Effectiveness of bacterial attachment media to reduce organic matter from wastewater of catfish aquaculture

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Abstract. Sangkuriang catfish aquaculture produces waste in the form of organic matter from the remaining feed and metabolic waste. Previous study revealed that the organic matter found in the outlet part of the fishpond was three times higher than the inlet. The attempt to decrease organic matter in that study was performed using freshwater clam and Bacillus subtilis bacteria; however, the decrease was less than 80% within 5 days. This study aims to obtain a maximum decrease level of organic matter with the fastest time of degradation. The experimental method with a Completely Randomized Plot Design (Split Plot Design) was employed in this study. The primary treatments (main plot) were time while the sub-plot was the surface area of bacterial attachment media. The time was set within 72 hours with an observation interval of 12 hours. The areas of the attachment media were 25%, 50%, 75%, 100% of the surface diameter of the experimental pail. The attachment media used in this study was synthetic coir. The control treatment was done without the attachment media (0%). The experiment was conducted in a laboratory under a facultative anaerobic condition. The level of organic matter and the total of bacterial suspension (TPC) in the water were observed every 12 hours. Organic matter was measured using a KMnO4 oxidizer. The results study indicate that adhering 100% of the surface of the attachment media decreased organic matter by 93.7% within 72 hours (three days). The total bacteria in water media within 12 hours was 0.5x10^2 CFU/ml, while the total bacteria within 72 hours was 26x10^2 CFU/ml. It can be concluded that the utilization of the bacterial attachment media has accelerated the decrease of organic matter in the wastewater of Catfish aquaculture with a stable amount of bacterial suspension.

1. Introduction

Aquaculture wastewater cannot be discarded directly to public waters since it contains a relatively high amount of organic matter. The amount of organic matter in the wastewater is approximately three times higher compared to organic matter content in inlet (before being used for aquaculture activities) [1]. The decomposition process of organic matter in water will decrease dissolved oxygen. Low oxygen concentration in public waters due to organic matter oxidation will affect the life of aquatic organisms. In this case, aquatic animals cannot get enough amount of oxygen. Therefore, organic matter from wastewater should be decreased as much as possible before being discarded into public waters. Organic matter in the wastewater can be in the form of remaining feed, urine and feces of fish or aquatic animals. The accumulation of organic matter can occur at the outlet of the aquaculture pond, following the shape of the pond structure, which allows the water flow.

An attempt to decrease organic matter in wastewater from catfish aquaculture was carried out by Budi et al., [2] using freshwater clam (Anodonta woodiana) as the biological agent. The results indicated that there was a reduction of organic matter by 30% from 102.7 mg/L to 70-80 mg/L within 9 hours with 75% shell density. However, using clam as the biological agent is less efficient due to its...
low ability in decreasing organic matter. Research to reduce organic matter in wastewater from catfish aquaculture using *Bacillus subtilis* as the bacterial agent with a density of 106 CFU/ml was also been done previously [3]. Water samples were obtained from catfish aquaculture ponds that have been used for about 3 months. Organic matter found in wastewater from catfish aquaculture was 80.26 mg/L. The results revealed that the organic matter content decreased and there was only 13.03 mg/L of organic matter left after 5 days of incubation time. Based on this result, the reduction in organic matter level could reach 83.8% after 120 hours. The decrease in organic matter also occurred before 5 days, but it has been not optimal yet.

*Bacillus subtilis*, as the organic decomposing agent in the previous study, was not able to decrease organic matter levels in a short period. Both of the studies from other research by Budi et al., [2] using freshwater clam and using *Bacillus subtilis* involved bioreactors that utilized organic matters suspended in the water [3]. Likewise, the bacteria that grew on the media were also suspended. The results from those studies show that they still need up to 5 days to get the lowest level of organic matter. The result of another research indicated that *Bacillus subtilis* bacteria could change cellulose organic matter into simple carbohydrates and the number of the bacteria was stable with the growth phase at 23 to 37 hours [4]. Carbohydrate, fat and protein were found in catfish aquaculture wastewater and the amount of these compounds decreased after 5 days in the media with *Bacillus subtilis* [5].

This study aims to analyze the effectiveness of bacterial attachment media to decrease organic matter in the wastewater of catfish aquaculture. The bacterial attachment media were provided in the experiment pails. The provision of attachment media was intended to provide growing media for the bacteria, and thus, the total amount of bacteria would be higher. Coir has narrow cavities that can be utilized by facultative anaerobic bacteria as the growing media. More bacteria were expected to increase the degradation of organic matter in the experimental media. Eventually, faster degradation time of organic matter can be obtained.

2. Materials and method

This study used 10 L pails filled with 4 L of wastewater from Sangkuriang catfish (*Clarias gariepinus*) aquaculture. The experiments were conducted without aeration. The bacterial attachment media were prepared with different areas (i.e., 25%, 50%, 75% and 100% of the pail diameter). Media were placed in the provided pails (in the middle/water column). The control treatment was carried out without bacterial attachment media (control 0%). The attachment media used in this study was synthetic coir (Figure 1 and Figure 2). The purpose of the attachment media addition into the experiment pail is to let the existing bacteria in the water to grow on the media so that bacteria did not only grow as a suspension in water. Coir has narrow cavities that can be utilized by facultative anaerobic bacteria as the growing media.

![Figure 1. Synthetic coir used as attachment media.](image1)

![Figure 2. Positioning of coir on a research pails.](image2)
Organic matter and the bacterial amount (TPC) were observed for 72 hours every 12 hours. Determination of the 72 hour observation time was based on the result of other research [6], which mentioned that the reduction of the organic matter would be effective within 3 days. Total organic matter (TOM) was measured by oxidizing KMnO₄. The study was carried out by employing an experimental method using Completely Randomized Design (CRD). The treatments of subplot were the differences in the areas of attachment media and time as the main plot.

3. Results and discussion

3.1. Total organic matter
Organic matter content in the wastewater of Sangkuriang catfish (Clarias gariepinus) aquaculture decreased by 93.7% after treatment with 100% attachment area from the initial level of 73.31 mg/L to 4.63 mg/L after 72 hours. The addition of attachment media could be utilized by the existing bacteria as a new place to grow. The growth of these bacteria could increase the utilization of organic matter, so the level of organic matter decreased.

Change in organic matter is a biochemical reaction catalyzed by extracellular enzymes and released by bacteria for breaking down complex compounds into simpler compounds [7]. In this study, organic matter could decrease from the initial treatment until the 72 hour. The decrease in organic matter can be explained as the presence of new media allows bacteria to grow in accordance with the initial growth process [8]. Bacterial growth in certain media requires a period called the maturation process. Organic matter stored in the media in a bioreactor will continue to be used by the bacteria until the organic matter runs out within a particular time. The decreasing level of the organic matter can be seen in Figure 3.

![Figure 3. The decreasing level of the organic matter.](image)

The attachment media can be used by various types of bacteria (heterogeneous) that exist in the water for growth. The bacteria will support the decomposition process of organic matter due to the ability of bacteria to utilize organic matter in water media. Bacteria associated with each other to form micro colonies. Some of the bacterial cells can be permanently attached to the surface of the material through the formation of Extracellular polymeric substance (EPS) consisting of large amounts of protein, polysaccharides, nucleic acids and phospholipids. EPS relates to cell surfaces in bacteria [9].

3.2. Total bacteria amount
The decrease in organic matter in wastewater from Sangkuriang catfish (Clarias gariepinus) aquaculture was due to the ability of indigenous bacteria from the wastewater itself, which were given some treatments of different areas of attachment media for bacteria to grow. Based on Figure 4, from 0
hour to 12 hour, indigenous bacteria were in the adaptation phase. In this phase, bacteria required
habitat and feed on the previous environment to the current environment.

![Figure 4. Total bacteria number during the experiment.](image)

Bacteria that are transferred into certain media will first undergo an adaptation phase to adapt to the
surrounding environmental condition [10]. Furthermore, bacteria will grow and use existing nutrients;
and the exponential or log phase can occur from the 24 hour to the 36 hour. Cells that have adapted to
the environment or new medium will begin to develop and divide exponentially (the exponential phase
or the log phase) [11]. The rate of bacterial growth depends on the environmental condition. If the
environment has a poor level of nutrition, then the bacterial growth will be slower. At the 36-hour, the
bacteria are in a stationary phase i.e., the number of bacterial cell populations will remain constant
because the number of growing cells is the same as the number of dead cells [12].

Furthermore, at the 36-hour to 48-hour, bacteria enter the phase of death, as evidenced by a
decline in the number of bacteria. The decline in the number of bacteria is due to a decrease in the
number of nutrients and dead microbial cells will undergo lysis [13]. In the next hours, the number of
bacteria increases again due to the presence of bacterial cells which grow back with nutrients from
dead bacterial cells and are reused by living bacteria [14].

3.3. Water quality parameters
Quality parameters of water media were observed every 12 hours during the observation period (72
hours). They included temperature, pH and dissolved oxygen. The results of temperature
measurements ranged between 25.3 to 28.9°C. The optimum temperature range during degradation
process is generally 25 to 30°C. The pH value during the study was relatively stable in the range of 7
to 8. The facultative anaerobic condition was implemented so that dissolved oxygen for 72 hours was
relatively low, ranging from 0.7 to 2.8 mg/L (Table 1). Facultative anaerobic microorganisms work in
a state of limited dissolved oxygen [15].

| No | Parameters                  | Treatment               | Observation Period (Hour) |
|----|-----------------------------|-------------------------|---------------------------|
|    |                             | Control                 | 12 | 24 | 36 | 48 | 60 | 72 |
| 1  | Temperature                 | Control                 | 26.8 | 25.6 | 25.5 | 25.3 | 26 | 25.6 |
|    |                             | Attachment Media 25%    | 26.8 | 25.4 | 25.6 | 25.3 | 26.3 | 25.7 |
|    |                             | Attachment Media 50%    | 28.7 | 25.8 | 25.7 | 25.3 | 26.6 | 25.5 |
4. Conclusion
This study analyzed the influence of bacterial attachment toward decreasing level of organic matter in the catfish aquaculture wastewater. The highest reduction of total organic matter (93.7%) was observed in the treatment of 100% area. The organic matter content decreased from 73.31 mg/L to 4.63 mg/L after 3 days (72 hours). It was suggested that the presence of the bacterial attachment media was fundamental in decreasing organic matter in the wastewater of catfish aquaculture.

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