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Submission date: 03-Oct-2018 09:10PM (UTC+0700)

Submission ID: 1013021582

File name: ga_2018_IOP_Conf._Ser._3A_Mater._Sci._Eng._316_012067-2-7_1.pdf (603.26K)

Word count: 3224

Character count: 16007

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Ozone Disinfection of *Vibrio vulnificus* in Shrimp Pond Water

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Abstract. One variety of shrimp, *L. Vanamei*, often uses brackish water during the operation in the shrimp pond. Chlorination and ultraviolet are usually used for disinfection of brackish water. However, it is ineffective and forms sediment in the water distribution. It can be a negative impact on the water quality cause a contamination on the shrimp, so the farmers might have loss of profit because *Vibrio vulnificus* causes infection and dead on the shrimp. It affects the safety of consumers and should be minimized. The purpose of this study is to reduce the number of *V. vulnificus* bacteria in the pond water. The water was put in the storage tanks then pumped to filter out the impurities of the water. Furthermore, the water set the flow rate in 1 LPM, 2 LPM, and 3 LPM. After that, the ozone was injected to the water flow to sterilize the *V. vulnificus* bacteria. Finally, the water was returned to the original tank. The water from the tank was taken through a valve and analyzed in 0, 3, 7, 12, 18, 24, 30 minutes. The sample was analyzed immediately using a Total Plate Count method to determine the number of *V. vulnificus* bacteria in the shrimp pond water. The flow rate shows that the longer time of ozone made a lower amount of *Vibrio v.* bacteria. In 2 LPM water, it shows the optimum results of *V. vulnificus.* bacteria reduction for 88.1% compared to the flow rate of 1 LPM and 3 LPM with the bacteria reduction of 68.8% and 70.6%. This study shows that the ozone with a flow rate of 2 LPM circulation is the most effective method to help reducing the number of *V. vulnificus* in brackish water distribution system in the shrimp environment and potentially as a disinfectant.

Keywords: Flow rate; Ozone; Shrimp pond water; *Vibrio vulnificus*

1. Introduction

Indonesia is a country of *L. vannamei* shrimp exporter in the world. State competitors China, Vietnam, Thailand have been out of business in exporting shrimp *L. vannamei*. Main export destination countries of Indonesia *L. vannamei* is America and Japan. This causes the shrimp production was decreased till 50% of production and the impact on exports. The production of shrimp in Indonesia and provide greater foreign exchange for the country. Many aquatic organisms are cultured experienced the mass death causing high economic losses caused by being infected with pathogenic microorganisms [1]. Various factors that cause crop failures that occur in shrimp ponds in Indonesia and resulted in the production of shrimp needs to be followed up. Problems often occur shrimp farm is an attack infectious *Vibrio vulnificus*. Vibriosis disease caused by the bacterium *Vibrio sp* cause of death in the shrimp in a short time. A pathogenic of bacteria comes from families Vibrionaceae with curved rod shape such as a comma. These bacteria has the properties gram negative with the size of 2-3 μm [2]. The shrimp were infected with these bacteria generally provide some clinical 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.

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Based on previous research, ozone has been demonstrated to exterminate a wide range of viruses, including Venezuelan equine encephalomyelitis, hepatitis, influenza, vesicular stomatitis, infectious bovine rhinotracheitis virus [3]. Ozone is used to treat the human pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Salmonella typhi*, and *Klebsiella pneumoniae* by using ozonizer [4,5]. Ozone is also used to analyze the ability of the virus to defend on the fungus *Alternaria solani* conidia Sorauer [6]. The results obtained proved lethal activity of ozone and provide precise data on ozone concentration times exposure. The research has been give that ozone technology can be applied to areas that have a virus, bacteria, also fungi. Because of its high oxidation potential, ozone oxidizes cell components of the bacterial cell wall to reduce the number of microorganisms become inactive. This is because ozone technology disrupts the integrity of the bacterial cell envelope through oxidation of lipoproteins and phospholipids.

Several methods to reduce the number of bacteria *Vibrio sp* has been carried out, among others, is a filtration method that has been used to reduce the number of bacteria *Vibrio sp* [7] and obtained the resulting decline in the number of *Vibrio sp* to 103 CFU / mL. Disinfection of *Escherichia coli* bacteria in water using ozone technology has also been done and prove that the technology can dilaplikasikan ozone in water treatment processes to inactivate bacteria. Further research to increase shrimp production again can be done by reducing the effect of Infection Myo Necrosis Virus (IMNV) using ozone technology. Based on these studies used ozone from the ozone generating devices are commonly referred to as ozone generator (ozonator). The success of ozone technology is further adopted to reduce the number of bacteria *Vibrio sp*. The ability to reduce the number of both bacterial or viral microorganisms using ozone is quite good, so in this study will be used the same technology that ozone technology. Ozone technology can be used as a disinfectant to inactivate bacteria, fungi, and other microorganisms. This research is used to reduce *V. vulnificus* artificial shrimp ponds of water, because it will determine a decrease in the number of theses bacteria alone. The system used in this study is continuous. Artificial pond water is passed on ozone by varying the contact time.

The aim of research conducted with using a variation of flow rate as well as the pond water disinfection time progresses. Observations were made on the number of *V. vulnificus* before and after disinfection and analyzed using the methods Total Plate Count (TPC). It is expected ozone technology can be a solution to overcome the problems of crop failures in shrimp aquaculture caused by pathogenic bacteria is *V. vulnificus*. In this research, the number of bacteria *Vibrio sp* contained in shrimp pond water vibriosis lowered to reduce diseases that attack the shrimp so that it can increase the harvest of shrimp.

2. Methodology/Experimental

Ozone technology is applied for disinfection of pathogenic bacteria in shrimp pond water. The *Vibrio vulnificus* was provided by the Balai Besar Perikanan Budidaya Air Payau (BBPBAP) in Jepara Central Java. The bacterial isolate was inoculated into the nutrient broth using a sterilized loop and was allowed to grow for 24 hours at 37°C in an incubator.

The disinfection process is performed to determine the effectiveness of ozone technology used in the disinfection of *V. vulnificus* bacteria. In this study, water sample containing *V. vulnificus sp* will be analysed in amounts of before and after the disinfection process. The experimental setup consisted of 1 L gas washing bottle with a tap for sampling at different time intervals. Disinfection is done using ozone technology. The process used continuously until the number of *V. vulnificus sp* reached 1000 CFU/mL. The laboratory scale installation used to apply the ozone treatment is shown in Figure 1.

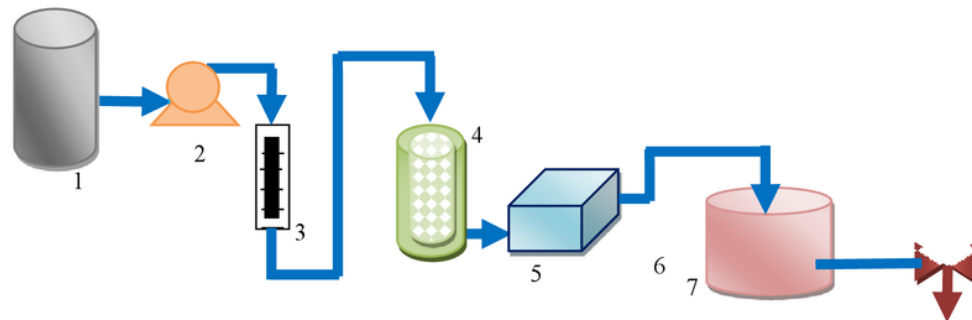


Figure 1 Research equipment system for *V. vulnificus*. Disinfection (1) Shrimp pond water tank, (2) pump, (3) flow meter, (4) micro filter, (5) ozonator, (6) sample valve (7) water tank after disinfection

In the research, oxygen from air is generated to ozone by ozonator (hanaco TSH-278) with flow around 0.0325 g/h. The tank is filled as much as 4 L of shrimp pond water. For the first step was take the initial sample before the disinfection process is carried out and inserted into the sample bottles that have been sterilized, then the ozone pass through to the pump **w** turned on at a total flow rate (ozone and water) of 1, 2, and 3 Liter/min (LPM) interchangeably. The **flow rate of incoming oxygen stream ranged from 1 LPM to 3 LPM**. The next step was turned on the stopwatch. Ozone output after disinfection process for taking sample after 3, 7, 18, 24, and 30 minutes and put in a sterile sample bottle.

Shrimp Pond water samples before and after (valve sample) the disinfection processes in sample bottle were tested using the Total Plate Count (TPC) method to determine the number of bacteria contained. Sterilization of tools used for TPC method and used Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) agar for the media. The decrease in the number of bacteria in the shrimp pond can be calculated by the percentage of bacterial amount after disinfection process to the number of early bacteria present in the shrimp pond.

3. Results and Discussion

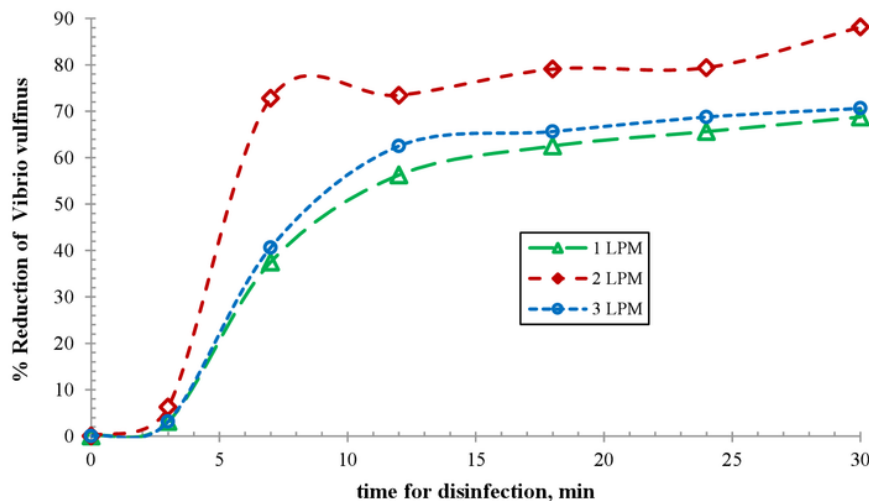
The disinfection process are performed on samples of shrimp pond water. The disinfection process performed using ozone generated by the ozone generator with the time variation of 0, 3, 7, 12, 18, 24, and 30 minutes. In this research using of pH 5. This study also uses a variation of the flow rate of the pump output which amounted to 1, 2, and 3 LPM. Table 1 shows that the total vibrio bacteria on variation of flow rate for time. The shrimp pond water from the tank flows along with the ozone at each flow rate indicates that there is a decrease in the number of bacteria within 3 minutes of the initial bacterial count in the shrimp pond water tank is 3200 CFU/mL. The highest decline occurred in 7 minutes and after that the decline was lower than before. With **1** 30 minutes the decrease of bacterial count reaches 1000 CFU/mL for the flow rate of 1 LPM, 380 CFU/mL for **flow rate of 2 LPM**, and 940 CFU/mL for flow rate of 3 LPM.

Flow rate increased **with the increase of flow rate from 1 LPM to 2 LPM** but decreased at 3 LPM flow rate. This is due to the decrease in ozone used at the LPM flow rate 3 and the ozone residence time in the reactor. However at 1 **1** M flow rate of high ozone level but the reaction damaging bacterial cell wall is still low. Although, dissolved ozone is more effective in reacting with bacteria, the ozone gas is less interactive because less time and much is wasted. Thus, at higher ozone concentrations, ozone may not participate proportionally in the inactivation process due to the constraints of the gas-ozone interaction with the bacteria.

Table 1 Decrease of *Vibrio sp* bacteria on variation of flow rate

Time (Minute)	Total Bacteria (CFU/mL)		
	flow rate 1 LPM	flow rate 2 LPM	flow rate 3 LPM
0	$3,2 \times 10^3$	$3,2 \times 10^3$	$3,2 \times 10^3$
3	$3,1 \times 10^3$	$3,0 \times 10^3$	$3,1 \times 10^3$
7	$2,0 \times 10^3$	$8,7 \times 10^2$	$1,9 \times 10^3$
12	$1,4 \times 10^3$	$8,5 \times 10^2$	$1,2 \times 10^3$
18	$1,2 \times 10^3$	$6,7 \times 10^2$	$1,1 \times 10^3$
24	$1,1 \times 10^3$	$6,6 \times 10^2$	$1,0 \times 10^3$
30	$1,0 \times 10^3$	$3,8 \times 10^2$	$9,4 \times 10^2$

Decrease of bacteria at flow rate 1, 2, and 3 LPM can be seen in Figure 2. Ability of ozone to disinfect water shrimp ozone pond in water at solute condition at pH = 7 indicates that at flow rate of 2 LPM, decrease of bacteria count reach 88,1% compared to 1 LPM reach 68,8% and for flow rate 3 LPM reach 70,6%. Ozone disinfectant power includes oxidation processes to kill bacterial protoplasm, membrane oxidation, and ozonolysis of unsaturated fatty acids which are external membranes. Low ozone concentrations and short contact times are sufficient to disinfect shrimp pond water. Extrapolation of fixed yields should also take into account the weather conditions preferring the adaptation and development of various strains of the most diverse genera. *Vibrio v.* causes red tailed disease in vanamei shrimp which is a severe acute and severe illness that has been detected as the cause of death problems because the shrimp are not resistant to attack from the vibrio bacteria. The dose administered at 2 LPM streams is 0.542 ppm ozone for 30 minutes can decrease the number of *vibrio v.* bacteria up to 88.1% according to the minimal minimum amount of *Vibrio v.* in shrimp ponds is 1000 CFU/mL.

Figure 2. Reduction *Vibrio Vulnificus* at variation flow rate

In Figure 3 shows the effect of ozone on the TPC results for the LPM flow rate of 2 LPM over a certain time interval. In the TPC test it appears that the number of bacteria still alive after the infectious process is evidenced by the presence of white round sign as a bacterium *Vibrio vulnificus*. At increasing time, the number of bacteria on the TPC is less and less. This is because the longer time the meeting between the bacteria and ozone during ozone is not turned into oxygen because the dissolved ozone can turn into oxygen back in 15 minutes [8].

The method for killing *Vibrio v.* bacteria can be done by determining the flow rate of the infected shrimp pond water flow appropriately. The mechanisms were predicted that the bacterial body was known to be protected by relatively dense cell membranes. If the ozone starts to come into contact with the cell wall as it flows with a certain flow rate it will disrupt the metabolism of bacterial cells. The cell wall is very important for bacteria because it is guaranteed the organism can maintain its shape. A sufficient amount of ozone permeates the cell membrane during this flow rate because the continuous system has a contact time between ozone and bacteria during the flow. This can cause bacterial damage. As the ozone molecule makes contact with the cell wall, the oxidative reaction that gives the structure of a small hole in the cell wall. At the same time there is inhibition and blocking the operation of the enzymatic control system. A newly created hole in the cell wall has injured bacteria and bacteria begin to lose its shape while the ozone molecules continue to make holes in the cell wall. If it has several times the ozone collision in a few seconds then the bacteria wall can no longer maintain its shape and eventually the bacteria cells die. The longer the contact time between the ozone produced then the bacteria will be damaged in the cell wall and then will die and found the result that the effective flow rate is 2 LPM. Chlorine is the decontamination agent is the most commonly used in the industry for frozen shrimp to kill potential pathogens. Chlorine completely eliminates this organism so that many dead organisms die. However, long contact with chlorine causes severe respiratory tract damage. There is research that serves to replace chlorine as a disinfectant natural materials chitosan. However, the ability of chitosan as a disinfectant is lower than shrimp pond treatment by using ozone, because with chitosan decrease the bacteria only reach 60% [9]. These results suggest the possibility of using ozone decontamination of pathogenic bacteria in seafood plants, a change that will reduce the health problems of workers.

E. coli bacteria have high sensitivity to ozone treatment and compare with other bacterial strains of *Salmonella typhi*, *Klebsiella pneumoniae*, and *Pseudomonas fluorescens*. The decrease in the number of *E. coli* bacteria reaches 88% for 15 minutes with ozone [5]. Direct count method indicates that after disinfection, the bacterial cell loses its ability to grow. This is almost the same as the bacterial sensitivity *V. vulnificus* against ozone.

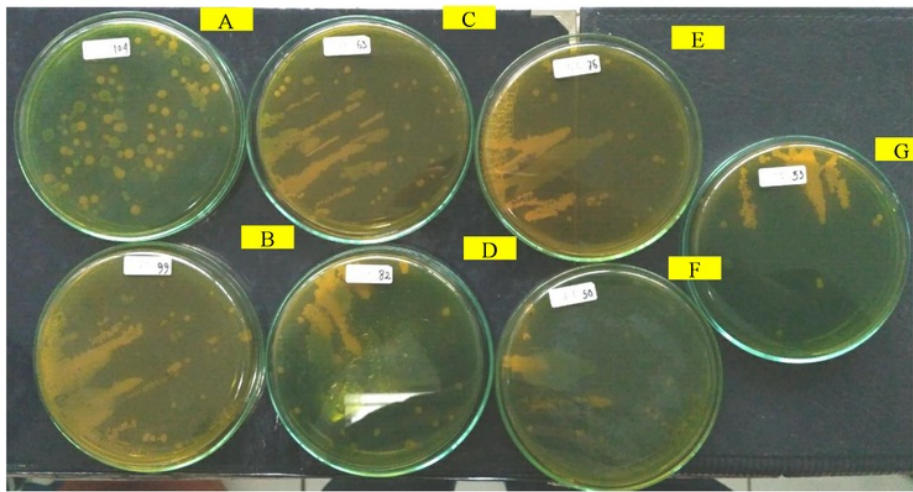


Figure 3. Effect of ozone concentration for *Vibrio vulnificus* population on a nutrient agar plate. [Ozone]=0,542 ppm; Flow rate=2 LPM; ozonisation duration=A: 0 minute, B: 3 minutes, C: 7 minutes,

D: 12 minutes E: 18 minutes, F: 24 minutes, G: 30 minutes.

4. Conclusion

Disinfection experiments were conducted with ozone treatment used shrimp pond water with *Vibrio vulnificus* bacteria. Decrease of a mount of bacteria is effective method for pathogenic bacteria of shrimp for flow rate of 2 LPM compared to 1 and 3 LPM.

5. Acknowledgments

Authors thankfully acknowledge for funding support received from the Chemical Engineering Department, Universitas Negeri Semarang, Ministry of Research, Technology and Higher Education of the Republic of Indonesia.

6. References

- [1] Elvis, A.M. and Ekta, J.S. (2011). *Ozone therapy: A clinical review. Journal of Natural Science, Biology, and Medicine*, Volume 2, Number 1, pp. 66–70.
- [2] Austin, B.D. and A. Austin. 1988. *Bacterial Fish Pathogen: Disease and Farmed and Wild Fish*. Ellis Horwood, Chichester. pp. 364.
- [3] Guzel-Seydim, Z.B., Greene A.K., and Seydim A.C. (2004). *Use of Ozone in the Food Industry*. *Lebensm.-Wiss. u.-Technol. Technol.* Volume 37: 453–460.
- [4] Kowalski, W.J., Bahnfleth, W.P., Striebig, B.A., and Whittam, T.S.(2003). *Demonstration of a Hermetic Airborne Ozone Disinfection System*’: *Studies on E. coli*, *AIHA Journal*, Volume 64, pp. 222–227.
- [5] Prabakaran M., Tamil Selvi S., Merinal, S., and Panneerselvam A. (2012). *Effect of ozonation on pathogenic bacteria. Advances in Applied Science Research*, Volume 3, Number 1, pp. 299-302.
- [6] He, H., Zheng, L., Li, Y., Song, W. (2015). *Research on the Feasibility of Spraying Micro/Nano Bubble Ozonated Water for Airborne Disease Prevention*. *Ozone: Science & Engineering*, 37, pp. 78–84.
- [7] Huq, B. Xu, M. A. R. Chowdhury, M. S. Islam, R. Montilla and R. R. Colwell. 1996. *A Simple Filtration Method To Remove Plankton-Associated Vibrio cholerae in Raw Water Supplies in Developing Countries*.
- [8] Viera, M.R., Guiamet, P.S., de Mele, M.F.L., Videla, H.A. 1999. *Use of dissolved ozone for controlling planktonic and sessile bacteria in industrial cooling systems*. *International Biodeterioration and Biodegradation* Vol 44, Issue 4, pg.201-207.
- [9] Chaiyakosa, S., Charernjiratragul, W., Umsakul, K, Vuddhaku, V. (2007) *Comparing the efficiency of chitosan with chlorine for reducing Vibrio parahaemolyticus in shrimp*, *Food Control*, volume 18, number 9, pp.1031–1035.

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