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RESEARCH ARTICLE

DISINFECTION OF WATER CONTAMINATED BY PATHOGENIC MICROORGANISMS USING FREE RADICALS GENERATED BY ZERO VALENT IRON POWDER

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ABSTRACT

A method has been provided for the disinfection of water polluted by bacteria comprising administering zero-valent iron to the water in the presence of oxygen. This administration lead to the hydroxyl free radicals generation assuring the disinfection of contaminated water. A method has been used to proof the generation of free radicals. This method is based on determining content of a thiobarbituric acid-reactive substance in a solution comprising administering zero-valent iron in the presence of oxygen and 2-thiobarbituric acid to the solution and measuring a concentration of a 2-thiobarbituric acid-complex. Several samples of polluted water were tested by treating using free radicals generated by zero valent iron powder. The results show that after 6 to 10 minutes the bacteria disappear completely.

INTRODUCTION

The goal of disinfection of public water supplies is the elimination of the pathogens that are responsible for waterborne diseases. The transmission of diseases such as typhoid and paratyphoid fevers, cholera, salmonellosis, and shigellosis can be controlled with treatments that substantially reduce the total number of viable microorganisms in the water. While the concentration of organisms in drinking water after effective disinfection may be exceedingly small, sterilization (i.e., killing all the microbes present) is not attempted. Chlorination is the most widely used method for disinfecting water supplies in the world. The near universal adoption of this method can be attributed to its convenience and to its highly satisfactory performance as a disinfectant, which has been established by decades of use. It has been so successful that freedom from epidemics of waterborne diseases is now virtually taken for granted. As stated in Drinking Water and Health (National Academy of Sciences, 1977), "chlorination is the standard of disinfection against which others are compared [National Academy of Sciences, 1977]". However, the discovery that chlorination can result in the formation of trihalomethanes (THM's) and other halogenated hydrocarbons has prompted the reexamination of available disinfection methodology to determine alternative agents or procedures (Morris, 1975) [Morris, 1975].

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The method of choice for disinfecting water for human consumption depends on a variety of factors (Symons *et al.*, 1977). For years, engineers have relied on various processes to make industrial wastewater “acceptably clean” by oxidation techniques, through which the contaminants are converted into carbon dioxide and water, which are ideal for treatment of wastewater containing one or more such organic pollutants. Each method, however, has its own drawbacks. The wet air oxidation process requires high temperature and high-pressure conditions and is, therefore, very energy consuming for diluted effluents. The ozonation techniques also demand a great deal of energy during operation. Other methods which involve $TiO_2$and metal oxides as photocatalystsare being developed to degrade phenolic compounds. Our method (Rima *et al.* 2006) herein include the use of iron (or in combination with other metals) coupled with oxygen gas, which can produce hydroxyl free radicals to degrade bacteria and organic chemicals in aqueous solutions. Free radical reactions can cause damage to lipids, proteins, membranes and nucleic acid, interrupt many biological processes, and lead to a variety of diseases. Among the reactive oxygen species, hydroxyl radical is one of the most active and strongest oxidant agents, and can react with almost any substance at a diffusion rate. We have utilized a wide range of applications of the radicals generated by Fe^0 for bacterial disinfection, domestic wastewater treatment, industrial water treatment, groundwater treatment, contaminated site remediation, etc. The hydroxyl free radical ($HO^{\cdot}$) was indirectly observed by its reaction with the deoxyribose to form malondialdehyde (MDA) (Rima *et al.* 2012). In one instance, this aldehyde can react with thiobarbituric acid in 15% acetic acid at about 100°C to produce a pink chromogen which can be measured at about 532 nm. Free radicals are very active molecules. They can react with practically all chemicals and substances and damage living organisms. We are interested in using possible applications of the free radical generation technology for disinfections of microorganisms in various matrices such as river water, waste water, and the like. Coliform bacteria exist naturally in the intestinal tract of warm-blooded mammals, including humans. Coliform bacteria can also be found in soil, other animals, insects, etc. Wide presence of coliform bacteria and their significance of the environmental pollution problems are main reasons to select them as testorganisms in the preferred embodiments. In addition, the total coliform group is relatively easy to culture in the laboratory, and therefore, has been selected as the primary indicator bacteria for the presence of disease causing organism. Most coliform bacteria are not pathogenic (disease causing) organisms, and are only mildly infectious. For this reason these bacteria are relatively safe to work with in the laboratory. If large numbers of coliforms are found in water, there is a high probability that other pathogenic bacteria or organisms, such as Giardia and Cryptosporidium may be present.

Typically, bacteria are removed by chemical disinfections, heat sterilization, photoradiation, and/or physical filtration. Filtration alone may not be completely effective, but can improve the performance of disinfectants by removing sediment that can shelter the bacteria. Methods of adding chlorine to water include solution feeders for dry chlorine or liquid chlorine or by feeding gas chlorine directly from 100, 150, or 2000 lb. cylinders. Gas chlorination is recommended only for larger systems that can support the services of a trained water treatment plant operator. Chlorine is normally dosed to a concentration sufficient to maintain a free residual of at least 0.2 parts per million (ppm). Other disinfectants include iodine, ozone, ultraviolet light, and physical methods such as boiling or steam sterilization. Chlorination is still the most common disinfection method in the United States, although recent concerns have been raised about the reaction of chlorine with organic matter in water. Such a reaction can result in the formation of small organohalogens such as trihalomethanes, which are suspect carcinogenic compounds. For most individual water supply systems, the most common form of treatment is ultraviolet disinfection. A new method for the disinfection of water and wastewater polluted by bacteria using free radicals generated from ZVIP has been developed.

MATERIALS AND METHODS

Nutrient media

Lactose broth, MacConkey Agar with 0.15% Bile Salts, CV and NaCl, Nutrient Agar, Bile Esculin Agar, iron powder, acetate buffer, oxygen.

Water samples: Surface river water was collected at Antelias, Beirut, Lebanon. Anteilas is located in a residential area of Beirut and the water is exposed to domestic effluents. All water samples were collected in 2-L autoclaved polycarbonate bottles, and used within 2 h after sampling.

Plating: River water samples were spread on lactose-MacConkey medium and incubated at 37 °C for 48 h to isolate E. coli in the river water. White or colorless colonies on the medium were isolated and pure-cultured on lactose-Mac Conkeymedium. This *E. coliculture* at 18-24 h of growth was utilized (without dilution) for the treatment experiments.

A presence-absence method was used to assess if a water sample is contaminated with bacteria. Aliquots of water samples after treatment were spread into nutrient medium (nutrient agar for river water, and lactose-MacConkey agar for E. *coli culture*) and incubated at 37 °C for 24 h.

Presence of coliforms in river water

In the presumptive test, the Most Probable Number (MPN) technique was used to enumerate microorganisms, presumably coliforms, in river water. Three replicates and 10-fold dilutions of river water samples in lactose broth with bromcresol purple as acid indicator were prepared. MPN tubes were incubated for 24 h at 37°C. Tubes with gas formation, indicated by displacement of liquid in the insert tube, were counted in each dilution, and the numbers of coliform organisms per milliliter of river water were then calculated by standard procedures (de Man 1975,1977, 1983)[6-8]. Another MPN tube containing the same medium and a non-diluted water aliquot was incubated at 44.5 °C for 24 h to identify the fecal conforms. In the confirmed test, cultures that show yellow color and gas production in the presumptive test were transferred onto lactose-MacConkey agar selective for the *coli* – aerogenes group, and incubated for 24 h at 37 °C.

Identification of group D Streptococci in river water: Bile Esculine Agar (Swan 1954) [9] was utilized for the isolation and identification of group *D. Streptococci* from river water**.** *Enterococci* and *Streptococci* hydrolyseesculin to esculetin and glucose, which reacts with ferric citrate producing a brownish black precipitate around the colonies.

6 Water treatment by zero-valent iron powder: Twenty-five mL of contaminated water was added to the same volume of a 0.2 M acetate buffer solution at pH 4.8 in a glass vial. Different masses (0.05-0.2 g) of metallic iron powder were added to study the effect of the amount of iron on the sterilization of water. The vials were shaken vigorously and oxygen gas was bubbling in the solution. Aliquots of 3 mL were withdrawn at different times of the reaction to assess the level of bacterial contamination.

RESULTS AND DISCUSSION

The total coliform bacteria test is a primary indicator of “potability”, suitability for consumption as drinking water. It measures the concentration of total coliform bacteria associated with the possible presence of disease causing organisms. Two kinds of samples were treated by free radicals, river water and 18-24 h *E. coli* culture. Table 1 shows the effects of iron mass and treatment time on the sterilization of contaminated water. CFUs decreased dramatically as the amount of iron increased from 0.05 g to 0.2 g. There is no cfu in the plates that were treated with 0.15 – 0.2 g of iron after 12 min.

Table 1. Effects of amount of zero valent iron powder and treatment time on removal of E.coli on culture (20 h) [a]

Iron mass, g	Time reaction			
	3 min	6 min	9 min	12 min
0.05	xxxxx[b]	xxx	xxx	xx
0.1	xxxx	xx	x	60 cfu[c]
0.15	xxx	xx	25 cfu[c]	0 cfu[c]
0.2	xxx	xx	10 cfu[c]	0 cfu[c]

[a]Samples were prepared by growing a high quantity of *E. coli*. Each test was done in duplicate.
[b]Highest bacterial growth
[c]0.1 mL of *E. coli* culture spread on the plate

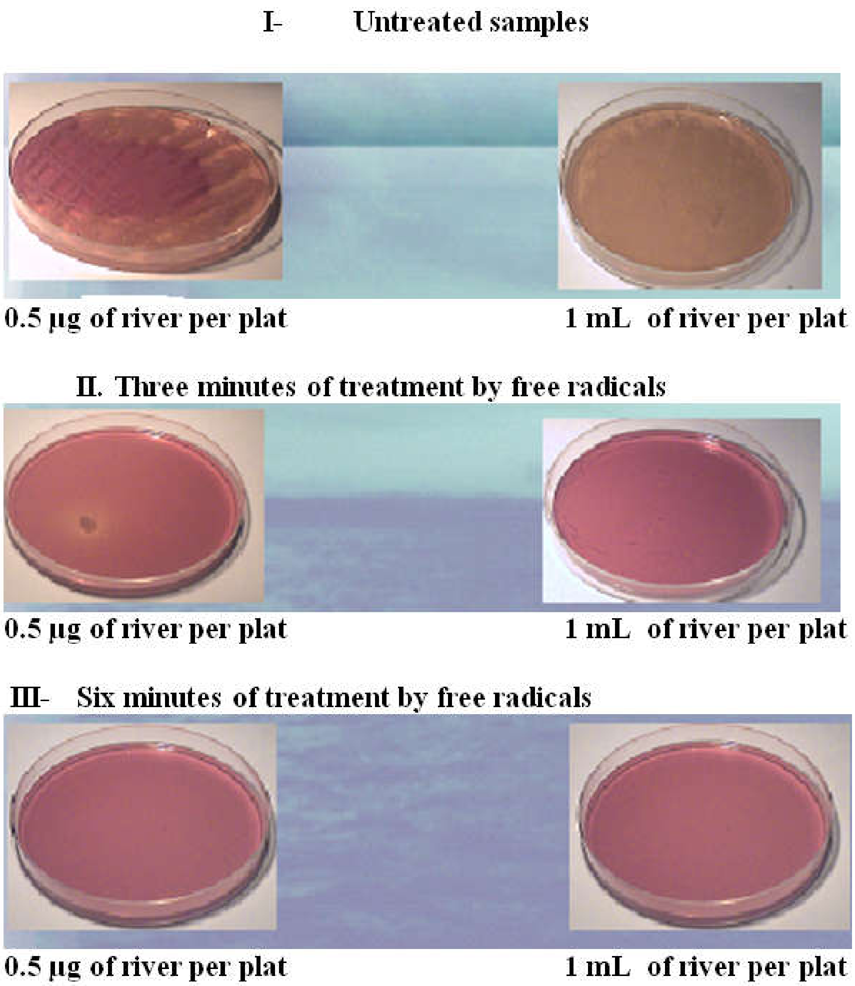

Figure 1. Disinfection of E-Coli by free radicals and generated by zero valent iron powder reaction. Municipality's wastewater

Polluted river water (Hawaii –USA)

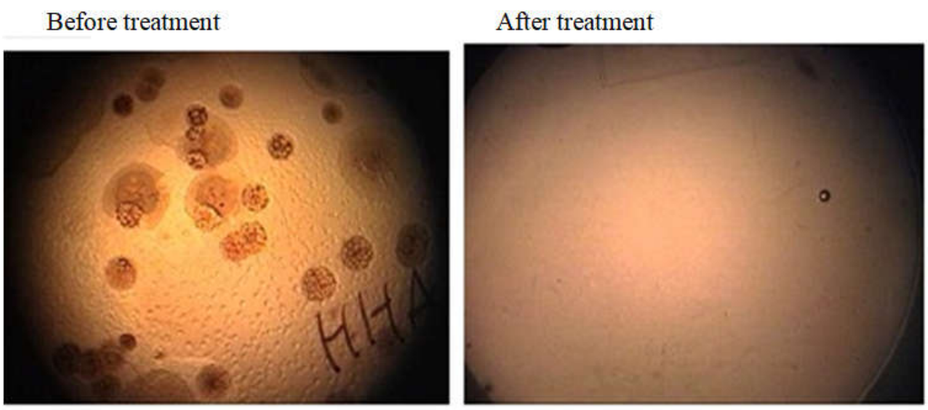

Figure 2. Example showing the disinfection of river water polluted by E coli using free radicals generated from zero valent iron powder reaction (25 ml of polluted water /0.2g of iron powder /air bubbling) River polluted (Hawaii –USA)

Figures 1-2 clearly show the efficiencies of bacterial disinfections by iron power. The results demonstrate that this new technique can effectively disinfect water contaminated with bacteria. Two samples were treated in the reaction, a pure 18-24 hour *E. coli* culture and polluted river water collected at a residential area in Beirut. The samples were treated at different conditions of free radical generation (iron mass, reaction time), and analyzed to determine the level of bacterial indicators (total conforms,*E. coli*). Treatment procedure data obtained in the experiments were analyzed to estimate the optimal parameters of complete bacterial destruction under conditions of free radical generation Polluted river water (Lebanon).

Conclusion

The results exemplify applications of ZVIP (and its combinations with other metals) to disinfect microorganisms, particularly pathogenic bacteria. The technology is applicable for medical sterilization of facilities and supplies, medical waste treatment and all water treatment and management. Many modifications and variations of the embodiments described herein may be made without departing from the scope, as is apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only.

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