 25-30°C. Whereas in Telukawur waters it has a pH ranging from 7.9-8.2, the highest salinity is 390 ppm, and the temperature range is 27-31°C. This value indicates that the pH of water is normal. The normal pH situation allows reducing ammonia and nitrate bacteria to grow. In the salinity parameter, the salinity concentration of Panjang Island waters is higher than that of Telukawur waters. This caused by Telukawur near the mainland of Java island, which has high rainfall and rainwater runoff from the mainland to the river and empties into the sea. In Pulau Panjang waters which are 4.73 Km from Telukawur, the salinity is higher but not too far from the salinity in Telukawur waters.

Table 2. Sticky Media Development

Location	Date		
	December 1, 2018	December 8, 2018	December 17, 2018
Panjang Island			
Teluk Awur			

It is seen from Table.2 that biofilms are thickening in the 2nd and 3rd week. And in the photo in Telukawur you can't get maximum and clear photos due to high turbidity so that visibility is limited. In the picture on Pulau Panjang waters look clearer than Telukawur waters because the sediment in Telukawur is more than in Pulau Panjang. This because Telukawur receives sediment from the land with rainwater runoff from the mouth of a small river in the vicinity of the Telukawur District.

Acclimatization was carried out using buckets containing substrate of sticky media in seawater moved in the outdoor laboratory of BBPBAP Jepara then fitted with aeration with smooth bubbles. Before shrimp pond wastewater is applied to the sticky media worn on the biofilm in the reactor the media water sample is taken first to determine the content of ammonium, nitrate and total organic to test the initial characteristics of the waste. Then the running process is carried out according to the time of water contact with the desired shrimp pond liquid waste. Observations were carried out for 15 days, and sampling was carried out every morning at 07.00 am. The waste is put into the reactor only once when the initial collection is 18 litres. The process of running is carried out constantly and continuously until the research is complete. Water samples that will be measured are taken from media water every day for 14 days. Taking water samples is taken directly from the reactor using a 300 ml plastic bottle. After that, it was taken using a styrofoam box and then sent to Semarang to be analyzed in the Environmental Engineering Water Laboratory of Diponegoro University.

At the acclimatization stage, the waste was carried out in the BBPBAP Artificial Feed Wet Laboratory, Jepara. The stage is to remove the biofilm first and then immediately apply it to a bucket containing shrimp pond liquid waste and given aeration for 14 days. For aerobic processing, it is necessary to add aeration, which is exhaled by air from the blower that is already available in the wet laboratory and only has to run the hose to the entire reactor using a spray aerator. There are 12 reactors used, and there are two types of reactors that do not use biofilm treatment using the only aeration as control whether the biofilm works optimally or the only aeration works to reduce ammonium. Plastic biofilm media that has been treated with seeding is dipped into wastewater that is already available in the reactor. Based on observations at the time of dyeing plastic media from the waters of Panjang Island, there were small shrimp that could not be removed and were seen seizures when in contact with the wastewater. This is because the wastewater contains toxic water which makes it unhealthy, so

it is thought to have a high toxic content for aquatic biota. Acclimatization is carried out for 14 days and tested for chemical and physical characteristics every day.



Figure 4. Acclimatization of Wastewater on Reactor, (a) Before Treatment, (b) After Treatment

The process of aerobic biological wastewater treatment is to utilize aerobic microbial activity in aerobic conditions to decompose organic matter contained in wastewater into inorganic substances in a stable state and reduce the impact of pollution on the aquatic environment. Basically, in the aquatic environment itself, there are aerobic bacteria to degrade organic matter naturally. Still, in a limited capacity and with a high quantity of pond wastewater from post farm ponds, wastewater treatment is needed to maintain water quality at the wastewater outlet that will be released in the water. In handling wastewater, bacteria play an important role because bacteria can be used to degrade organic materials and unwanted minerals from this water. In general, bacteria are chemoautotrophic, namely, bacteria that use organic matter as a source of energy and carbon. These bacteria are those that oxidize reduced inorganic compounds such as NH for energy and use CO₂ as a carbon source. Chemoautotrophic bacteria are the most important bacteria in wastewater treatment because these bacteria will break down organic matter, oxidize ammonium, nitrogen to nitrogen nitrate by nitrifying bacteria.

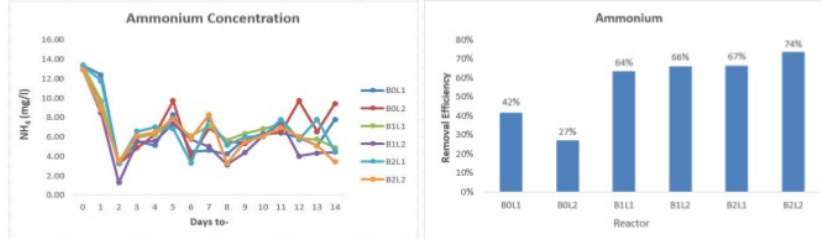


Figure 5. Ammonium Result Data: 1.Efficiency Ammonium. 2. Ammonium Concentration.

High levels of ammonium in waterbody can endanger aquatic biota. Oxygen supply is needed to convert ammonium to nitrate so that this process can reduce DO in water. Ammonium is very toxic to biota in water, but normal amounts of nitrate do not harm organisms that live in water. High levels of ammonium in waterbody can endanger aquatic biota. Oxygen supply is needed to convert ammonium to nitrate so that this process can reduce DO in water. Ammonium is very toxic to biota in water, but normal amounts of nitrate do not harm organisms that live in water.

There are many chemicals and biological factors that can affect growth and affect the performance of nitrifying bacteria. The most important factors are biochemical processes such as pH, temperature and salinity. Then the supply of nutrients to biofilms such as substrate and DO also affects the rate of nitrification (Chen et al. 2006 in Sudarno, 2012).

Data collection resulted from testing for ammonium allowance as the dependent variable. While the independent variable is in the form of variations, location of the immersion media attached to the sea waters of Pulau Panjang and Teluk Awur waters. Ammonium concentrations have different treatments giving a significant effect on ammonium concentration. Different waste treatments have no significant effect on the concentration of ammonium substances. The interaction of different biofilm and wastewater treatments gave a significant effect on ammonium concentration.

Recommendations for waste treatment installations consist of 4 processing units, namely sedimentation units, anaerobic units, aerobic units, and effluent units. This processing is explained in figure 6.

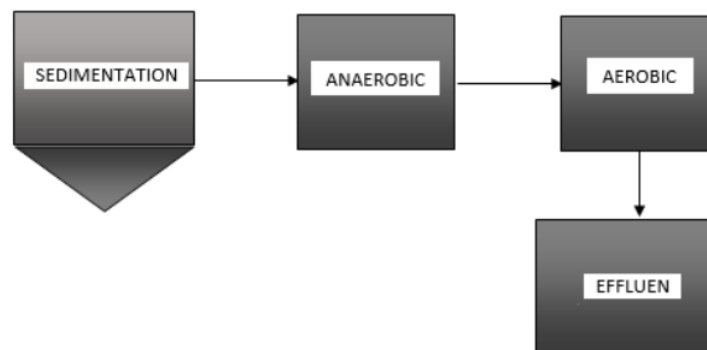


Figure 6. Flowchart of the Wastewater Treatment Plant

The flow chart in Figure 6 explains that waste treatment through 4 units is sedimentation, anaerobic, aerobic and effluent. The discharge of sewage entering the processing of $225 \text{ m}^3 / \text{sec}$ is taken from 10% of discarded pond waste per 1-3 days. So that the dimensions and residence time needed by each processing unit are found. A first unit is a sedimentation unit with dimensions $p \times l \times t$, which is $15.5 \text{ m} \times 7.7 \text{ m} \times 1.5 \text{ m}$ with a detention time of fewer than 2 hours. A second unit is an anaerobic unit with dimensions of $p \times l \times t$, which is $10.5 \text{ m} \times 3.5 \text{ m} \times 1.5 \text{ m}$ with a detention time of less than one day. A third unit is an aerobic unit with dimensions of $p \times l \times t$, which is $13 \text{ m} \times 13 \text{ m} \times 1.5 \text{ m}$ with a detention time of 1.5. A last processing unit is an effluent unit with dimensions $p \times l \times t$, which is $15.5 \text{ m} \times 7.7 \text{ m} \times 1.5 \text{ m}$ with a detention time of fewer than 2 hours. This design was made as a recommendation for planning a waste treatment plant in brackish water pond wastewater.

4. Conclusions

Based on the results of the analysis and discussion of the effect of the inoculant variations on the sticky media in the waters of Panjang Island and Telukawur by the removal of organic ammonium and total on native shrimp farm waste it can be concluded that Variations in the source of inoculant adherent media have an influence on the removal of ammonium and organic total for 14 days. The inoculant source of the adhesion media of Telukawur waters has the effect of removing ammonium from vaname shrimp pond waste with an efficiency of 74% with a reduced rate $0.68 \text{ mg} / \text{l}$ per day. Then it can also remove ammonium in marguensis waste by 74% with a reduced rate of $0.68 \text{ mg} / \text{l}$ per day. Analysis of the effect of biofilm adherent media inoculant is sourced on the efficiency of organic ammonium and total removal in shrimp pond waste the inoculant source of Pulau Panjang watershed

media has a thickness of biofilm that is not too thick but has controlled nitrate concentration, it is thought that biofilms have a balanced ability to process nitrification and denitrification. So it is assumed that the denitrification process does not work well even though the biofilm looks very thick. The application of brackish water aquaculture is designed with four processing units namely sedimentation bath 15.5 mx 7.7 mx 1.5 m, like anaerobic 10.5 mx 3.5 mx 1.5, like an aerobic 13 mx 13 mx 1, 5 m, and effluent bath 15.5 mx 7.7 mx 1.5 m.

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