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# Reduction of Thermotolerant Coliforms Present in the Sea Water by Means of Micro-Nanobubbles of Air-Ozone of the Beach Los Pavos, Lima, Peru

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The marine pollution in Latin America and the Caribbean is due to domestic sewage discharges into the sea, which do not receive previous treatment, thus generating health problems for the population. This is due to the presence of thermotolerant coliforms and solid waste on the beaches in the Peruvian coast. The beach Los Pavos, as well as other beaches in Lima, is a very popular recreation area especially in summer. This beach complies with the parameters of beaches cleaning quality and presence of hygienic services, thus qualifying as a healthy beach. However, the analysis carried out in this research have found the presence of thermotolerant coliforms of 1400 NMP / 100mL at pH 7.13 and 44.5 °C (or 112.1 °F), exceeding the environmental standard values. In order to reduce this considerable amount of thermotolerant coliforms, sea-water microbubbles (MNBs) of air-ozone were tested. These micro-bubbles were generated by a device for the generation of Micro Nanobubbles invented by Dr. Jhonny Valverde Flores. The air / ozone volume ratio was 3/1, the size of the MNB was 7 µm, with a water flow of 4.67 L / min and a pressure of the air-ozone mixture of 90 PSI.

The volume ratio of sea water / water with MNBs for sample A was 3/1, for sample B of 1/1 and for Sample C of 1/3. As a result of research, the presence of thermotolerant coliforms of sample A decreased to 56 NMP / 100mL, of sample B decreased to 79 NMP / 100mL and of sample C decreased to 130 NMP / 100mL, achieving an efficiency of 96%, 94.36% and 90.71%, respectively.

## 1. Introduction

The marine pollution in Latin America and the Caribbean is due to domestic sewage discharges into the sea. This is due to the presence of thermotolerant coliforms and solid waste on the beaches in the Peruvian coast. There is a new concept to treat pollution into the environment. It is called the environmental nanotechnology. The environmental nanotechnology is a technological discipline which study properties of natural and man-made nanomaterials, applications, techniques for their characterization, integration processes and transformation into ecosystems (Valverde, 2016). Microbubbles have the ability to change the normal characteristic of water. Bubbles are filled with gas with an internal equilibrium pressure that is less than atmospheric pressure (Tsuge, 2014). The Microbubbles (MBs) have diameter more than 100 µm, the micro-nanobubbles (MNBs) have diameter between 1 to 100 µm and the nanobubbles (NBs) have diameter less than 1 µm within the fluid field (Valverde, 2016). Microbubbles (MBs) possess special properties such as the capacity for generating free radicals (Sadatomi et al., 2007), self-pressurization, and carrying a negative charge. Such properties have attracted much attention in the fields of food science and agriculture (Sumikura and others 2007; Takahashi and others 2007b). MNBs have now attracted attention for applications in engineering areas such as the sewage treatment of wastewater by air flotation (Choung et al 1993, Fan et al. 2010). Ozone (O<sub>3</sub>) is a strong oxidizing agent commonly used for sterilization, virus inactivation, deodorization, bleaching (decoloration), and decomposition of organic matter (Takahashi and others 2007a). The specie of thermotolerant coliforms is principally *Escherichia coli* and it is the most common fecal contamination index (BVSDE, 2006).

*Escherichia coli* and thermotolerant coliforms are of major importance as indicators of fecal contamination of water. Due to its negative surface charge and relatively low die-off or inactivation rate coefficient, *E. coli* is able to travel long distances underground and is therefore also a useful indicator of fecal contamination of groundwater. (Foppen & Schijven, 2006). Fujioka *et al.*, (1981) reveals that in most studies the coliform bacteria were reported that survive for days in seawater. The presence of *E. Coli* (or thermotolerant coliforms) is an index of fecal contamination (BVSDE, 2006).

Air-ozone micronanobubbles can reduce coliforms in domestic residual water (Cruz & Valverde, 2016). The marine water has salts and different physical properties than fresh water ( Cifuentes, Torres & Frias, 1997)

The micro-bubble generation technology in water is applied in the following situations:

- Aqua-life culture: fish and oyster farming (Englehardt, 1994).
- Industrial cleaning: in processing of industrial effluents (Ikeura *et al.*, 2011) and sterilization (Nakashima *et al.*, 2012).
- Agriculture: removal of residual food pesticides (Serizawa, 2003).
- In medicine: drug delivery to human organs, diagnosis using ultrasonic cavitation (Tsuge, 2014).
- In Health: cardiovascular problems (Valverde, 2016).
- Pollution control: prevent growth of blue-green algae in water bodies (Nakashima *et al.*, 2012) absorption of CO<sub>2</sub> gas (Rijk *et al.*).
- Separation process: treatment of oil/water emulsion (Takahashi *et al.*, 2007); gas liquid contactors and algal separation (Chu *et al.*, 2008).

## 2. Materials and Methods

### i) Collection of marine water sample

The marine water samples of the beach Los Pavos, Lima were collected, following the indications of the Quality Monitoring National Protocol of Water.

### ii) Sample analysis in laboratory

The thermotolerant coliforms were analyzed by an accredited laboratory. Also field parameters as pH, electrical conductivity, temperature, dissolved oxygen and Turbidity were measured.

### iii) Fabrication of air-ozone micro-nanobubbles (MNBs)

The research was carried out ex-situ, in laboratory, where the whole system was first assembled to generate and to use the air-ozone micro-nanobubbles in marine water. The patent obtained by Dr. Eng. Jhonny Valverde Flores was used to generate MNBs. The schematic diagram of experimental apparatus for preparation of MNBs solutions is shown in Figure 1.

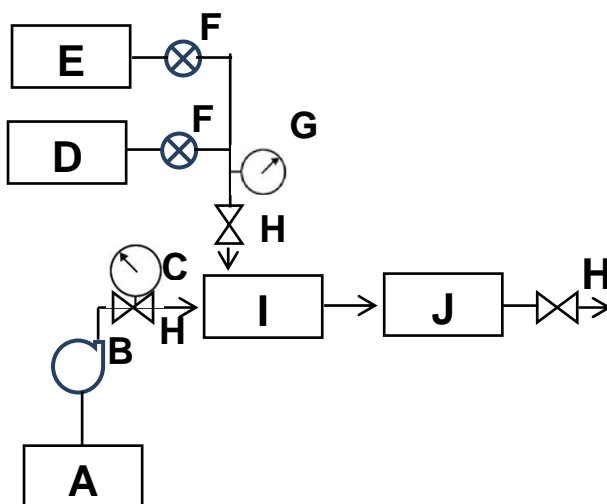


Figure1. Schematic diagram for the marine water treatment. Where, A: water tank, B: pump, C: flowmeter, D: air generator, E: ozone generator, F: pressure valve, G: pressure manometer, H: valve (general), I: MNBs generator, J: marine water with MNBs.

The obtained average diameter of a MNB was 7  $\mu\text{m}$ , the water flow was 4.67 L/s, the gas pressure was 90 PSI (Air: 80 % and Ozone: 20 %).

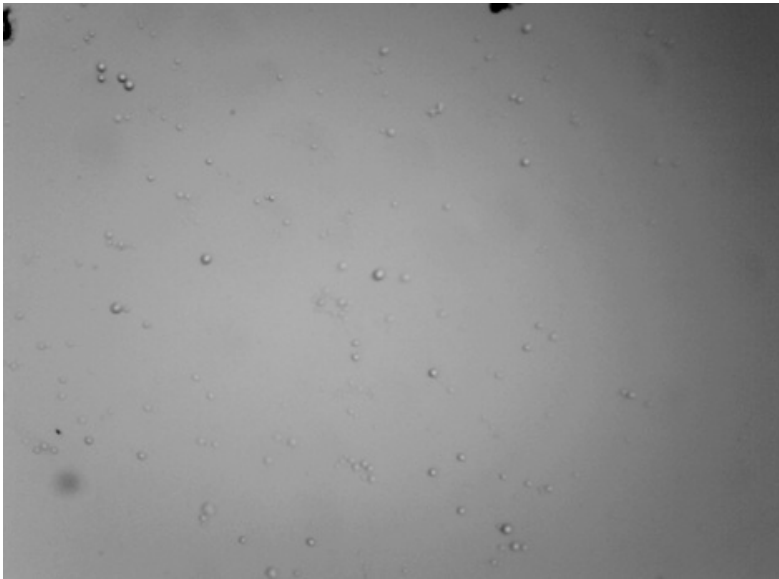


Figure 2. Average diameter of a MNB size is 7  $\mu\text{m}$

#### iv) Treatment of the samples

Three different treatments were dosed in order to evaluate the best reduction of thermotolerant coliforms into the marine water sample. Treatment 1 is called T1 [750 mL (marine water) + 250 mL (water with MNB)], Treatment 2 is called T2 [500 mL (marine water) + 500 mL (water with MNB)], and treatment 3 is called T3 [250 mL (marine water) + 750 mL (water with MNB)].



Figure 3. Preparation of treatment 1, treatment 2 and treatment 3.

#### v) Treatment's efficiency in laboratory

Results of the three treatments in different concentrations were analyzed after of using air-ozone micro-nanobubbles.

To measure the MNBs treatment's efficiency on thermotolerant coliforms (FC) was used the equation 1:

$$\% \text{Remotion(FC)} = \frac{[\text{Fecal coliforms}]_{\text{mittal}} - [\text{Fecal coliforms}]_{\text{end}}}{[\text{Fecal coliforms}]_{\text{mittal}}} * 100 \quad (1)$$

### 3. Results and discussion

#### 3.1. Physical parameters from marine water with thermotolerant coliforms before of treatment.

It was obtained by multiparameter Hatch.

Table 1. Field parameters before of treatment.

	Value	Unit	EQS*
pH	7.13	-	6 a 9
Electrical Conductivity	36.6	mS/cm	-
Dissolved Oxygen	10.33	mg/L	5 mg/L
Turbidity	14.54	CFU	100 CFU

\*EQS is Environmental Quality Standard.

Comparing the results of field parameters before the treatment, the pH parameter is within the EQS, and the dissolved oxygen has twice the EQS.

Table 2. Field parameters after of treatment.

Parameter	Unit	PRE TEST		POST TEST	
		INITIAL	Treatment 1 750 mL (marine water) + 250 mL (water with MNB)	Treatment 2 500 mL (marine water) + 500 mL (water with MNB)	Treatment 3 250 mL (marine water) + 750 mL (water with MNB)
pH	-	7.13	7.58	7.66	7.95
Electrical Conductivity	mS/cm	36.6	15.42	27.89	39.90

The pH in treatment 3 has increased than in treatment 1 and treatment 2. The electrical conductivity has increased in treatment 3 than in treatment 1 and treatment 2.

Table 3. Comparison from initial sample of thermotolerant coliforms vs. treatments (1, 2 and 3)

	Dosis (mL)		thermotolerant coliforms (CFU/100 mL)	EQS
	Sample	MNB		
initial sample			1400	
Treatment 1	750	250	56	Yes
Treatment 2	500	500	79	Yes
Treatment 3	250	750	130	Yes

At the beginning, the initial sample had 1400 CFU/100 mL. After of applying MNBs into the treatment 1, treatment 2 and treatment 3, the results gave within EQS.

#### Treatment Efficiency

To calculate the MNBs treatment's efficiency on thermotolerant coliforms in T1 as % Remotion was used the equation1:

$$\% \text{ remotion} = (1400 - 56) * 100 / 1400 = 96 \%$$

then is calculated the treatment 2 and treatment 3. As a resume the efficiency is seen in Table 4.

Table 4. Efficiency of the treatments (1, 2 and 3)

	Dosis (mL)		thermotolerant coliforms (NMP/100 mL)	EQS	EF (%)
	Muestra	MNB			
initial sample			1400	No	
Treatment 1	750	250	56	SI	96.00
Treatment 2	500	500	79	SI	94.36
Treatment 3	250	750	130	SI	90.71

The efficiency in treatment 1 is 96%, in treatment 2 is 94.36% and in treatment 3 is 90.71%

#### 4. Conclusions

- There are reductions of thermotolerant coliforms presents in marine water. The initial thermotolerant coliform concentration was 1400 CFU /100 mL, after applying the air-ozone micro-nanobubbles, the coliforms concentration was reduced. The reduction of thermotolerant coliforms in Treatment 1 was 56 CFU / 100 mL, in Treatment 2 the reduction was 79 CFU / 100 mL and in Treatment 3 the reduction was 130 CFU / 100 mL respectively.
- The thermotolerant coliforms in T1, T2 and T3 were within the Water's Environmental Quality Standard (EQS), which is 1000 CFU/100mL.
- The best treatment reduction Efficiency of thermotolerant coliforms in marine water was in T1 by applying air-ozone micro-nanobubbles. It was obtained 96%.

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