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Biocidal Efficacy of Dissolved Ozone, Formaldehyde and Sodium Hypochlorite Against Total Planktonic Microorganisms in Produced Water

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Abstract: The performance of three biocides (dissolved ozone, formaldehyde and sodium hypochlorite) in eliminating the bacteria and fungi in produced water is investigated experimentally. The analysis involves monitoring the microbial population in nine conical flasks each containing the same volume of a mixture of produced water, culture medium that sustains the growth of microorganisms and a known concentration of biocide. The concentrations of each biocide used in the study are 0.1, 0.2 and 0.5 ppm. It is shown that dissolved ozone exhibits the best biocidal characteristics and a concentration of 0.5 ppm eliminated all the microorganisms in the produced water after 150 min contact time.

Key words: Dissolved ozone, formaldehyde, sodium hypochlorite, produced water, microorganisms, biocides

INTRODUCTION

Microorganisms such as sulphate reducing bacteria (*Desulfovibrio* species), sulphide oxidizing bacteria (*Thiobacillus ferroxidans*), iron bacteria (*Gallionella* species), nitrogen utilizing bacteria (*Pseudomonas* species) and filamentous fungi (*Cladosporium resina*), are detrimental and can be found in closed systems such as fuel storage tanks, heat exchangers, cooling towers and pipelines (Beech and Sunner, 2004). System failures resulting from corrosion influenced by these microorganisms have been widely reported in petrochemical and gas industries (Ibe, 1989; Abu, 1992), nuclear power stations (Videla, 1996), underground pipelines (Bowman, 2003; Rim-Rukeh and Awatefe, 2006), geothermal plants (Pryfogle, 2002), fire protection systems (Mittleman, 2003) and wastewater treatment plants (Iversen, 2001). It is estimated that about 20% of all corrosion damages are caused by microorganisms (Booth, 1971). Damages resulting from microbial corrosion in production, transport and oil storage facilities, amount to hundreds of million US dollars per year in United States of America (Costerton and Boivin, 1991).

Biofilms are slimy, mucous-like substances formed on the surfaces of materials in aquatic environment where nutrients are available for bacteria utilization. Biofilms consist of microbial cells immobilized on the surfaces of a material together with Extra-cellular Polymeric Substances (EPS) which bind the cells together (Characklis and Marshall, 1990; Costerton *et al*., 1995). Cases of contamination of surgical equipment by biofilms are reported in the literature (de Carvalho, 2007). It is estimated that about 750,000 surgical equipment are contaminated by biofilms annually in United States of America, resulting in more than 1.6 billion US dollars in hospital expenses (Gupta *et al*., 2002).

Microorganisms exist in the soil and are often found in oil pipelines (EIA, 2005). Strategies such as use of biocides (to counteract the actions of microorganisms), keeping the pipeline clean (pigging) and prevention of direct contact between the metal and its environment, have been employed to prevent biocorrosion of pipelines (Videla, 1996; Guiamet and de Saravia, 2005). Biocides are anti-microbial chemicals that have the potency to kill microorganisms or inhibit their growth and reproductive cycles by poisoning the microbial enzyme and causing protein denaturation, cell leakage and lysis (Gaylarde, 1995). Examples of biocides are chlorine, sodium azide, dissolved ozone, formaldehyde, sodium hypochlorite, chloramine, bromine, hydrogen peroxide, glutaraldehdye, peracetic acids, isothiazolones and quaternary ammonia compounds (Videla, 1996).

Produced water (or formation water) is one that accompanies crude oil and gas from a producing well. It is an integral component of hydrocarbon recovery process and is usually produced during drilling and production phases of a well. Naturally, the crude oil and produced water contain various microorganisms which must be

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removed to avoid microbial corrosion of internal surfaces of pipes in which the crude oil is transported after separating the oil from the produced water. The produced water obtained from the separation process is discharged into the receiving water body after treatment, which necessitates elimination of the microorganisms in the produced water. Thus, the presence of microorganisms in both the crude oil and produced water is detrimental to the operations of oil companies and the ecosystem. In this paper, we compare the biocidal efficacy of sodium hypochlorite, formaldehyde and dissolved ozone, against total planktonic microorganisms in produced water; the results which may be applied in biocorrosion prevention and control programmes (Total planktonic microorganisms is the total number of free swimming microorganisms in water environment). The choice of these biocides is based on their reported environmental friendliness and stability over a wide range of pH (Videla, 1996).

MATERIALS AND METHODS

Characteristics of biocides used in the study: Sodium hypochlorite (NaOCI) is a pale yellow liquid. In its pure form, sodium hypochlorite is unstable and reacts with water to form hypochlorous acid (HOCI) and sodium hydroxide (NaOH). At a pH value ranging from 7 to 9, hypochlorous acid can be converted to hydrogen ion (H^+) which is not an effective biocide and hypochlorite anion (OCl^-). At a pH of 7.5, about equal amount of hydrogen ion and hypochlorite anion are present. At a pH of 8.5, the split is about 10% hydrogen ion and 90% hypochlorite anion (Aieta *et al*., 1980).

Ozone is a bluish gas with a characteristic pungent odour. It is an oxidizing biocide that is partially soluble in water and highly unstable as it readily reverts to oxygen. The solubility of ozone in water is related to the amount of ozone in the carrier gas stream. Thus, it is important to produce a gas stream containing a relatively high amount of ozone. For example, the maximum solubilities at 25°C for gas stream containing 1 and 3% ozone are 2.7 and 8.1 ppm, respectively (Rice and Wilkes, 1991). These maximum levels are not obtained in practice because of out-gassing of the carrier gas which removes some of the dissolved ozone. Solubility of ozone also decreases with increasing temperature. Ozone degrades with pH, being fairly stable under certain conditions at pH of 6 and stable at pH of 10 (Rice and Wilkes, 1991).

Formaldehyde (HCHO) is a non-oxidizing biocide. It is a liquid that is readily soluble in water because of the presence of polar carbonyl group. It is an effective biocide that acts both on proteins and lipopolysaccharides of the bacterial cell envelope. It is known to be effective even at low concentration of 0.1 ppm (Longley *et al*., 1980).

Samples preparation

Culture medium: The culture medium (petroleum agar) used in the experiments contains 0.5 g of ammonium chloride (NH_4CI), 2.5 g of hydrated sodium hydrophosphate ($NaHPO_4{\cdot}12H_2O$), 5 g of potassium hydrophosphate (K_2HPO_4,) and 15 g of Diefo agar powder, per litre of engine oil. The pH of the culture medium was adjusted to a range from 7.0 to 7.2 for stability of the medium by addition of 50 mL of 1 M NaOH solution. The culture medium was autoclaved at a temperature of 121°C and pressure of $1.2{\times}10^4$ Pa. After sterilization, the medium was allowed to cool to room temperature of the laboratory (about 28°C). This method of preparation of the culture medium is reported in the literature (Steelhammer *et al*., 1995) and the choice of petroleum agar is due to its suitability as substrate for a broad spectrum of microorganisms (Steelhammer *et al*., 1995).

Biocide concentrations: The concentrations of each biocide used in the study are 0.1, 0.2 and 0.5 ppm. In a typical experimental design, the concentration of biocide should increase or decrease gradually, perhaps with equal intervals. We note that 0.2-0.4 ppm give very close results, hence the choice of the three concentrations (0.1, 0.2 and 0.5 ppm) used in the present analysis. Higher dosage of biocide (i.e., concentration greater than 0.5 ppm) has a lot of environmental consequences including greenhouse effects (Meitz, 1991). Effective concentration of biocide for control of microbial growth in a system ranges from 0.1 to 0.5 ppm. (Guiamet and de Saravia, 2005; Rice and Wilkes, 1991; Videla *et al*., 1995). The various concentrations of dissolved ozone used in the study were prepared by NEK Technical, Port Harcourt, Nigeria; 0.1 ppm of sodium hypochlorite and 0.1 ppm formaldehyde were prepared by mixing 0. 1 mL of the pure liquid with 1 L of water; 0.2 ppm was prepared by mixing 0.2 mL of the pure liquid with 1 L of water and 0.5 ppm was prepared by mixing 0.5 mL of the pure liquid with 1 L of water. The pH of the prepared sodium hypochlorite solutions were adjusted to 8.5 (as indicated in the preceding section) by the addition of 50 mL of 1 M NaOH solution in order to obtain high concentration of hypochlorite anion in the medium (Aieta *et al*., 1980) and consequently high concentration of sodium hypochlorite.

Experimental method: The produced water used in the study was obtained from a gas plant in Rivers State, Nigeria, in October 2006 and taken to Postgraduate Microbiology Laboratory, University of Port Harcourt, Nigeria, for analysis. Microbial population in the produced water sample contain four groups of planktonic microorganisms (EIA, 2005): (i) Hydrocarbon Utilizing

Bacteria (HUB) such as *Pseudomonas* sp. and *Bacillus* sp., (ii) Heterotrophic Bacteria (HB) such as *Pseudomonas* sp., *Bacillus* sp. and *Norcadia* sp., (iii) Hydrocarbon Utilizing Fungi (HUF) such *Saccharomyces* sp., *Penicillium* sp. and *Candida* sp. and (iv) Heterotrophic Fungi (HF) such as *Saccharomyces* sp., *Penicillium* sp. and *Candida* species.

Two grams of petroleum agar were added to 900 mL of produced water to sustain the growth of microorganisms and the microbial population of the mixture was determined after 1 h using the rapid agar dipstick (time-kill) technique which is widely used in the literature (Bloomfield *et al*., 1998; Wang *et al*., 2006; Willinger *et al*., 2005; Nato *et al*., 2003; Olsen *et al*., 2004). The rapid agar dipstick method simply involves dipping the dipstick in the produced water such that appropriate volume of the liquid adheres to it. After resealing the wet stick in its container, it is incubated at a temperature of about 30°C for 24 h. The number of microorganisms in the produced water was determined by matching the microbial growth on the incubated agar dipstick against a calibration chart provided by the manufacturer (Boots Micro-Check Company, Nottingham, UK). The inoculated medium (that is, the mixture of petroleum agar, produced water and microorganisms) was then divided equally into nine (9) conical flasks labeled according to the different concentrations of each biocide (i.e., 0.1, 0.2 and 0.5 ppm of sodium hypochlorite; 0.1, 0.2 and 0.5 ppm of dissolved ozone and 0.1, 0.2 and 0.5 ppm of formaldehyde). The same volume of biocide was added to each flask as labeled and the microbial population in each flask was determined at intervals of 30 min using the rapid agar dipstick technique.

RESULTS AND DISCUSSION

Figure 1-3 show the effects of different concentrations of each biocide on total planktonic microorganisms in the produced water.

It may be seen from Fig. 1-3 that the total planktonic microorganisms (or microbial population) in the produced water is 10^9 cells mL^{-1} initially; that is, in the absence of biocide. On application of a biocide, the total planktonic microorganisms in the produced water decreases with time due to the effect of the biocide. The degree of reduction of total planktonic microorganisms increases as the concentration of each biocide increases and this effect is more pronounced with dissolved ozone, followed by sodium hypochlorite and then formaldehyde. For example, Fig. 1 indicates that after 120 min contact time, 0.1 ppm dissolved ozone reduces the total planktonic microorganisms in the produced water from 10^9 cells mL^{-1} to 10^3 cells mL^{-1} (i.e., 6 log reduction of total planktonic

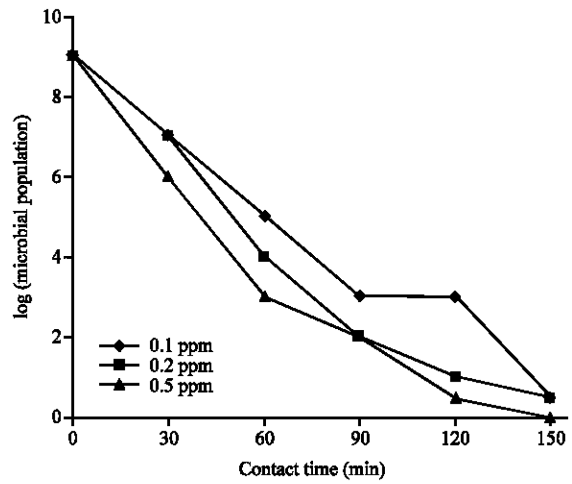

Fig. 1: Effects of different concentrations of dissolved ozone on total planktonic microorganisms in produced water

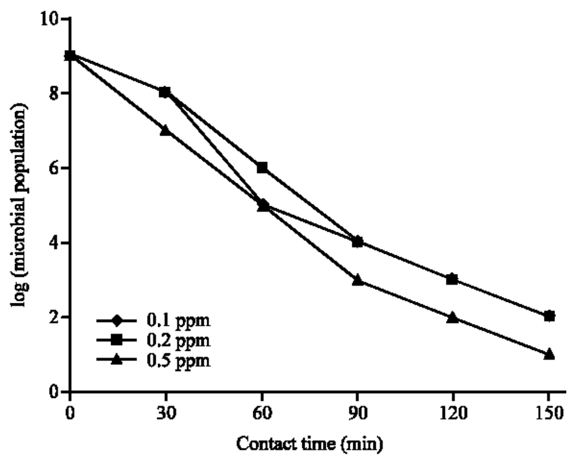

Fig. 2: Effects of different concentrations of sodium hypochlorite on total planktonic microorganisms in produced water

microorganisms); 0.2 ppm reduces the microorganisms from 10^9 to 10^1 cells mL^{-1} (8 log reduction) and 0.5 ppm reduces the microorganisms from 10^9 to about $10^{0.5}$ cells mL^{-1} (8.5 log reduction). After 150 min contact time, 0.1 and 0.2 ppm dissolved ozone reduce the total planktonic microorganisms in the produced water to about $10^{0.5}$ cells mL^{-1} while 0.5 ppm eliminated all the planktonic microorganisms in the produced water.

In the case of sodium hypochlorite as shown in Fig. 2, 0.1 and 0.2 ppm reduce the total planktonic microorganisms in the produced water to 10^3 cells mL^{-1} after 120 min contact time, while 0.5 ppm reduces the total planktonic microorganisms to 10^2 cells mL^{-1} for the same

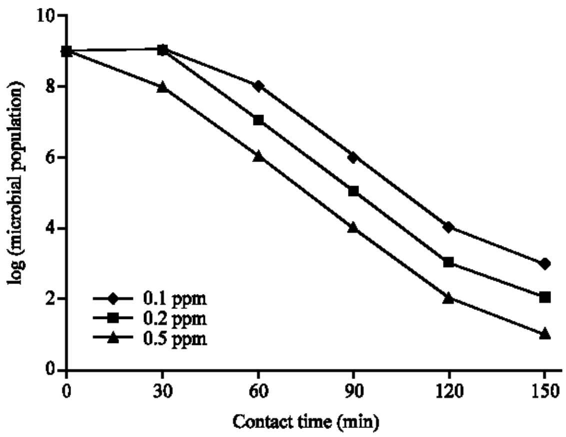

Fig. 3: Effects of different concentrations of formaldehyde on total planktonic microorganisms in produced water

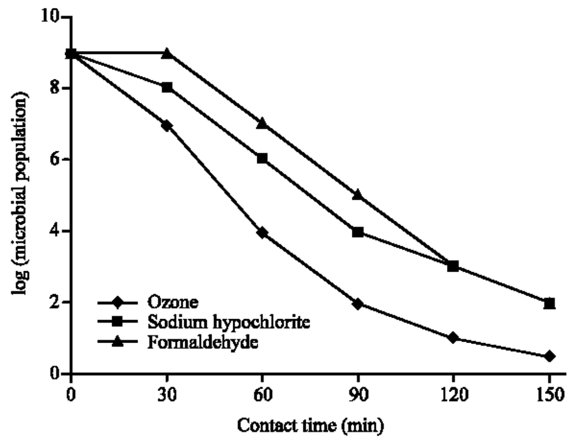

Fig. 5: Effects of 0.2 ppm of dissolved ozone, sodium hypochlorite and formaldehyde, on total planktonic microorganisms in produced water

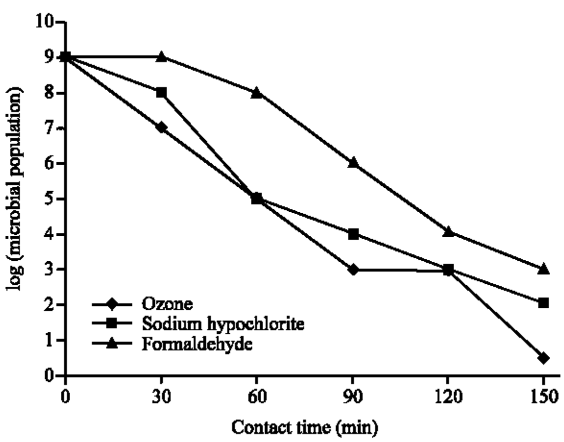

Fig. 4: Effects of 0.1 ppm of dissolved ozone, sodium hypochlorite and formaldehyde, on total planktonic microorganisms in produced water

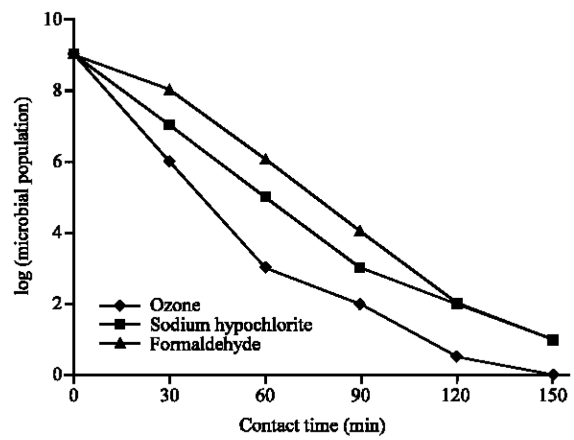

Fig. 6: Effects of 0.5 ppm of dissolved ozone, sodium hypochlorite and formaldehyde, on total planktonic microorganisms in produced water

contact time. After 150 min contact time, 0.1 and 0.2 ppm sodium hypochlorite reduce the total planktonic microorganisms in the produced water to 10^2 cells mL^{-1} while 0.5 ppm reduces the total planktonic microorganisms to 10^1 cells mL^{-1}. Figure 3 shows that after 120 min contact time, 0.1 ppm formaldehyde reduces the total planktonic microorganisms in the produced water to 10^4 cells mL^{-1}; 0.2 ppm reduces the total planktonic microorganisms to 10^3 cells mL^{-1} and 0.5 ppm reduces the microorganisms to 10^2 cells mL^{-1}. After 150 min contact time, 0.1 ppm formaldehyde reduces the total planktonic microorganisms to 10^3 cells mL^{-1}; 0.2 ppm reduces the microorganisms to 10^2 cells mL^{-1} and 0.5 ppm reduces the microorganisms to 10^1 cells mL^{-1}.

Figure 4-6 show the performance of the same concentration of the three biocides on the total planktonic microorganisms in the produced water. Thus, at a given concentration of the biocides, dissolved ozone exhibits the best biocidal efficacy against the total planktonic microorganisms in the produced water, followed by sodium hypochlorite, with formaldehyde as the least effective of the three biocides, which is consistent with Fig. 1-3. As the concentration of a biocide increases, its performance also increases while the contact time decreases and conversely.

Although 0.5 ppm dissolved ozone eliminated all the planktonic microorganisms in the produced water after 150 min contact time, 0.1 and 0.2 ppm dissolved ozone would also eliminate the microorganisms in the produced water but would require longer contact time depending on the concentration used. Since a biocide is said to be effective if it can achieve at least 4 log reduction of total planktonic microorganisms (Costerton and Lashen, 1984; Gaylarde and Videla, 1992), 0.1, 0.2 and 0.5 ppm dissolved ozone became effective as biocide after about 60, 50 and 40 min contact time respectively as may be read from Fig. 4-6. Dissolved ozone as low as 0.01 ppm can result to between 2 and 4 log reduction of *E. coli* bacteria (Brooke and Puckorius, 1991; Videla, 1996). It is also indicated that a constant concentration of ozone within a range from 0.02 to 0.05 ppm in cooling water systems will avoid formation of biofilms (Brooke and Puckorius, 1991). For biofouled metal surfaces, the presence of 0.2 to 1.0 ppm dissolved ozone results in rapid separation of sessile bacteria. Ozone is also said to be environmentally friendly because of its rapid decomposition to oxygen (Meitz, 1991).

CONCLUSION

Laboratory investigations of the effects of different concentrations (0.1, 0.2 and 0.5 ppm) of each of three biocides (dissolved ozone, sodium hypochlorite and formaldehyde) on the total planktonic microorganisms (bacteria and fungi) in produced water have been presented. It is shown that the performance of each biocide increases as its concentration increases resulting in a shorter contact time of the biocide. Dissolved ozone exhibits the best biocidal efficacy against total planktonic microorganisms in the produced water, followed by sodium hypochlorite, with formaldehyde as the least effective of the three biocides.

REFERENCES

Abu, G.O., 1992. Biotechnological and physico-chemical strategies on the monitoring and control of biocorrosion due to sulphate reducing bacteria activities in petroleum production systems. NICA/ICON/PAPER 1/92/6.

Aieta, E.M., J.D. Berge, P.V. Roberts and R.C. Cooper, 1980. Comparison of chlorine and chlorinated substances on disinfections. J. Water Pollut. Control Fed., 52: 810-822.

Beech, I.B. and S.T. Sunner, 2004. Biofilms on Corroding Materials. In: Biofilms in Medicine, Industry and Environmental Biotechnology-Characteristics. Analysis and Control, Lens, P., A.P. Maron, T. Mahony, P. Stoodly and V. O'Flaherty (Eds.). IWA Publishing House. Greenford, Middlesex, England, pp: 115-131.

Bloomfield, S.F., G.S. Stewart, C.E. Dodd, I.R. Booth and G.M. Power, 1998. The viable count of rapid agar dipstick explained. Microbiology, 144: 1-2.

Booth, G.H., 1971. Microbiological Corrosion. Mills and Boon Limited, London.

Bowman, C.W., 2003. New developments in the use of chemicals for pipeline corrosion control. J. Corrosion Sci. Eng., 6: 21-27.

Brooke, M.J. and P.R. Puckorius, 1991. Ozone for Cooling Tower Systems: Is it a Panacea? In: Corrosion 91/Paper No. 212, NACE. International. Houston, Texas.

Characklis, W.G. and K.C. Marshall, 1990. Biofilms. John Wiley and Sons, New York.

Costerton, J.W. and E.S. Lashen, 1984. Influence of biofilm on efficacy of biocides on corrosion-causing bacteria. Mater. Performance, 23: 17-22.

Costerton, J.W. and J. Boivin, 1991. Economics of microbial corrosion in water systems. In: International Congress on Microbially Influenced Corrosion, Knoxville.

Costerton, J.W., Z. Lewandowski, D.E. Coldwell, D.R. Korber and H.M. Lappin-Scott, 1995. Microbial biofilms. Ann. Rev. Microbiol., 49: 711-745.

de Carvalho, C.C.R., 2007. Biofilms: Recent developments on an old battle. Recent Patents Biotechnol., 1: 49-57.

EIA, 2005. Environment Impact Assessment Report of OB-OB Gas Plant Revamping Development Project Report No. S-0106, Nigerian Agip Oil Company, Port Harcourt.

Gaylarde, C.C. and H.A. Videla, 1992. Biocide Action on Metal Biofilm. In: Proceedings of the Pan American Congress on Corrosion and Protection, Videla, H.A. (Ed.). Cambridge University Press, Cambridge, UK., pp: 327-332.

Gaylarde, C.C., 1995. Design, Selection and Use of Biocides in Bioextraction and Biodeterioration. Biofouling and Biocorrosion, Gaylarde, C.C. and Videla, H.A. (Eds.). Cambridge University Press, Cambridge, UK., pp: 327-332.

Guiamet, P.S. and S.G.G. de Saravia, 2005. Laboratory studies of biocorrosion control using traditional and environmental friendly biocides: An overview. Latin Am. Applied Res., 35: 295-300.

Gupta, R., O.K. Beg and P. Lorenz, 2002. Bacterial alkaline proteases: Molecular approaches and industrial applications. Applied Microbiol. Biotechnol., 59: 13-32.

Ibe, G.E., 1989. Practical field experiences with SRB activities and their influences on corrosion in crude oil production systems. In: Proceedings of NICA Seminar on Corrosion in the Petroleum and Related Industries. Port Harcourt, Rivers State, Nigeria.

Iversen, A., 2001. Microbially influenced corrosion of stainless steels in wastewater treatment plants: Part 1. Br. Corrosion J., 36 (4): 277-283.

Longley, L.E., B.E. Moore and C.A. Sorber, 1980. Comparison of chlorine and chlorine dioxide. Pollut. Control Fed., 52: 2098-2105.

Meitz, A., 1991. Environmental Concerns and Biocides. In: Corrosion 91/Paper No. 306. NACE International, Houston, TX.

Mittleman, M., 2003. Microbially influenced corrosion of sprinkler piping. Corrosion, 49: 13-17.

Nato, F., A. Phalipon, L. Nguyen, T. Diep, P. Sansonetti and Y. Germani, 2003. Dipstick for rapid diagnosis of *Shigella flexneri* 2a in stool. Bull. World Health Org., 78 (7): 601-607.

Olsen, S.J., J. Pruckler, W. Bibb, N.T. Thanh, T.M. Trinh, N.T. Minh, S. Sivapalasingam, A. Gupta, P.T. Phuong, N.T. Chinh, N.V. Chau, P.D. Cam and E.D. Mintz, 2004. Evaluation of rapid diagnostic tests for Typhoid fever. J. Clin. Microbiol., 42 (5): 1885-1889.

Pryfogle, F.A., 2002. Geothermal biocorrosion. Corrosion, 42 (64): 2-10.

Rice, R.G. and J.F. Wilkes, 1991. Fundamental Aspects of Ozone Chemistry in Recalculating Cooling Water Systems. In: Corrosion 91/Paper No. 205. NACE International, Houston, TX.

Rim-Rukeh, A. and J.K. Awatefe, 2006. Investigation of soil corrosivity in the corrosion of low carbon steel pipe in soil environment. J. Applied Sci. Res., 2 (8): 466-469.

Steelhammer, J.C., C.F. Watson and R. Youmans, 1995. Biocontrol-Trends in industrial water treatment methods for microbiological control. Ind. Water Treatment, 7: 49-55.

Videla, H.A., M.R. Viera, P.S. Guiamet, M.F.L. de Mele and A.C. Staibano, 1995. Biocidal activity of dissolved ozone on sessile and planktonic bacteria: Effects of corrosion behaviour of cooling water systems structural materials. In: Proceedings of the 1995 International Conference on MIC, NACE International, Houston, Texas.

Videla, H.A., 1996. Manual of Biocorrosion. CRC Lewis Publishers, Florida.

Wang, X., M. Ansaruzzamon, R. Vaz, C. Mondlane, M.E. Lucas, L. von Seidlein, J.L. Dien, S. Ampuero, M. Puri, T. Park, G.B. Nair, J.D. Clemens, C. Chaignat, M. Rajerison, F. Nato and J. Fournier, 2006. Field evaluation of a rapid immunochromatographic dipstick test for the diagnosis of cholera in a high-risk population. BMC. Infect. Dis., 6: 17-26.

Willinger, B., S. Wein, A. Hirschl, L.R. Manfred and M. Manafi, 2005. Comparison of a new commercial test GLABRATA-RTT with a dipstick test for rapid identification of *Candida glabrata*. J. Clin. Microbiol., 43 (1): 499-501.