Available online at www.ijrp.org

International Journal of Research Publications
Volume 2 – Issue. 1, April 2018

Study on Antibacterial Ag-TiO_2 Nanocomposite for Water Purification

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Abstract

The aim of this study is to increase the efficiency of a low cost, low energy and low environmental impact drinking water purification system by using synthesized silver-titanium dioxide(Ag-TiO_2) nanocomposite materials that exhibits strong antibacterial activity. Composite Ag-TiO_2 was synthesized by one-pot sol-gel method using silver nitrate ($AgNO_3$) as silver (Ag) source and titanium isopropoxide as titania (TiO_2) source. Ag-TiO_2 nanocomposites were characterized by x-ray diffraction (XRD), scanning electron microscopy (SEM) and size distribution analysis (Zeta sizer). Prescreening anti-E.coli bacterial activity of the obtained nanocomposites was tested using agar-well diffusion method and plate count method.AgTiO_2 nanocomposite that has strongest antibacterial activity was selected and applied to ceramic water filter pot(CWP) and then compared with the activity of current antibacterial substance colloidal silver. Atomic absorbance spectroscopy (AAS) was used to detect silver ion leakage from this nanocomposite coated pots. Negligible Ag ion leakage was observed in filtered water from nanocomposite coated filter pot. Contaminated water samples were collected from Hlaingriver, Ngamoeyeik creek and Hlaw- gar water distribution pipe-line, and flowed through the nanocomposite coating ceramic water filter pots to be tested for bacteria removal effectiveness. E.coli/coliform petrifilms were used to determine the bacterial removal efficiency by comparing the concentrations of target organisms before and after treatment. The result showed that higher removal of contaminated E.coli/coliform was obtained by synthesized Ag-TiO_2 nanocomposite than colloidal silver. It is seen that TiO_2 served as solid support to maintain the dispersion of Ag clusters that could contribute to the better antibacterial performance in CWF.

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Keywords: Ag-TiO_2 nanocomposite, drinking water filtration system, antibacterial activity, ceramic water filter pot(CWP)

1. Introduction

Water is a fundamental human need. Each person on earth requires at least 20 to 50 liters of clean, safe water a day for drinking, cooking, and simply keeping themselves clean [1]. Water is the most important natural resources and the scarcity of safe and clean drinking water is a major concern [2]. An estimated 663 billion people worldwide (about 9%) can access safe drinking water (WHO/UNICEF 2015). According to the World Health Organization's 2017 report, safe drinking-water is water that "does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages" [3]. As drinking water sources have been severely polluted by various organic and inorganic matters, turbid materials and pathogens, more than half a million deaths per year was caused by contaminated water [4].

Diarrhoea, one health consequence of consuming unsafe drinking water accounts for 1.8 million deaths mainly among children under five years of age [5]. Along with poor sanitation and hand hygiene, drinking-water contamination is one of the routes by which there can be transmission of the bacterial, viral or protozoal pathogens responsible for diarrhea [6]. Low-income countries such as Myanmar, particularly affected by deficient water systems and services, and poor sanitation and hygiene are urgently needed to access the provision of microbiologically safe water at both community and household levels.

One of the most promising point-of-use water-treatment technologies is ceramic water filter pot manufactured with local labor using clay, water and rice husk as a combustible organic material and impregnated with colloidal silver. The ceramic water filter consisting of a porous pot-shaped filter element that can hold 10 liters of water is placed in a larger plastic container with a lid and a spigot. The filter element is manually filled with water from a contaminated source, which seeps through the pore at a rate of 2 to 3 liters per hour. The filtering effect of the clay eliminates a large portion of water-borne pathogens but laboratory tests indicate that colloidal silver is necessary to achieve complete disinfection. Colloidal silver used as an antibacterial agent is obtained from an international supplier, yet represent a small fraction of the total cost of the filter (about US $ 1 per filter). One of the main concerns about the application of silver compounds to ceramic filters is the release of silver (ionic or colloidal) from the filters over time. The loss of silver from the filters has two main disadvantages: 1) potentially undesirable health effects for filter users and 2) reduction of disinfectant efficiency. Some recent studies have reported that the efficiency of ceramic filter decreased after loading multiple batches of highly contaminated water and the impregnation of silver is not enough to sustain high efficiencies for a long period of time [7]. To overcome these problems, fixing silver nanoparticles on various supports method can be used. These are called silver-doped nanocomposite materials that are chemically durable and release silver ions for a long time period and can perform as an excellent antibacterial coating in the food industry, water disinfection and other disinfection related fields [8].

In order to investigate integrated performance of bacterial inactivation for rapid antibacterial treatment of drinking water, Ag–TiO_2 nanocomposites were fabricatd by two-step approach via sol–gel method followed by calcination. A common challenge to engineering successful antimicrobial composite materials is to obtain highly dispersed, small (sub-10 nm) Ag particles while maintaining high Ag loading. To meet this requirement, we used a novel method to synthesize Ag-TiO_2 nanocomposite powders that consist of TiO_2 nanoparticles supporting high-loading and well dispersed Ag nanoclusters. Chitosan that have been used as stabilizing agents for synthesis of pure metallic nanoparticles serve as the dispersers and stabilizers to control Ag cluster growth. To the best of our knowledge, this is the first study to demonstrate the stabilizing effect of chitosan on metal particle size control in composite nanomaterials during synthesis. We expected to get good result in a lower risk to human and environmental exposure from Ag and a lower cost by substituting this synthesized antibacterial nanocomposite materials. Potent antibacterial activity against Escherichia coli (E. coli), a typical gram-negative bacterial strain, has been achieved using this material. The main aim of this study is to provide a sustainable and long term water treatment solution which helps to build local economies that has low capital costs and uses local resources.

2. Materials and Methods

2.1. Chemicals and Materials

Titanium (IV) isopropoxide (TIP), N,N-dimethylformamide (DMF), the silver nitrate ($AgNO_3$), acetonitrile and ethanol used in our experiments were of analytical grade. Colloidal Ag (Argenol, Collargol) and Chitosan(Degree of Deacetylation 80) were used as received. Deionized water was used throughout the experiments except for in the antibacterial tests, when sterilized water was used. All experiments were conducted in high-grade glassware. Ceramic water filter pots (CWP) supplied by Thirst aid association were used as received (pore size, 0.2 μm). Three type of polluted water were collected from Hlaingriver, Ngamoeyeik creek and Hlaw-gar water distribution pipe-line to test the efficacy of the water treatment with environmental samples. The collected water was allowed to pass through CWF and indicator bacteria count of treated water and untreated water was comparatively determined by using E.coli/coliform petrifilm.

2.2. Methods

2.2.1. Synthesis of Ag-TiO_2 nanocomposites

Five grams of chitosan powder was poured into a vessel containing 100 ml of deionized water and 5 ml of acetic acid. The mixture was stirred for 12 h at 90 °C and finally cooled naturally to room temperature. 20 ml of this as-synthesized chitosan solution was added drop wise into a vessel containing 180 ml of DMF, in which 5% $AgNO_3$ was dissolved. TIP (20 ml) was slowly introduced into the solution with vigorous stirring. Deionized water (5 ml) was added drop wise to hydrolyze the TIP and form a gel. After overnight aging, the gel was washed with acetonitrile repeatedly to remove the entrapped chitosan, vacuum-dried at 80 °C, and calcined in air at 550°C for 2 h to produce Ag/TiO_2 powders. Another Ag/TiO_2 sample was also prepared as the same procedure without using chitosan for comparisons. The nanocomposite materials thus synthesized were characterized using x-ray diffraction (XRD), scanning electron microscope (SEM) and size distribution by Nano-ZS Zetasizer dynamic light scattering detector (Malvern Instruments,UK) equipped with a 4.0 mW internal laser.

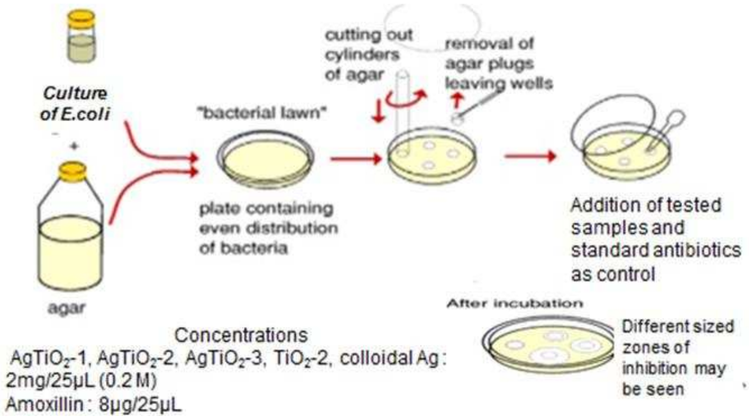
2.2.2. Antibacterial Activity Test

2.2.2.1. Agar-well diffusion method

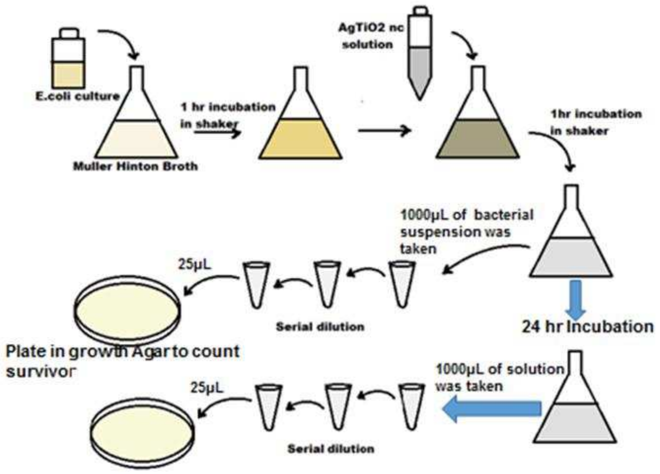
Synthesized nanocomposite powders, colloidal silver and TiO_2 powder were weighed separately and dissolved in a known volume of distilled water, to obtain a final concentration 2 mg / 25µl of each. Antibiotic amoxillin was used as negative control. Gram-negative E. coli (ATCC) were used as model organisms to perform antibacterial tests and it was prepared as culture broth solution. Muller Hinton agar solution was prepared and sterilized in the autoclave at 121ºC and 20 min. After cooling, the solution was poured into sterile petri dishes.10 µl of E. coli broth solution were spread on the whole medium surface. Agar plate was punched with a sterile cork borer of 8 mm size and 25lL of each sample was poured with micropipette in the bore. The plates were allowed to standby for 30 min and then incubated at 37°C for 24 h. After incubation, the diameter of the growth inhibition zones was investigated [Fig. 1(a)].

2.2.2.2. Plate Count Method

Nanocomposite stock solutions(1000 mg/l) used for bacterial viability assay were prepared by stirring Ag-TiO_2 nanocomposite vigorously in ultrapure deionized water followed by 30-min sonication (FS30H, Fisher Scietific, 100W, 42 kHz). The suspensions were sonicated again for at least 5 min before use. E. coli suspension was inoculated into fresh sterilized Muller Hinton broth of 80 ml in a 150 ml conical flask. These stock agar slants were incubated at 37˚C on a rotary shaker at 150 rpm for 1 h under aerobic conditions [9]. Nanocomposite stock solution was added into bacteria agar slants and incubated again as above. 25µl of serially diluted bacteria suspensions and permeate solution were spread onto Muller-Hinton agar plates. All plates were incubated at 37˚C for 16 h, and the numbers of colonies on the plates were then determined by the plate count method. The same experiments were repeated three times [Fig.1 (b)].



(a)



(b)

Fig.1 (a) Screening of antibacterial activity by agar-well diffusion method and (b) Screening of antibacterial activity by Plate count method

2.2.3. Nanocomposite application to CWF

Forty six milligram of approximately 200ml of synthesized nanocomposites are applied to the inside of the ceramic water filter pot using a paint brush. 23mg of 100 ml of mixture are applied to the outside of the pot. A higher volume is applied to the inside because there is more water in contact with this surface. Additionally, as water passes through, it will impregnated the nanocomposite deeper into the ceramic wall. The application on the outside of the pot also helps to prevent pathogens growing on the outside of the filter wall [10]. At the same time, colloidal silver supported by Thirst aid association was also applied to CWF as their standard concentration. Once the filters have been painted, these are allowed to dry for a short time. After disposing 30 l of water (3 pots full), first filter water was checked for silver ion leached out by AAS method.

2.2.4. Measuring microbiological efficacy of nanocomposite coated CWF

The consumption of drinking water contaminated with human and animal excreta is the greatest risk for infection from microbes in water. The non-pathogenic organisms that are always present in the intestines of humans and animals are excreted along with the pathogens, but in far greater numbers. Several of these coliforms are easily isolated and are ideal for use as indicators of fecal contamination. E. coli, a member of the coliform group, can survive for several weeks under ideal conditions and are far more easily detectable than the other indicator bacteria. The dimensions of E.coli are 0.5 x 1.0-3.0 µm. E.coli are almost exclusively of fecal origin and their presence confirms fecal contamination. The WHO guideline value for all water directly intended for drinking water is that the E.coli concentration must not be detectable in any 100 ml sample [11]. Microbiological efficacy of nanocomposite coated CWF was measured by reduction in indicator bacteria count of treated water. The bacterial removal efficiency was obtained by comparing the concentrations of target organisms before and after treatment. The kill percentage was calculated by the following equation [12]

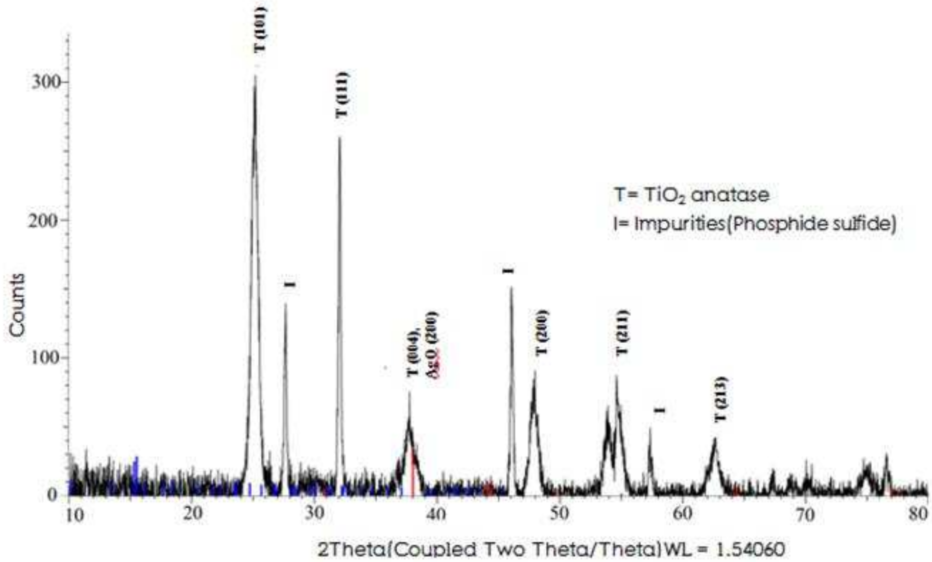
$$\text{The kill \%} = 100 - \frac{\text{Survivor count}}{\text{Initial count}} \times 100$$

3. Results and Discussion

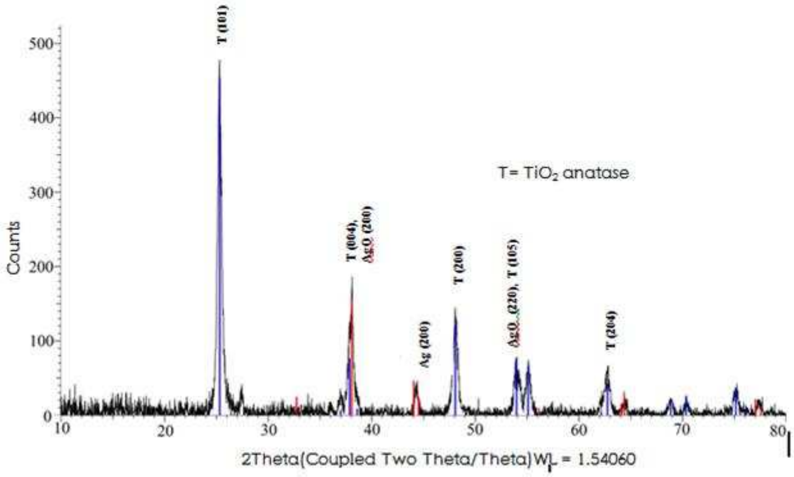
3.1. Characterization of Ag-TiO_2 nanocomposite

The X-ray diffraction pattern of the synthesized Ag-TiO_2 nanocomposites using chitosan as stabilizing agent was shown in Fig 2(a). The crystalline phase of silver and titanium were observed with the presence of secondary or impurity peaks. It indicates that more purification steps are needed to get pure Ag-TiO_2 nanostructure.

The XRD pattern of the sample synthesized without chitosan was indicated that the material includes mainly the TiO_2 anatase phase and some peak combining with Ag. This might be due to nanosized Ag particles were embedded within TiO_2 matrix. The presence of Ag or $AgNO_3$ phase confirms the imcomplete doping of Ag in TiO_2 matrix. Therefore, hybridization of TiO_2 with Ag clearly leading to a composite phase with both anatase and metallic Ag diffraction patterns was observed in Fig. 2(b).



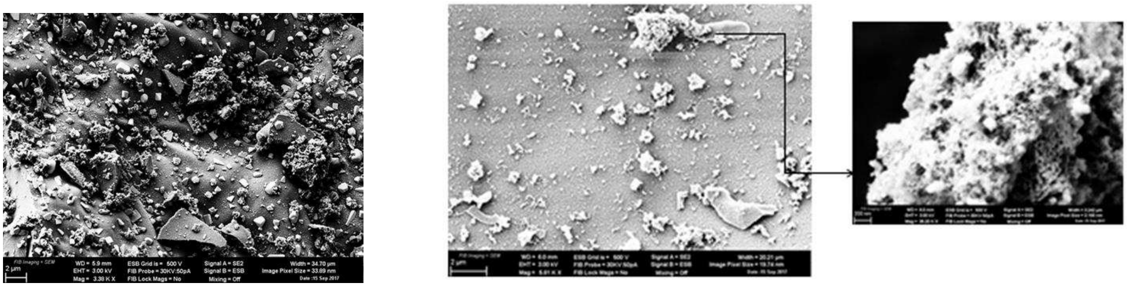
(a)



(b)

Fig.1 XRD pattern of Ag-TiO_2 nanocomposite synthesized by (a) using chitosan as stabilizing agent and (b) without chitosan

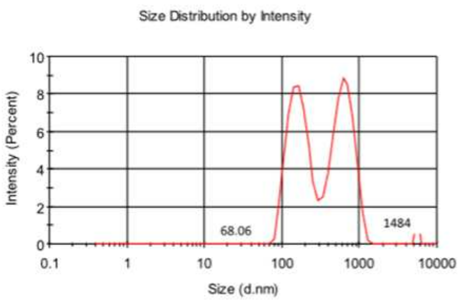
From SEM results of the TiO_2-Ag composite prepared with chitosan (Fig 3.a), it can be deduced that many impurities are contained and the titania particles with and without pores embedding Ag are spreading inconsistently. Fig.3(b) shows the micrographs of Ag-TiO_2 nanocomposites prepared without chitosan as stabilizing agent. As it is clearly indicated from the presented micrographs, the aggregated growth of spherical particles with the presence of metallic silver on mesoporous surface of the TiO_2 nanoparticles can be seen. And also some TiO_2 particles without pores and Ag are observed. The obtained composition is in good agreement with the experimental result from XRD analysis.

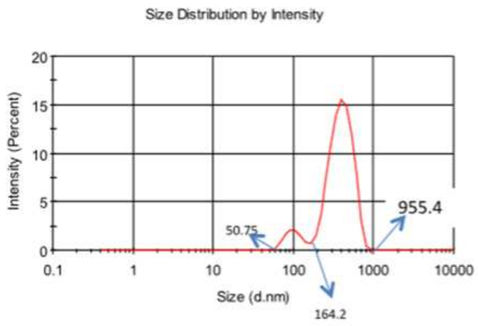


(a) (b)

Fig. 3 Scanning electron micrographs of (a) Ag-TiO_2 nanocomposite prepared with chitosan as stabilizing agent and (b) Ag–TiO_2 nanocomposite prepared without chitosan

Fig. 4(a) and 4(b) show the size distribution of synthesized Ag-TiO_2 nanocomposite, wide size distribution with two peaks ranging from 68.06 to 1484 nm was obtained from preparation method with chitosan. The Z-average size of 453 nm in diameter ranging from 50.75 to 955.4 nm was observed in size distribution result of Ag-TiO_2 synthesized by method 2. These data shows that there are two size populations present in both samples.





(a) (b)

Fig.4 Size distribution of Ag-TiO_2 nanocomposite synthesized by (a) using chitosan as stabilizing agent and (b) without chitosan

3.2. Antibacterial Activity Tests

The bactericidal activity of synthesized Ag-TiO_2 was studied by well diffusion method and plate count method. In agar-well diffusion method, after 24 hours of incubation, the inhibitory effect of Ag-TiO_2 nanocomposite was significant as compared to colloidal silver and pure TiO_2(Fig. 5). Zone of inhibition (ZoI) was used as a measure for comparing bactericidal activity of these nanocomposites. Ag-TiO_2 showed about 13 mm zone against the test organisms: E. coli.

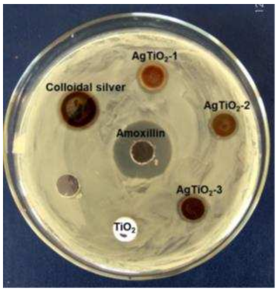


Fig.5 Comparison of antibacterial activity of synthesized Ag-TiO_2 nanocomposite against E. coli by agar well diffusion method

The plate count result of Ag-TiO_2 sample with 1×105cells/mL of E. coli was examined after 24 hours. For comparison, pure TiO_2 sample was also tested and the images are presented in Fig. 6. The results reveal that, Ag-TiO_2 sample shows complete inhibition of the bacterial growth under the present incubation condition of 24 h (Fig. 6), whereas, pure TiO_2 sample shows the presence of bacterial colonies. The enhancement of antibacterial property of Ag-TiO_2 compared to pure TiO_2 is mainly due to the following reason. When Ag-TiO_2 particles are dispersed in the growth media, the Ag atoms present in these particles interact with the bacterial cells and adhere to the bacterial cell walls. The electrostatic forces between bacteria and Ag atoms may be the reason for their adhesion. Due to the excess number of carboxylic and other groups, the overall charge on the bacterial cell surface at biological pH values is negative [13]. Further, Djokic and Burrel reported that ionic Ag strongly interacts with thiol groups of vital enzymes and inactivates them and once treated with Ag ions the DNA loses its replication ability which results in cell death[14,15]. Thus in our present study, the solid supporter TiO_2 significantly enhanced the antibacterial property of Ag.

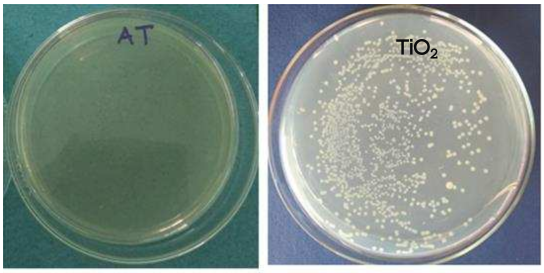


Fig. 6 Plate count results of (a) Ag-TiO_2 samples and (b) pure TiO_2 after 24 h with 1x 105 cells/ml of E. coli

3.3. Silver Loading and silver leaches out Test

The resulting silver concentrations in both Ag-TiO_2 samples were quantified by atomic absorption spectroscopy analysis. According to the results, the amount of the loaded silver on Ag-TiO_2 sample 1 is 0.5 g/kg and that of sample 2 is 1.3g/kg.

AAS analysis of released silver concentration from ceramic water filter pot was also carried out. Silver ions resident within the metal oxide TiO_2 or Ag-TiO_2 nanocomposite coated to CWF can leached to the filtered water. The silver release in the treated water was observed in the first flowed water through colloidal silver coated CWF pot and the concentration was a little bit higher than the WHO guideline of 100 µg/l[16]. Silver leached out result from CWF pot in comparison with WHO standard guideline was described in table-1.

Table 1. Silver leach out result

Description	Silver leached out concentration
Colloidal silver coated CWF pot	0.0143 mg/l
Acceptable level of silver in drinking water (WHO Guideline)	0.01 mg/l

3.4. Microbiological efficacy of nanocomposite coated CWF

Removing pathogenic indicator microorganisms from the water is one of the main tasks of nanocomposite coated ceramic water filter pot. E.coli/coliform in the influent and effluent are measured to determine the removal efficiency of the filters. Three types of polluted water sample were collected and passed through these CWF pot and indicator bacteria removal concentration in influent and effluent was comparatively determined by using petrifilm.

The main parameters that determine the quality of drinking water is to be analyzed in the water quality analysis. Water quality analysis of the water samples (pre and post treatments) such as pH, total dissolved solids, total coliform, E.Coli are determined by using standard methods. From the results, it was found that the total dissolved solids, coliform count etc. of the samples were not within the permissible limit as specified by WHO Drinking water quality standard(Table 2). Hence the water samples are not suitable for direct utilization. So the usage of this water without proper treatment leads to harmful diseases [19].

Table 2.Physicochemical parameters of raw water samples

Parameters	Sample A	Sample B	Sample C	WHO Guideline standard
pH	8.25	8.5	7.2	6.5-8.5
Total dissolved solids	5500	3200	720	1000 mg/l
Total coliform	120×10^{-3}/100 ml	25×10^{-3}/100 ml	3×10^{-3}/100 ml	0/100 ml
Fecal coliform	37×10^{-3}/100 ml	10×10^{-3}/100 ml	0/100 ml	0/100 ml

whereas Sample A = Ngamoeyeik creek water sample, Sample B = Hlaing river water sample, Sample C = Hlawgar pipe water sample

The results of removal effectiveness tests for E.coli/coliform bacteria in polluted water samples are shown as graph in Fig.7. According to the graph, nanocomposite Ag-TiO_2 0.069g coated CWF pot has the strongest removal efficiency to total bacteria and the kill percentage is 99.97%. So it has better removal efficiency than commercial colloidal silver coating CWF pot. Tested E.coli/coliform petrifilm photo are shown in Fig.8.

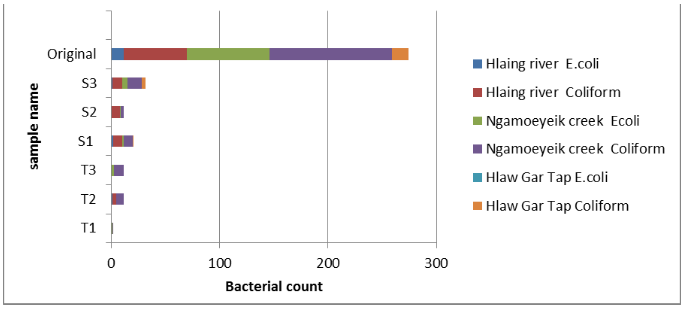
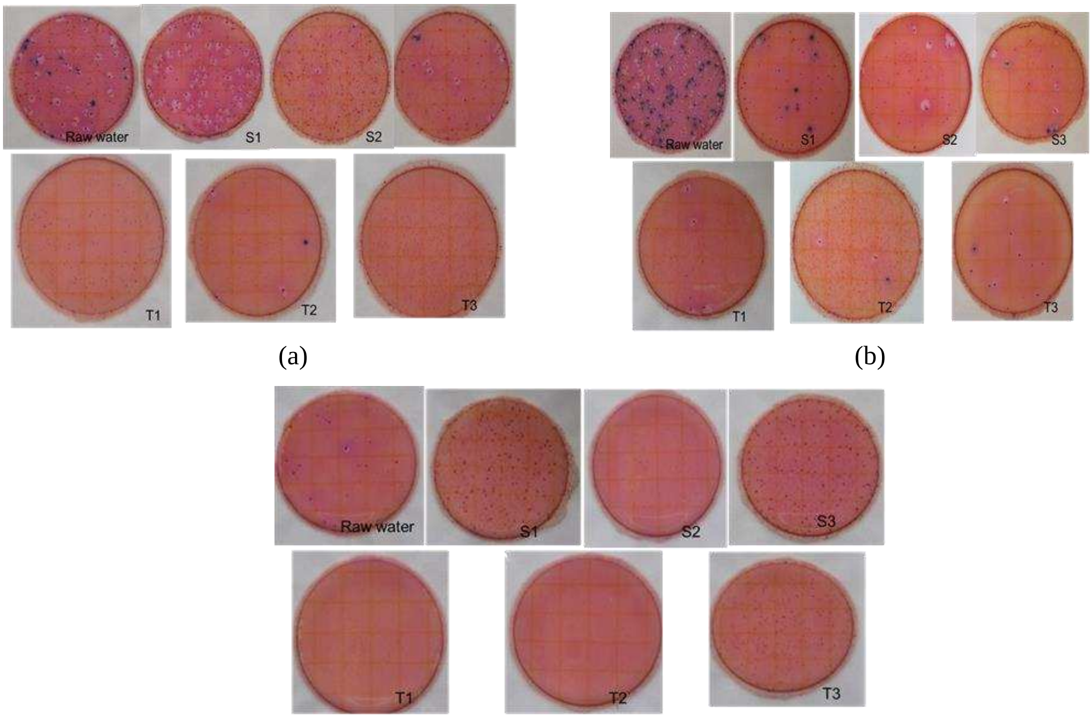


Fig.7 Graph showing the comparative analysis of ceramic water filter pot for E.coli and Coliform count whereas T is test sample and S is standard sample, [T1= $AgTiO_2$ (0.069 g) coating CWF pot, T2 = $AgTiO_2$ 0.012 g coating CWF pot*Thirst aid formula, T3 = colloidal silver 0.012 g coating CWF pot, S1= no materials included CWF pot, S2 = commercial Twantae pot , S3 = CWF pot that contain $AgNO_3$]



(a) (b)

(c)

Fig. 8 E.coli/coliform petrifilm showing microbiological efficacy of nanocomposite coated CWF for (A) Hlaing river water sample (B) Ngamoeyeik creek water sample and (C) Hlawgar pipe line water sample[Each red or blue dot indicates a CFU (colony forming unit), red for total coliform and blue for E. coli.]

4. Conclusion

Silver-Titanium dioxide (Ag-TiO_2) nanocomposite powders were synthesized successfully by sol-gel method using titanium isopropoxide (TIP) as titanium precursor. Detailed characterization of the nanocoposite was carried out using SEM, particle size distribution and XRD analyses, which confirmed the presences of silver loading on the titanium substrates. Although silver concentration in synthesized nanocomposite is lower than colloidal silver that have been already used as antibacterial agent in CWF pot, in vitro antibacterial activities of these two materials were not different. This may be due to very small amount of Ag loading in TiO_2 was sufficient to inactivate bacteria and better antibacterial performance of Ag cluster dispersed on TiO_2 surface. Bacteria removal efficacy of Ag-TiO_2 nanocomposite in disinfection of water by coating to ceramic water filter pot was analyzed. According to the comparison result of the colloidal silver effectiveness, Ag-TiO_2 nanocomposite were found to be more effective for disinfection of collected water sample contaminated with E. coli/coliform bacteria. The observations from this study suggest that the silver ion leached out from colloidal silver coated CWF pot may be the point of decreasing microbial disinfection. Therefore, the application of these antibacterial Ag-TiO_2 nanocomposite that maintain well dispersed Ag loading in CWF pots provide a sustainable and long term water treatment solution which helps to build local economies that has low capital costs and uses local resources.

Acknowledgements

We would like to thank Department of Research and Innovation (DRI), Ministry of Education, Myanmar for opportunity to do this research and we would also express our gratitude towards Thirst aid Myanmar association for their support. Furthermore, we would like to thank the laboratory staff of National Analytical Laboratory (DRI) for measurements of SEM, XRD and AAS analysis.

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