CHEMCONFLUX[20] Special Issue

J. Indian Chem. Soc.,
Vol. 97, No. 10a, October 2020, pp. 1699-1702

Microbial inactivation of groundwater using ultrasound and chemical additives (H_2O_2 and TiO_2)

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Manuscript received online 20 July 2020, revised and accepted 15 October 2020

Ultrasonication is promising intensified technique for chemical processing applications, advance oxidation processes and water disinfection. Water disinfection is major concern in water treatment technologies due to high risk of water borne diseases. Present study investigated the bacterial disinfection of groundwater using ultrasound and chemical additives such as hydrogen peroxide (H_2O_2) and titanium dioxide (TiO_2). Combined use of ultrasound and chemical additives enhances the rate of disinfection. The experiments were performed on groundwater collected from the hand pump, has shown ≥99.9% bacterial inactivation using ultrasonication in 60 min. Use of H_2O_2 and TiO_2 improves the disinfection rate. Combination of ultrasound and H_2O_2 achieved complete disinfection within 20 min.

Keywords: Ultrasonication, water disinfection, groundwater.

Introduction

Waterborne diseases are major challenge for human health, millions of people using unsafe contaminated water die due to waterborne diseases[1]. Waterborne diseases like cholera, diarrhea and typhoid are major threat for the children health, in rural areas of developing and under developing countries. Waterborne diseases are responsible for the more numbers of death of children[2]. In India, waterborne diseases affect 37.7 million and major concern is that more than 1.5 million children die due to diarrhea. Loss of 73 million working days occurs because of waterborne diseases that is responsible for loss of $600 million per year in economy[3]. Chlorination is most prominent method for the disinfection but over the years researchers have found that by-products of chlorination are carcinogenic[4,5]. Groundwater quality is also under threat due to extensive exploitation and increasing number of bore well digging. It is reported that urban as well as rural areas of Gorakhpur have high TDS and hardness[6]. India mark-II hand pump water is safe for drinking purposes but shallow water hand pump water is contaminated in Gorakhpur city[7]. Family of *Enterobacteriaceae bacteria* such as *Salmonella shigella proteus* and *Klebsiella*, they cause diseases like typhoid, dysentery, cholera and gastroenteritis[8].

Several studies showed that ultrasonic disinfection is useful for bacterial inactivation and chemical additives reduce the time of disinfection. In this research water disinfection was performed by using acoustic cavitation and combined effects of ultrasound and chemical additives were studied.

Materials and methods

In this study, groundwater from shallow water hand pump of Madan Mohan Malaviya University of Technology, Gorakhpur, India campus was used for the bacterial disinfection. Bacterial count of ground water varies with the season, bacterial count varies from 50 to 100 CFU/ml. Hand pump was operated for 30–50 strokes before the collection of sample water to avoid sampling of water contained in pipeline of hand pump.

LR grade chemicals were used for the experimental analysis; three different kind of culture media were used for the colony counting were purchased from the Himedia. Nutrient agar (Himedia-M001) was used for the cultivation of less fastidious microorganism (Total plate count, TPC). Plate count agar (Himedia-M091) was used for the plate count of the microorganism in water (Plate count) and potato dextrose agar (Himedia-MH096) used for the growing yeasts and moulds present in water sample. Hydrogen peroxide (H_2O_2)

and titanium dioxide (TiO_2) were used as additional chemical disinfectant additives were purchased from the Rankem (H0130 and T0070) (LR grade).

Three different kind of Powder Agar were used for the enumeration of the bacteria: nutrient agar, potato dextrose agar and plate count agar. Nutrient Agar was used for the enumeration of less fastidious bacteria present in water sample. Typical composition of nutrient agar is peptone, sodium chloride, HM peptone Ba, yeast extract and agar (solidifying agent). Nutrient agar does not separate different types of bacteria, it grows colony of bacteria such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes, Salmonella enteritidis, Salmonella typhimurium, Yersinia enterocolitica and Yersinia enterocolitica* in good luxuriant way. Potato dextrose agar was used for the enumeration of the yeasts and moulds from the sample, which consist of potatoes (infusion form), dextrose agar. It grows *Aspergillus brasiliensis, Candida albicans and Saccharomyces cerevisiae* in luxuriant way. Plate count agar is also considered for enumeration of bacteria present in the water sample and known as Plate count. Typical composition of plate count agar is tryptone, yeast extract, dextrose and agar. Plate count agar also used for the estimation of the number of heterotrophic bacteria in water. *Bacillus subtilis*, *Escherichia coli, Enterococcus faecalis, Lactobacillus casei, Staphylococcus aureus and Streptococcus pyogenes* are organism which can be grown using plate count agar.

Ultrasonic bath (Labman LMUC-3) of 2.5 L (240×140×100 mm^3 Tank size) capacity with 100 W ultrasonic power and 40 kHz frequency was used. In experiment, 2 L of collected groundwater was sonicated for the 40 min. Samples were collected carefully to avoid external contamination after every 5, 10, 15, 20, 30 and 40 min. Ultrasonic bath generates high frequency using Magneto-strictive or piezoelectric transducers. High frequency vibration cause compression and rarefaction in medium which is responsible for the generation of cavity[9]. Operation of ultrasonic bath continuously increase significant temperature. High temperature is also responsible for the bacterial inactivation, but it is observed that the temperature does not increase above 50°C. Container of ultrasonic bath was cleaned properly using alcohol and then dried to remove bacteria present in container.

Bacterial population was enumerated by the pour plate method, procedure was carried out according to the recommendation of American Public Health Association 2015 (APHA)[10] and Sanders[11]. All glassware's (petri dishes, conical flask, L shape glass spreader, 1 ml pipette etc.) and utensils were sterilized before conducting experiment. Sterilization of glassware was performed in autoclave at 15–20 bar pressure and 120°C for 15 min. All three agar is prepared according to the instruction provided by the manufacturer. For making nutrient agar 28 g of nutrient agar dissolved in 1000 ml distilled water and then boiled with continuous stirring until it completely dissolved. 39 g of potato dextrose agar and 17.5 g of plate count agar was dissolved in 1000 ml respectively and boil and stirrer until dissolved completely. All three dissolved agar autoclaved for 15 min. When temperature of agar reduces to 45–50°C then it taken out from autoclave to pour in the petri dishes. 1 ml of sample taken after every 0, 5, 10, 15, 20 and 30 min and poured in sterilized petri dishes then sterilized agar poured. After agar get solidify petri dishes put in incubator for 24 h. Colonies grown counted manually and reported as Colony forming unit per ml (CFU/ml). Two duplicate petri dishes were taken for the same sample, it was observed that colony in all three dishes varies between ±15 colonies. Average number of colonies reported as CFU/ml.

Results and discussion

Ultrasound was used to kill the microbes in water. Continuous contraction and expansion of wave produce cavity bubbles from interaction of ultrasound with aqueous medium; these bubbles grow to certain size then burst in the medium[1,12,13]. Violent bursting of bubbles generates high shear stress, shock waves, local hot spots (~5000 K), high velocity fluid jets (mechanical effects) and oxidising radicals (chemical effect) shown in Fig. 1. Mechanical and chemical effects of bursting cavity is collectively responsible for rapturing of microbial cell wall[12,14].

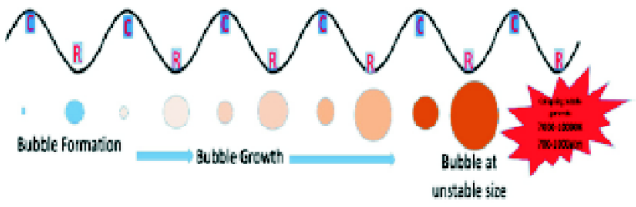

Fig. 1. Growth and collapse of cavities.

Percentage of bacterial deactivation (disinfection) is calculated using formula

$$\%\text{Disinfection} = \frac{C_0 - C}{C_0} \times 100$$

where C_0 is number of colony (CFU/ml) before treatment, C is number of colony (CFU/ml) after treatment. Calibration curve (Fig. 2) was drawn for ultrasonic bath at 1.5 L, 2.0 L and 2.5 L. Using ultrasonic only 100% microbial inactivation obtained for Fungi, Yeast and moulds (PDA) ~80% microbial inactivation obtained for the less fastidious microorganisms known as Total plate count TPC (NA) and 92% microbial inactivation obtained for plate counts of microorganisms (PCA) (Fig. 3).

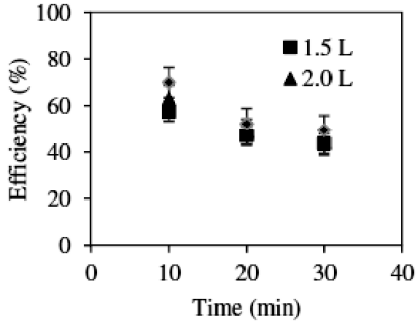

Fig. 2. Calibration curve of ultrasonic bath.

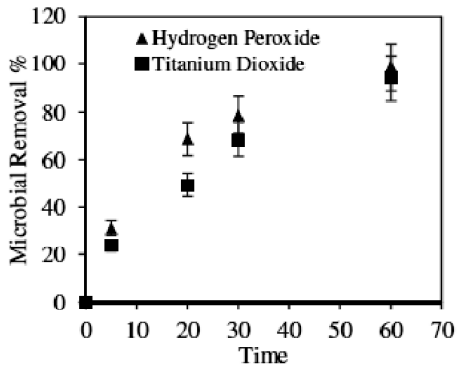

Fig. 3. Microbial inactivation using H_2O_2 and TiO_2 only.

Control study were performed using PCA agar for H_2O_2 (1 ml/L) and TiO_2(1 mg/L) results are shown in Fig. 4. H_2O_2 only gives 78% disinfection and TiO_2 gives 74% disinfection in 30 min, respectively.

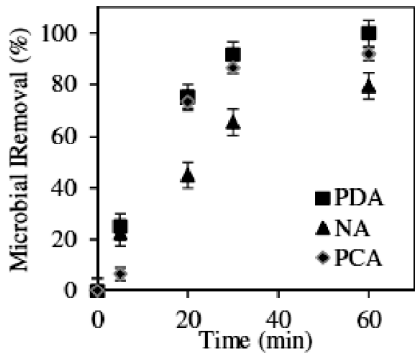

Fig. 4. Microbial deactivation using ultrasonication only.

Using ultrasonication and hydrogen peroxide (US/H_2O_2 (1 ml/L)), ≥99.9% microbial inactivation achieved for Fungi, Yeast and moulds (PDA). Upto 98% microbial inactivation obtained for the less fastidious microorganisms known as Total plate count TPC (NA). ≥99.9% microbial inactivation obtained for plate counts of microorganisms (PCA) (Fig. 5.).

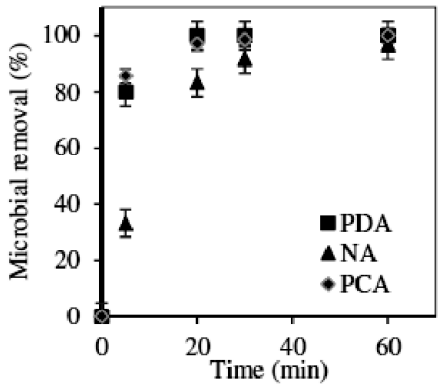

Fig. 5. Microbial deactivation using ultrasonication + H_2O_2.

Combination of ultrasonication and TiO_2 (1 mg/L) ≥99.9% microbial inactivation for Fungi, Yeast and moulds (PDA) 98% microbial inactivation obtained for the less fastidious microorganisms known as Total plate count TPC (NA) 98% microbial inactivation obtained for plate counts of microorganisms (PCA) (Fig. 6.).

Ultrasonication and combination of chemical additives able to kill more than 90% bacteria within half an hour. In US + H_2O_2 98% and US + TiO_2 91% bacteria killed in first half an hour of treatment (Figs. 7a and 7b).

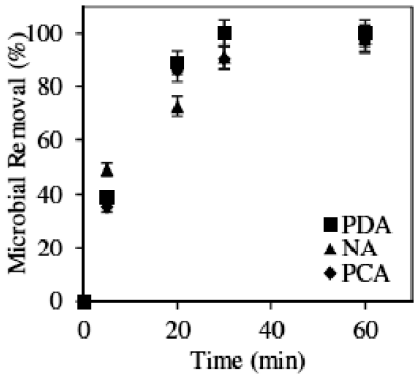

Fig. 6. Microbial deactivation using ultrasonication+TiO_2.

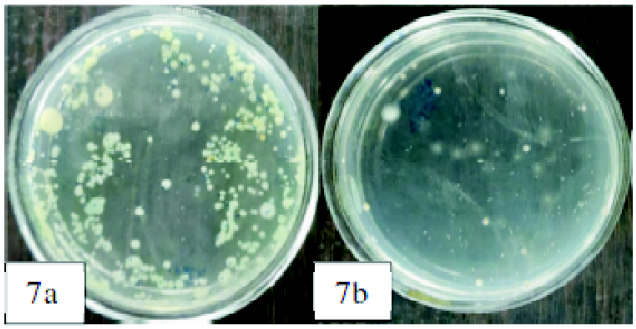

Fig. 7. (a) Colony counted on nutrient agar plate before treatment, (b) colony counted on nutrient agar plate after 30 min treatment using ultrasonication + TiO_2.

Conclusions

Water disinfection using acoustic cavitation is promising techniques to reduce the use of chemical disinfectant. Acoustic cavitation alone can kill ≥99.9% bacteria in 60 min. Use of H_2O_2 and TiO_2 improves the disinfection rate. Combination of ultrasound and H_2O_2 achieved complete disinfection within 20 min. It can be concluded that use of chemical disinfectant reduce the time of disinfection.

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