# Ozone Technology for Pathogenic Bacteria of Shrimp (Vibrio sp.)

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## Ozone Technology for Pathogenic Bacteria of Shrimp (Vibrio sp.) Disinfection

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#### Abstract

One of important marine commodities in Indonesia, shrimps are susceptible with Vibrio sp bacteria infection. That infection must be cleared. One of the technologies for disinfecting Vibrio sp, is a zone technology. In this research, Vibrio sp, is a pathogenic bacterium which infects Penaeus vannamei. Ozone technology is applied for threatening Vibrio sp. In this research, ozonation was performed in different pH. Those are neutral, acid (pH=4), and base (pH=9). The sample was water from shrimp embankment from Balai Besar Perikanan Budidaya Air Payau (BBPBAP) located in Jepara. That water was the habitat of Penaeus vannamei shrimp. The brand of ozonator used in this research was "AQUATIC". The used ozonator in this research had 0,0325 g/hour concentration. The flow rate of sample used in this research was 2 L/minute. The ozonation process was performed in continuous system. A tank, pipe, pump, which was connected with microfilter, flowmeter and ozone generator were the main tools in this research. It used flowmeter and valve to set the flow rate scalable as desired. The first step was the insert of 5 L sample into the receptacle. Then, by using a pump, a sample supplied to the microfilter to be filtered and passed into the flow meter. The flow rate was set to 2 LPM. Furthermore, gas from ozonator passed to the flow for the disinfection of bacteria and then was recycled to the tank and the process run continuously. Samples of the results of ozonation were taken periodically from time 0, 3, 7, 12, 18, 24 to 30 minutes. The samples of the research were analyzed using Total Plate Count (TPC) test in BBPBAP Jepara to determine the number of Vibrio sp. bacteria. The result of this research was the optimal condition for pathogenic bacteria of shrimp (Vibrio sp.) ozonation was in neutral condition.

Keywords: pH, ozone, vibrio sp, shrimp, disinfection, shrimp embankment, pathogenicic bacteria;

#### 1. INTRODUCTION

Indonesia is a maritime country which has abundant marine commodities. One of the important export commodities in Indonesia is shrimp. There are two species of shrimp which are cultivated in Indonesia; they are Penaeus vannamei and Penaeus monodon (the popular name in Indonesia is Windu shrimp). Some regions sizable shrimp producer in Indonesia are Lampung, Maluku, Jepara, and Kendal. Those shrimps are susceptible with bacteria infection. Bacteria that attack is Vibrio sp, and viruse that attack shrimp is Myo. The cause of shrimp diseases discussed in this study is the Vibrio sp bacteria. The bacteria are pathogenic bacteria, comes from the family Vibrionaceae with curved rod shape such as a comma. The bacteria are gram-negative has the properties to the size of 2-3 µm [1]. The shrimp were infected with these bacteria generally can be identified by some of the symptoms that can be seen with the naked eye, namely: shrimp looks weak, dark red or pale, and on the antennae and legs of shrimp swimming in red. Pathogenesis of vibriosis, bacteria enter through the blood and circulation of tissue causes damage and inflammation of the blood vessels, skin, and base of the fins and followed by infection of the heart [2].

In the shrimp farm in the Sidorukun Village, Kendal found the Vibrio sp bacteria of  $3.5 \times 10^2$  CFU/mL in 2014 (Report on Laboratory Test Results BBPBAP Jepara 2014), and increased in 2016 with the number of bacteria  $7.8 \times 10^3$  CFU/mL (Report of Laboratory Test Results BBPBAP Jepara in 2016) caused by embankment water contaminated with Vibrio sp bacteria. The maximum of Vibrio sp. in embankment water is  $1\times10^2$  CFU/mL [3]. The Vibrio sp. bacteria arises because water quality in embankments that are not good.

The presence of bacteria and viruses on shrimp make shrimp die and will have an impact on the harvest of shrimp. That causes Indonesia's per capita income will be reduced and will reduce the level of the Indonesian economy. Hence the need for a technology to disinfect shrimp embankments water systems

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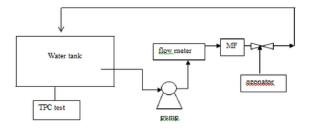
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containing bacteria and viruses so that the water quality of embankment where shrimp live to be more qualified. This causes the shrimp survival and better quality so that more abundant harvests shrimp and the Indonesian economy will increase. In this research, microorganism that cause disease in shrimp is Vibrio sp. bacteria.

Several methods to reduce the number of Vibrio sp bacteria has been carried out, among others, is a filtration method that has been used to reduce the number of Vibrio sp bacteria [4] and obtained the resulting decline in the number of Vibrio sp from 10<sup>4</sup> to 10<sup>3</sup> CFU/mL. Disinfection of Escherichia coli bacteria in water using ozone technology has also been done by Wulansarie [5] and prove that the technology can be applied in water treatment processes to inactivate bacteria by 10<sup>6</sup> to 10<sup>1</sup> CFU/mL. Additionally, Mukti [6] has also been conducting research to increase shrimp production return 100% of the previous began to fall due to virus Infection Myo Necrosis Virus (IMNV) technology using ozone. Based on background of study, ozone technology will be applied to reduce the number of bacteria Vibrio sp. Ozone technology acts as a disinfectant to inactivate bacteria, fungi, and other microorganisms. The sample was water from shrimp embankment from Balai Besar Perikanan Budidaya Air Payau (BBPBAP) located in Jepara. The system used in this study is continuous. The study was conducted using a variety of disinfection and pH conditions of embankment water. Observations were made on the number of Vibrio sp before and after disinfection and analyzed using the methods Total Plate Count (TPC), which was conducted in the BBPBAP Laboratory, Jepara. It is expected ozone technology can be a solution to overcome the problems of crop failures in shrimp aquaculture caused by pathogenic bacteria is Vibrio sp.

#### 2. METHODOLOGY/ EXPERIMENTAL

Ozone technology is applied for threatening pathogenic bacteria of shrimp, Vibrio sp. In this research, ozonation was performed in different pH. Those pH are neutral (water shrimp embankment without treatment), acid (pH=4), and base (pH=9). The sample was water from shrimp embankment from Balai Besar Perikanan Budidaya Air Payau (BBPBAP) located in Jepara. That water was the habitat of Penaeus vannamei shrimp. The brand of ozonator used in this research was "AQUATIC". The used ozonator in this research had 0,0325 g/hour concentration. The flow rate of sample used in this research was 2 L/minute. The ozonation process was performed in continuous system. A tank, pipe, pump, which was connected with microfilter, flowmeter and Ozone generator were the main tools in this research. Microfilter containing membrane 10 micron used to filter pollutant. This research used a form of variable pH of sample. It used flowmeter and valve to set the flow rate scalable as desired. The first step was the insert of 5 L sample into the receptacle. Then, by using a pump, a sample supplied to the microfilter to be filtered and passed into the flow meter. The flow rate was set to 2 LPM. Furthermore, gas from ozonator passed to the flow for the disinfection of bacteria and then was recycled to the tank and the process run continuously. Samples of the results of ozonation were taken periodically from time 0, 3, 7, 12, 18, 24 to 30 minutes. In this case the samples tested at acidic and alkaline conditions, to determine the optimal pH of embankment water for disinfection using ozone. Condition of pH was 4 for acidic and 9 for alkaline. The research equipment system can be seen in Picture 1.



Picture 1. Equipment system for Vibrio sp. disinfection

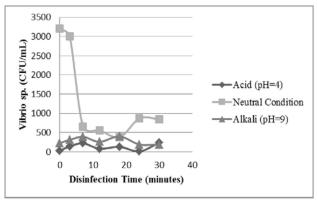
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After disinfected by ozone, the sample will be analyzed. The samples of the research were analyzed using Totat Plate Count (TPC) test in BBPBAP Jepara to determine the number of Vibrio sp. bacteria in the samples.

#### 3. RESULTS AND DISCUSSION

The result of this research can be seen in Picture 2. From the picture we can see the effect of ozone for Vibrio sp.



Picture 2. The result of disinfection Vibrio sp. using ozone

From Picture 2 we can see the optimum degradation of Vibrio sp. bacteria. That condition was in neutral condition, the condition that there wasn't no treatment in the samples. That neutral condition has pH=6,7. In that condition degradation of Vibrio sp. reach until 73.4%. The number of Vibrio sp. in final disinfection process was 850 CFU/mL. That number of Vibrio sp. still exceed the maximum limit [3]. According Peratitus [7] there are three ozonation; direct ozonation (in acid condition/pH<4), ozonation by ozone and OH radical (in pH= 4-9), and ozonation by OH radical (p>9). According Manley and Niegowski [8], ozonation by OH radical is more powerfull rather than ozonation by ozone.

Number	Active species	Redox Potential (mV)
1	Fluorin	+3,06
2	Radikal hidroksil (OH*)	+2,80
3	Ozon (O <sub>3</sub> )	+2,07
4	Hidrogen peroksida (H <sub>2</sub> O <sub>2</sub> )	+1,77
5	Permanganat	+1,67
6	Klor dioksida	+1,50
7	Asam Hipoklorit	+1,49
8	Gas Klorin	+1,36

Table 1. Reduction and Oxidation Potential [8]

The reduction and oxidation potential of ozone and OH radical can be seen in Table 1. So from that table we can see that the ozonation process will be better in neutral condition (pH= 4-9) because OH radicals and ozone had synergy for degradation of Vibrio sp. bacteria. After disinfection the number of bacteria was decrease so that it is suitable with Dahuri [9] "water quality is one of the keys to success in shrimp farming". The research result also suitable with Graham [10] that say "ozone, UV rays, microfiltration is

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effective for removal of E. coli bacteria and all coliforms. Ozone can be produced by corona discharge [11]. Ozone can act as a disinfectant against pathogens, reducing taste and odor and the ability to oxidize compounds [12] which suitable with the research.

#### 4. CONCLUSION

From this research can be conclude that the optimal condition for pathogenic bacteria of shrimp (Vibrio sp.) ozonation was in neutral condition of samples (pH=6,7).

#### 5. ACKNOWLEDGEMENT

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