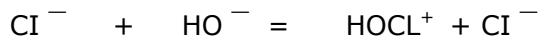


Water Pollution & Treatment

INTRODUCTION AND LITERATURE REVIEW

Water pollution is a major global problem particularly in the world, where the facilities for proper hygienic conditions are poor, where the sewage pipe lines in the absence of proper monitoring surveys. Water quality has been a vigorous research area since water is an important vehicle for the transmission of various microorganisms, notably parasites, bacteria and viruses. Thus, water treatment projects were indicated to improve its quality using various means including physical and chemical methods of disinfection including in chlorine compounds such as hypochlorites, chlorine dioxide, and inorganic chloramines.

Chlorine was widely used as a disinfectant by Semmelweis in 1846. In 1881, Koch demonstrated the bactericidal activity of disinfectants since they are economical, nonpoisonous, easily manipulated, deodorized, and last but not least, is their strong germicidal power (Lesser, 1949). Chlorine can exist either as free or combined form. The free form includes chlorine. (HOCL and OCL). The combined is present in the form of nitrogenous compounds to form chloramines. Chlorine demand is the difference between applied and remaining chlorine. The free residual chlorine concentration is related to super-chlorination beyond the needed level to assure a safety limit for any sign of presence of microorganisms. Chlorine in water exhibits the following series of reactions:



Hypochlorous acid (HOCL) Is a weak acid; thus at low pH values HOCL will dominate in solutions, while at high pH values Hypochlorite ion (HOCL-) will dominate. It was recognized in several studies that the disinfecting efficiency of chlorine decreases in pH and vice versa (Weidenkopf 1985 and Clark 1956).

At pH = 6.98% of Cl was in the HOCl form and 2% as OCl- Chlorine is a strong oxidizing agent capable of reaction quickly with Fe^{+2} Mn^{+2} , NO_2 , H_2S and organic materials. The oxidizing property explains the mechanism by which chlorines assure their action (Knox 1958 and Friberg 1957). Several factors assure the stability of chlorine solutions which include a low concentration, high alkalinity, free of catalysts (CO, Cu, Ni), low temperature and strage and absence of organic materials (Chlorine Bleach Solution 1957). The biocidal activity of chlorine is affected by several variables including pH, concentration of chlorine and temperature.

Rudolph (1941) showed that at a concentration of 25 ppm, 99% of Bacillus spores are killed in 2.5 minutes at pH = 7. Mercer in 1957 showed that 15 ppm hypochlorite kills 99% of bacillus spores at pH = 6 in 8.5 minutes. In 1941 Rudolph showed that increasing HOCl concentration 4 times will lead 50% reduction in killing time while a 2 fold increase in concentration will increase reduction by 30%. Weber (1944) showed that an increase in temperature of 10°C leads to 50-60% reduction in killing time.

The main pollution of water is fecal and, therefore, *E. coli* was considered as a determinant of fecal pollution as recommended by WHO standard guidelines for drinking water quality. Among the first reports done on biocidal effect of free residual concentration on *E. coli* was that of Butterfield who showed that at pH = 7, 0.05 ppm of residual chlorine can kill completely in 1 minute at ambient temperature 2×10^5 microorganism. *E. coli* was also considered as an indicator organism for viral pollution (Craun and McCaken 1973) taking into account that viruses were the main cause of epidemics (HAV, NANBV, polio) (Dennis 1959 and Wong 1980). However, resistant organisms to chlorination were still present despite the death of *E. coli*. This is why it was found that the validity of fecal coliforms, total coliforms and fecal streptococci as indicator organism are highly questionable and does not assure viral absence (Berg 1978). Viral outbreaks are believed to occur at higher rates than what reports present. This is attributed to difficulties in viral diagnosis and detection. Several studies showed a minimal infection dose (MID) of around 205 for the polio virus (Kate and Plolkin 1967, Akin 1983). However 1 PFU (Plaque forming unit) is believed to cause disease in susceptible individuals. However from a practical point of view, a microbiological cost study showed that an enteric virus test costs 300\$ compared to 2.34\$ for a total plate count and 2.75\$ for a total coliform test (Geldreich and Kennedy 1978). Therefore; other indicator organisms have been searched for and proposed to ensure complete viral disinfection. The indicator organism should be present wherever pathogenic organisms are present in similar or higher numbers and should be at least as resistant to treatment processes as well as detectable by practical techniques (Englebrecht 1980). Grabon in 1980 found that acid fast bacteria are exceptionally resistant organisms and their absence after chlorination stage ensure the inactivation of vegetative bacteria and viruses, while *P. aeruginosa* is relatively resistant. However, *Candida albicans* displayed no indicative value. Viruses usually show resistant to chlorine about 10 times greater than that of enteric bacteria. Petson et al on 1983 showed that 2 – 2.5 mg. of free residual chlorine per liter destroyed the infectivity of HAV completely while a report in 1979 showed that disinfection of drinking water required 1.0mg. of FRC per liter for at least 3 minutes at water pH values of less than 8.0 to ensure inactivation.

In 1983 Grabon et al showed that if a mixture of HAV and other indicator organisms are present, free residual chlorine of 1-2 mg./1 for 1-2 hours at pH less than 8 should be maintained to ensure disinfection. Englebrecht et al showed in 1980 that at a pH of 6 less time is needed for the inactivation of viruses having coxakie B virus to need a free residual concentration of 0.51-2.52 mg/1 for 3.5 minutes. Of course, these studies show higher resistance of viruses than coliforms. A study done by Brazis et al shows that *Bacillus anthracis* are killed at a free residual concentration of 2.4 ppm in 120 minutes at room temperature and pH = 7.2. spores are the most resistant of all organisms, and studies on *Chlostridium* and *Bacillus* spores have shown that they should be used as indicator organism of complete disinfection of treated water since they are present in large numbers in sewage (Bisson and Cabelli 1980).

In 1988 Sally Zierler et al have proved a strong correlation between drinking the chlorinated Mississippi River water and urinary bladder cancer among the residence of Massachusetts, due to the formation of trihalomethanes (THMs) enhanced by Chlorine reactivity with the organic substances present present in the water. Zierler states that the concentration of THMs in the disinfected Mississippi River water amounted 4 ug/1 compared to 1 ug/1 in undisinfected water.

The issue relates to our water quality. The question to be asked is whether our water is safe to drink especially in our present chaotic situation. We suppose it is not since we are not sure of the complete control of the microorganisms. The second question relates to the high concentration of the chlorine residual and its inactivation by solar radiation; as well as to find alternatives such as solar energy and its evaluation for complete inactivation of free residual chlorine after complete water disinfection. This is highly recommended especially after the associated urinary bladder cancer with the chlorine. A big investigation is needed for proper evaluation of the situation of our present drinking water quality and conditions.

MATERIALS AND METHODS

A. Materials

For testing the sporocidal activity of disinfectants, soil extract nutrient broth was used after filtration and was dispensed in 10 ml portions into 25 x 150 mm tubes and autoclaved for 20 minutes at 121°C. This broth was used to propagate test cultures of *Bacillus subtilis*.

Nutrient agar was used in the form of slants to maintain stock culture of *Bacilli*.

Modified fluid thioglycolate medium to subculture spore exposed to 2.5 NHCL.

The organism tested for was *Bacillus subtilis*.

Dilute hydrochloric acid 2.5 N was used to determine the resistance of dried spores.

Suture loop carriers were prepared from spool of size 3 surgical silk suture (Ethicon) in a way providing 65 mm of suture in a 2 – loop coil that can be conveniently handed in ordinary aseptic transfer procedures. The loops were extracted and placed in HCL, rinsed with water and dried.

For the determination of free residual chlorine, we used a commercial visual comparator technique using a commercial N, N-paraphenylenediamine (DPD) as a reagent.

The disinfectant used was a commercial sodium hypochlorite solution.

For testing the germicidal activity of sunlight, series of dilutions of *E. coli* colonies were prepared by dissolving respectively 5, 10, 20, 25, 50 and 100 colonies in 100 ml of distilled water. MacConkey agar was used to culture the prepared dilutions.

B. Techniques and methods

Bacilli were grown in soil extract nutrient broth. Three tubes were inoculated using one loop stock culture and incubated for 72 hours at 37°C. The 72 hour culture was poured into a tissue grinder and macerated to break up the pellicle and was filtered through a sterile funnel. We placed 10 suture loops in each of the three tubes. Four contaminated loops were transferred into a thioglycolate medium to serve as a viability control. After 2, 5, 10 and 20 minutes individual loops were transferred into substance media and incubated for 21 days at 37°C.

The ml of a serial dilution preparation of 5,25% sodium hypochlorite solution into 6 x 150 mm tubes. 5 suture loops were placed in the six tubes leaving a 2 minute interval for seeding each tube. Then, loops were removed from disinfectant and were transferred into a fresh tube of thioglycolate medium and incubated for 21 days at 37°C. Results were reported as growth or no growth.

Further information is present in the AOAC official methods of analysis.

The free residual chlorine was determined for tubes showing no growth. A time study was done to study the effect of sunlight on the residual chlorine inactivation.

Another group of *Bacillus subtilis* spores was inoculated into transparent noncolored glass bottles, and were exposed to sunlight for 1 hour, 2, 3, 12 and then for 24 hours, and were afterwards subcultured and incubated for 21 days at 37°C for any further growth.

Series of *E. coli* dilutions were exposed to sunlight for 1 hour, 2, 3, 12, 24 and 48 hours respectively for 24 hours at 37°C.

Bacillus subtilis and *E. coli* in high concentrations were exposed also to sunlight to study the solar effect on their growth showed a complete disinfection of spores after a one minute exposure using a 0.34 N NaOCL, but due to the high alkalinity of the solution, a diluted 0.6 x 10 N solution was used.

Spores were exposed to sunlight using various dilutions and time of exposure.

N.B. All experiments were repeated 5 times.

C. Results

None of the *Bacillus subtilis* spores exposed to sunlight, irrespective of the concentration of colonies and irrespective of time of exposure, were killed.

Only low concentration preparations of *E. coli* colonies, i.e. up to 10 colonies/100 ml revealed no growth after a 1 hour exposure to sunlight.

Therefore, solar radiation cannot ensure complete disinfection of all biological agents that may cause waterborn infections.

However, a diluted 0.6 x 10 N solution was insured a complete destruction of the bacteria after a two hour exposure. Free residual chlorine was determined, and the results showed that it was all eliminated after 1 hour of exposure sunlight.

DISCUSSION

Since low coliform count can be affected by sunlight while high concentrations cannot. And since it is not always feasible in the Third World or in the far areas to assess regularly for water bacteriological and viral counts, and since coliforms have a limited virucidal and sporadically inductivity, we assume that the highest possible contamination level, and we use a resistant organism such as *B. subtilis* spores to

assess for vegetative bacteria and viruses in water. Spores that are not affected by sunlight, the disinfection of water a 67×10^{-4} N.

NaOCl and exposure to sunlight, will assure the complete destruction of the spores. The free chlorine, known to be carcinogenic by initiating the formation of trihalomethanes, in addition to its bad odor and taste, will be completely inactivated within 45 minutes of exposure to sunlight. In this way water will be safe to drink under any circumstances where a nearby laboratory is not available for the bacteriological and the disinfection validity assessment.

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