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Disinfection of Water Contaminated with *Vibrio cholerae* by Electrical Current

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Abstract: In this study, at the first stage microbial cell suspension of *Ogawa* and *Inaba* was prepared. Next, various dilutions of 10^{-1}, 10^{-3} and 10^{-5} CFU/ml of microorganisms were cultured in TCBS medium. After passing a direct electrical current through the cultures, they were incubated for 24 hours. To conduct the electrolysis, an electrolysis system with variable voltages and gold electrodes was used. Microbial liquid volume was 75 ml and its resistance was about 7 kΩ. The voltage was 12 V and the current intensity passing through the system was found to be 12 mA. The maximum time required for destroying all the bacteria of *Vibrio cholerae* was 15 min, thus the force of the system was 0.144 W. The *Ogawa* strains were disappeared later in comparison to *Inaba* strains. It was concluded that to destroy one liter of microbial cell suspension by the density of $10^4 \times 30$ bacteria per ounce, supply of an electrical energy of 2.4 W.h was needed. This method was found to be cheaper and also more practical than chlorination; it can be used easily in rural and urban areas for disinfecting drinking water.

Key words: *Vibrio cholerae* · Electrical current · *Ogawa* · *Inaba* · Disinfection · Contaminated water

INTRODUCTION

In spite of rapid developments in medical field and also vaccination against infectious diseases, there are still some kinds of bacteria which endanger human's health seriously. One kind of bacteria is *V. cholerae* that cause cholera disease. According to documents, no incident is more horrible than cholera's epidemic. Although the method of curing and treatment is obvious, mankind is not able to control *Vibrio* till now. The estimations show that 7.5 million cases were infected with cholera annually in the world and in approximately 120,000 cases, it causes death. These bacteria have some special characteristics first, they possess high pathogenesis and second, they can survive in surface waters for a longtime. Although, *V. cholerae* may has other alternative to treat; generally the treatment is similar (using antibiotics especially tetracycline) [1]. Knowing the exact number of infected cases and being able to prevent their unhygienic evacuation, it's obvious that the life cycle of this dangerous bacterium could be destroyed and prevention can be placed to the continuance of the disease in the society [2, 3]. Owing to this, a method is suggested to attain the aim. The purpose of present papers is to introduce a new method which involves the use of electrical current and electrolysis of the bacteria for disinfection of water. This can be an important factor in prophylaxis of *V. cholera*.

MATHERIALS AND METHODS

An important factor in this study is the relation between the number and type of the bacteria in the stable mass of water. Also, type and the electrical quantity as well as the time needed to disinfect the infected water are important parameters. In order to study these factors, first the microbial cell suspension of *Ogawa* and *Inaba* strains was prepared. Then, various dilutions of 10^{-1}, 10^{-3} and 10^{-5} CFU/ml were retained. Several cell suspension samples were applied direct current for the period of 5, 10, 15, 20 and 30 min. Then treated samples with electrical current were incubated for 24h. The incubated samples were carefully examined for any cell survival. The analysis was carried out for both *Ogawa* and *Inaba* strains. The appropriate medium in this study was TCBS. After culturing and growing the bacteria, they were carefully scanned and analyzed. Some samples of *V. cholerae* in a defined amount of water were sterilized by heating for half

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an hour. After preparing the various dilutions, the electrical current with different voltages was passed through them and the possibilities of growth of *V. cholerae* in different environments was studied [4, 5]. The electrical resistivity was measured using an ohmmeter. In the next step, the V=I.R formula was used to attain the electrical energy and also the amount of consumption. After the electrical current was passed through the microbial cell suspension at different times and intervals without stopping the electrical current, some other samples were collected from microbial cell suspension. Finally after 24 to 48 hours, the results of culturing were studied. The electrolysis system with variable voltages consisted of a cylinder with length of 2.5 cm and radiuses of 2.5 cm which two flat electrodes were attached to its surface. The electrodes were made of high quality gold, because of the possibility of chemical reactions which might destroy or neuter the bacteria. In fact, the cylinder was divided into two parts and one of them was used as electrolysis container. This special shape of electrolysis system was on the basis of an accepted physical formula and rule that the electrical current generally passes through the shortest path with little resistance. By the use of only two bars in a container of microbial cell suspension instead of cylinder, the electrical current could have passed in the space between the two bars and not in the whole container. Thus, by means of two flat electrodes in the electrolysis container, the electrical current would have led to the all parts of the microbial cell suspension. Before passing the electrical current through the samples, two samples of cultured microbial cell suspension were separated from others. The biomass of these separated samples was 2% ml. Then, connected the current was connected and just after 1 min, two other samples were separated. This process was continued without stopping the current and in the intervals of 2 and 3 min. After 3 min and after collecting data, the residues of the sample were thrown away and the container of the experiment was washed with alcohol (90%) and then with sterilized cold water, finally i was dried by a heat gun. It was needed to fulfill this procedure not to destroy the propriety of density of bacteria in the next experiments. White alcohol was used instead of industrial alcohol, because the industrial one have toxic and chromatic substance (pyridine) which might had severe effects on other experiments if it remained in the container by accident. This process was performed using other volumes of 5 and 10 ml and at various voltages 24 and 36 V in order to form different current intensity. The reason for changing microbial cell suspension volume was to construct different contact surfaces between cell suspension and electrodes. Consequently, different current intensities were passed through the circuit. All of these maneuvers were respect to the special shape of the electrolysis container.

RESULTS AND DICCUTIONS

In this study, the effects of electrical current on the strains of *V. cholerae* (*Ogawa* and *Inaba*) were investigated and it was found that the electrical current between the voltages of 4 to 36V was capable to destroy the bacteria. So, in the electrical experiments against *V. cholerae* bacteria, the direct current of 12 V was used. When the direct current was passed through them, the ions which existed inside the cell were forced to move to the outside of it and the anions and cations were directed to the electrodes of the container. Due to this, the bacterial cells were emptied of ions and consequently they were expired. This study shows that the electrical current which was passed through the sample for a longer time had a better effect on destroying the bacteria; this is another important element which helps better disinfection. If the microbial density becomes minor, the process of disinfection will have better results. The method of the study is laboratorial and it's full of interference. The volume of the microbial liquid which was used in every stage was 15 ml. The resistance of the system was 1 kΩ and the voltage was 12 V. According to the V=I×R Formula, the current intensity which passes through the system is equal to 12 mA or 0.012 A. By studying the chart of the results it was estimated that after 15 min, the electrical current completely destroyed the *V. cholerae* bacteria in the two under experiment systems.

A simple calculation was performed to achieve some preliminary results. The electrical system had the capacity of 15 ml of liquid and it was known that 0.012 A of electrical current passed through the circuit. So, the force is calculated as (F) = (12)(0.012) = 0.744 V.A. The maximum time to destroy the bacteria in this experiment was 15 min, so: 0.144 × 0.03W.h for 15 ml of electrical energy was calculated as follows: The power used = (0.03) (1000ml) /15ml=2.4W.h/l.

Thus, in order to destroy one liter of the microbial cell suspension with the density of 104 × 30 bacteria per unit, it was needed to supply 2.4 watt hour of electrical energy, so in order to clarify 1000 liter (1 square meter) of infected water with the same microbial density, 2.4 kilowatt hour of electrical energy was required [6].

The results of experimentations on the *Ogawa* and *Inaba* strains of *V. cholerae* bacteria which are shown Table 1, 2, imply that two subordinate facts can be discussed:

Table 1: The results of experimentations on the *Ogawa* strain of *V. cholerae*

OA (10^{-1})				OB (10^{-3})		OC (10^{-5})	
Time, min	Number of cells	Time, min	Number of cells	Time, min	Number of cells	Time, min	Number of cells
O_0	24×10^4	OA_0	23×10^3	OB_0	24×10^2	OC_0	25
O_5	64×10^3	OA_5	4×10^3	OB_5	25×10	OC_5	0
O_{10}	0	OA_{10}	2×10^3	OB_{10}	0	OC_{10}	0
O_{15}	0	OA_{15}	0	OB_{15}	0	OC_{15}	0
O_{20}	0	OA_{20}	0	OB_{20}	0	OC_{20}	0
O_{30}	0	OA_{30}	0	OB_{30}	0	OC_{30}	0

Table 2: The results of experimentations on the *Inaba* strain of *V. cholerae*

IA(10^{-1})				IB(10^{-3})		IC(10^{-5})	
Time, min	Number of cells	Time, min	Number of cells	Time, min	Number of cells	Time, min	Number of cells
I_0	30×10^4	IA_0	28×10^3	IB_0	35×10	IC_0	4×10
I_5	10×10^3	IA_5	5×10^3	IB_5	0	IC_5	0
I_{10}	0	IA_{10}	20×10	IB_{10}	0	IC_{10}	0
I_{15}	0	IA_{15}	0	IB_{15}	0	IC_{15}	0
I_{20}	0	IA_{20}	0	IB_{20}	0	IC_{20}	0
I_{30}	0	IA_{30}	0	IB_{30}	0	IC_{30}	0

- In the dilution of 10^{-1} CFU/ml, more time was needed to destroy both kinds of strains. The cause of this is unknown to the researcher.
- The *Ogawa* strains were killed later in comparison to *Inaba* strains. By studying the OB and IB columns in the Tables, it can be understood that this difference is due to the different structures of these strains. The electrical alternate current had no influence on disinfection but the electrical direct current was efficient for killing the bacteria because when D.C was used, the poles did not change. One of them was positive, the other one was negative continuously, thus, the ions could move toward the opposite poles. The cells emptied out ions at the moment and were killed. But in alternate current, each pole converts to too many positive and negative frequencies per second, so there is no time to empty ions from the cells. Consequently, the ions will remain untouched and they will not be destroyed. During experimentation, it was found that if the rate of electrical energy in a circuit preponderates and if the time of passing increases, then the effects of electrical current on disinfection of *V. cholerae* will be more visible.

This method of disinfection is better than the other method called chlorination, because of the following reasons: First, the cost of disinfection with electrical current is lesser than disinfection with cholera. Second, electrical current is able to destroy all of the microbes in the container but chlor can't destroy them completely, unless in high densities. Third, making use of electrical current is simpler than chlor. Forth, in rural areas where healthy water is rare, a gelatin battery can be simply used, for example an automobile battery is enough to refine a large amount of contaminated water and even by means of a small generator healthy and disinfected water could be supplied [7, 8]. On the other hand, for disinfecting hospitals' wastewaters, a large amount of chemical materials are needed that may pollute the environment, thus this method can be used to disinfect hospital's wastewater. It can safely be claimed that the *cholera* bacteria can be vanished and there should be no fear of it. Special containers of disinfection should be supplied to rural areas and people should be taught how to use it. Accordingly, the disinfected water can be used confidently.

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