Iranian Journal of Fisheries Sciences 21(2) 500-515 2022
DOI: 10.22092/ijfs.2022.126496

Research Article

Investigating the effect of water filter made using polyurethane foam containing silver nanoparticles on controlling *Yersinia ruckeri* in *Oncorhynchus mykiss* water tanks

Sanchooli N.[1*]; Sanchooli E.[2]; Khandan Barani H.[1]

Received: July 2017 Accepted: September 2017

Abstract

The aim of this study was to evaluate the efficiency of polyurethane foam containing silver nanoparticles synthesized by *Verbena officinalis* leaf extract in controlling of *Yersinia ruckeri* in treatment of water tanks of rainbow trout farming. Biosynthesis of silver nanoparticles is done with 2mM silver nitrate solution and *Verbena officinalis* extract. To study antibacterial effects of the produced foams in vitro methods were used, including antibiogram tests on a petri dish, the diameter of inhibition and tube test at different times. Also In vivo methods were used consisting of treatment of water contaminated with bacteria in the presence and absence of fish. Results of mean of diameter of inhibition zone of foam containing 50 and 100mg of Ag-NPs on *Yersinia ruckeri* were 15.33±1.6 and 14.83 ±0.76mm respectively. *Yersinia ruckeri* was completely removed after 2h contact with foams of 50 and 100 mg Ag-NPs in water treatment without fish, and after 10min in test tubes at different times. Antibiogram tests showed no colonies in 50 and 100mg Ag-NPs at the bottom of the foam. Water treatment in the presence of fish showed a significant reduction in density of bacteria after 48h contact with filters containing 50 and 100mg silver nanoparticles. Results of this study, as a new production of nano-biofilter, can be used to reduce and control *Yersinia ruckeri* bacteria in rainbow trout fish farms.

Keywords: Polyurethane foam, *Yersinia ruckeri*, Green synthesis, *Oncorhynchus mykiss*

1- Department of Fisheries, Hamoun International Wetland Research Institute, University of Zabol, Zabol. Iran.
2- Department of Chemistry, University of Zabol, Zabol, Iran.
*Corresponding author's Email: narjes.sanchooli@uoz.ac.ir

Introduction

Silver (Ag^+) has been regarded as a strong poison for a wide range of microorganisms for a long time. Because of these widespread antimicrobial properties, silver has been widely used for centuries in biomedical applications and other disinfectants processes and in disinfection of the living environment (Liau *et al.*, 1997; Nomiya *et al.*, 2004). So far, a wide variety of antibacterial agents are identified and used to control infectious diseases in aquaculture to the extent that due to excessive and prolonged use of antibiotics, many bacteria become resistant to them, therefore, because of development of bacterial resistance and limited use of antibiotics, with regard to drug remaining and environmental problems, applying alternative methods seems necessary (Rai *et al.*, 2009). One of such methods is using silver nanoparticles on different surfaces of metal, plastic and polymer. Polyurethane foam (PUF), an organic insulating material, is of interest for many consumers due to its excellent properties including good mechanical properties, low cost and effective thermal insulation (Hu and Li, 2014; Ramezani Kakroodi *et al.*, 2015). Polyurethane coated with silver nanoparticles can be used as a water filter to remove bacteria from water (Jain and Pradeep, 2005). High reactivity of nanoparticles because of their large surface area relative to their volume is shown to be stable. Any condition leading to increase in the surface area, such as introduction of nanoparticles on porous materials such as polyurethane, may lead to an increase in biological activity (Mulongo *et al.*, 2011). For this purpose, polyurethane foams were selected with regard to carbamate group (-NH-(C=O)-O-) that is expected to facilitate the connection with nanoparticles and to show antibacterial properties (Jain and Pradeep, 2005). Synthesis of silver nanoparticles is done in different ways, including chemical (Sun *et al.*, 2002), electrochemical (Yin *et al.*, 2003), radiation (Dimitrijevic *et al.*, 2001), photochemical processes (Callegari *et al.*, 2003), the Langmuir-Blodgett (Zhang *et al.*, 2006) and biological techniques (Naik *et al.*, 2002). Disadvantages of synthesis of different nanoparticles include, use of harmful toxic chemicals, low rate of conversion and high energy consumption, which can have negative effects on water and fish. Green synthesis techniques or using live microorganisms, such as fungi, bacteria or plant extracts are regarded as simple alternative and practical method for physical and chemical synthesis (Palanivelu *et al.*, 2015). Biological method is mainly applied through enzymatic and sometimes non-enzymatic processes, and due to production of nontoxic substances in the environment, it is also called green technology. *Yersinia ruckeri* is an opportunistic pathogen that is usually available in water and has shown resistance to antibiotic treatment (Ross *et al.*, 1966). Toxicity of silver nanoparticles synthesized by chemical methods on fish and other aquatics is reported in several studies (Asharani *et al.*, 2008; Chae *et al.*, 2009; Bilberg *et*

al., 2012; Monfared and Soltani, 2013). Regarding observed damage from exposure to silver nanoparticles in fish, direct use of nanoparticles in aquaculture as an antimicrobial agent and releasing them to the environment is not allowed. Thus polyurethane foam containing silver nanoparticles synthesized by biological method is used as a new effective method, since it has less pollution and it does not use chemicals and toxins in synthesis of nanoparticles it was used as a filter to remove *Yersinia ruckeri* bacteria from water tanks of rainbow trout.

Materials and methods

Biosynthesis of silver nanoparticles with Verbena officinalis extract

Verbena officinalis leaf extract was obtained from fresh leaves collected in Agricultural Research Center of University of Zabol located in Zabol city and based on Kumarasamyraja and Jeganathan' method (2013). Synthesis of silver nanoparticles is done as a result of reaction of 2mM silver nitrate solution and extracts of *Verbena officinalis*. 1mL of extract was combined with 19mL of distilled water and then added to 400 µl of 0.1 M silver nitrate solution and placed for 24h at 37°C in a constant position in the dark. To determine physicochemical properties of nanoparticles, spectrophotometer UV-Visible, Fourier Transform Infrared (FTIR) Spectroscopy and scanning electron microscopy (SEM) were used (Palanivelu *et al.*, 2015).

Synthesis of nano-bio-filter

Raw materials of polyurethane foam, such as polyols (polyether polyols, ρ=1.1 g/cm^3), polyisocyanates (polymeric MDI, ρ=1.23 g/cm^3 diphenylmethane diisocyanate) were purchased from Axon panah, Iran. The ratio of 6 to 1 polyol to isocyanate was used to create more porosity, then 50 and 100mg of silver nanoparticles synthesized was blended with polyol and then isocyanate was added to it. After stirring at 1000rpm, the foaming process as a result of production of CO_2 began. After formation of foams they were washed and dried at room temperature (Gooch, 2007). Foams replaced in aquarium filters, foams without silver nanoparticles were used as a control group. After foams were made FT-IR and SEM analysis were performed. In order to investigate antibacterial effects of produced foams two laboratory (in vitro) and experimental methods (in vivo) were used.

In vitro methods for testing antimicrobial properties of produced water treatment filters

Antibiotic susceptibility testing of foam piece on petri dish

First 10µl of bacterial suspension containing 10^5Cfu/mL *Yersinia ruckeri* (KC291153) on nutrient agar medium cultivated surface was prepared. After that, pieces of 2×2cm of foam containing silver nanoparticles were placed in the middle of Petri dishes, after 24h of incubation at 30°C, the number of cells in sub foam areas was counted (Jain and Pradeep, 2005).

Testing inhibition zone diameter of foams containing silver nanoparticles

Yersinia ruckeri with the density of 10^5Cfu/mL was cultured on nutrient agar media, then, polyurethane foams containing silver nanoparticles or without them (control) with the approximate size of 6.5-8mm were placed in the middle of petri dishes and diameter of growth inhibition zone was measured after 24h.

Test tube tests

10mL suspension of *Yersinia ruckeri* with the density of 10^5Cfu/mL was prepared in distilled water. Then, a piece of 0.5×1×9 cm of foam containing AgNPs and foam not containing AgNPs (control) were added into each pipe. After 10min, foams were removed and squeezed in a clean and empty tube with no contaminated water. Then they were diluted to 10^{-1}, 10^{-3} and 10^{-5} from each dilution, 10µL was cultured on nutrient agar medium and after 24h the number of grown colonies was counted (Mulongo *et al.*, 2011).

Test tubes at different times

In this experiment, to each test tube containing 5mL of sterile distilled water 1.5 g of foam containing AgNPs (50 and 100 mg) and control foam was added. Then, 100µL of *Yersinia ruckeri* (1.5×10^5CFU/mL) was inoculated to each tube. To determine the density of bacteria, 100µL was removed from each tube in 0, 2, 24h after inoculation, first dilution was carried out. After dilution, 10µL from each dilution (10^{-1}, 10^{-3} and 10^{-5}) was cultured on nutrient agar medium, and then density of bacteria after 24h of incubation at 30°C was determined using total count.

The method to evaluate treatment of contaminated water with bacteria in presence of Oncorhynchus mykiss

In this study, 90 *Oncorhynchus mykiss* specimens with an average weight of 34g were used. First, the density of bacteria was set with a solution of 1 McFarland (3×10^8 Cell/mL). In order to contaminate water of each aquarium, a part of bacterial suspension was used so that a concentration of 1×10^5 *Yersinia ruckeri* in each mL of aquarium water was achieved. Sampling of aquarium water was carried out at 2, 24, 48 and 96h after contamination. After sampling, first the broth dilution was performed, then 10µL from each dilution (10^{-1}, 10^{-3} and 10^{-5}) was cultured on nutrient agar medium and after incubation for 24h at 30°C number of grown colonies was counted (Shahim *et al.*, 2015).

The method to evaluate treatment of water contaminated with bacteria in absence of Oncorhynchus mykiss

To measure the amount of bacterial removal by each filter, sampling water from each aquarium was done at 0, 2 and 24h after inoculation of bacteria. After the sampling was done, first broth dilution was performed and then 10µL of dilutions (10^{-1}, 10^{-3} and 10^{-5}) was added to nutrient agar medium and kept in incubator for 24h at 30°C.

Statistical Analyses

All of the collected data are processed using Excel software. For statistical analysis SPSS software package and to detect differences among treatments one way analysis of variance (One way-ANOVA) were used. If any significant difference was detected at (p<0.05), Duncan's Multiple Range Test was used to assess differences between means.

Results

AgNPs characterization

UV-vis spectra analysis

As *V. officinalis* leaf extract was added to silver nitrate solution, the color of the solution changed from light yellow to reddish brown after the process of reduction of Ag+ to Ag nanoparticle that indicates the AgNPs formation. The results of optical density showed that maximum measured absorption solution containing nanoparticles was at around 420nm.

Fourier transforms infrared spectroscopy

FTIR spectrum of synthesized Ag nanoparticles using *Verbena officinalis* leaf extract is shown in Figure 1.

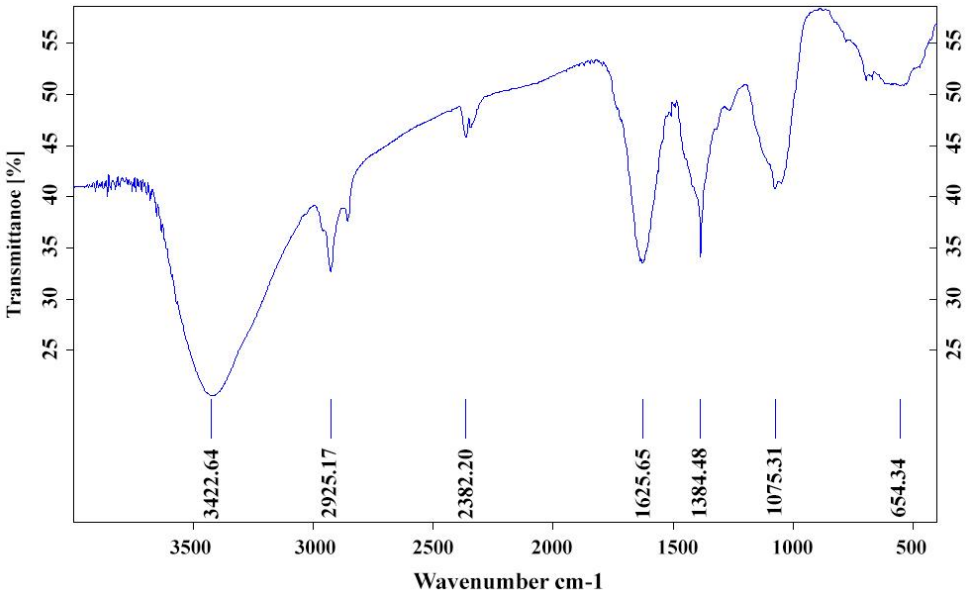


Figure 1: FTIR analysis of AgNPs obtained from *V. officinalis* leaf extract.

Prominent bands of absorbance were observed at around 1075.31, 1384.48, 1626.65, 2925.17, and 3422.64 cm^{-1}. The observed peaks at 1075.31, 1384.48 denote stretching vibration of aliphatic and aromatic amines, respectively (Khalil *et al.*, 2014). The strong peak in 1626.65 was related to stretching vibration of the C=O that usually exists in proteins and indicates the presence of protein in plant extract as a reducing agent and a stabilizer (Veerasamy *et al.*, 2011). The relatively broad peak in 3422.64 shows the presence of hydroxyl functional groups (O-H). These peaks show compounds in plant extract. C=H aliphatic bonds, intense peak in the range of 2850 to 3000 that the presence of

these peaks are observed in the frequency of 2925.17 in structure of plant extract.

Scanning electron microscopy

SEM image of AgNPs is shown in Figure 2. Nanoparticles were formed with an average size of 42.57±5.34 nm.

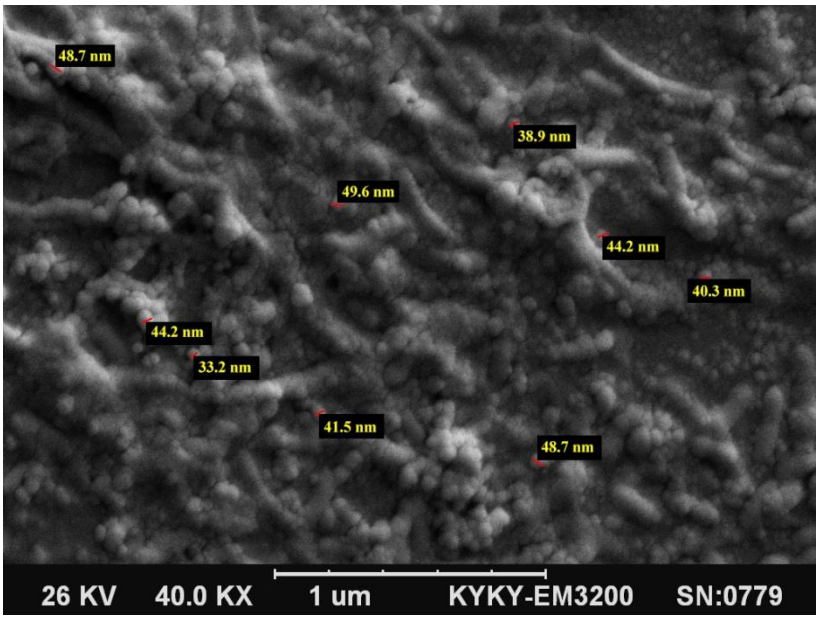


Figure 2: SEM image of silver nanoparticles formed by the reaction of 2mM silver nitrate and 1ml leaf extract of *V. officinalis*.

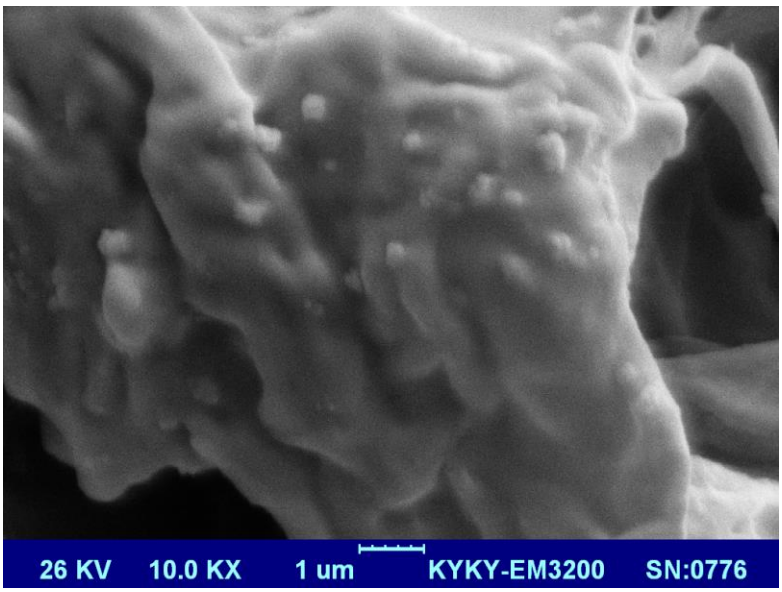
SEM of polyurethane foam containing biologically synthesized silver nanoparticles

In order to study the morphology of silver nanoparticles and their effects on the size and shape of pores of the foam, electron microscope images of them were prepared (Fig. 3). The results of microscopic analysis showed that the cells were transformed with addition of silver nanoparticles synthesized by biological methods. This means that the number of cavities was increased compared to the shape of foam without nanoparticles and the size of pores was reduced. As it is clear nanoparticles are visible on the surface of polyurethane foam (Fig. 3- top).

FTIR analysis

The FTIR spectrums that were made with control foam and foam containing silver nanoparticles are shown in Figure 4. Peaks in the area of 400-1725 are a bit different because of the presence of silver nanoparticles in foam which is coated with silver nanoparticles. The index peaks in the listed area can be noted to peak in the area of 1725 that is related to stretch of carbonyl (C=O) that is the characteristic of formation of foam from isocyanate and polyol and is related to the Carbamate functional group (-NH-(C=O)-O-), and also in the 1600 area is related to stretching of C=C of benzene ring. Most the changes were related to stretching of N-H bond that appeared in the control foam in its

real form in the area of 3300-3400, however, due to interaction of this factor group with silver nanoparticles in the foam containing silver nanoparticles the peaks in this area are decreased and C-H stretch of aromatic and aliphatic region was also somewhat weakened.



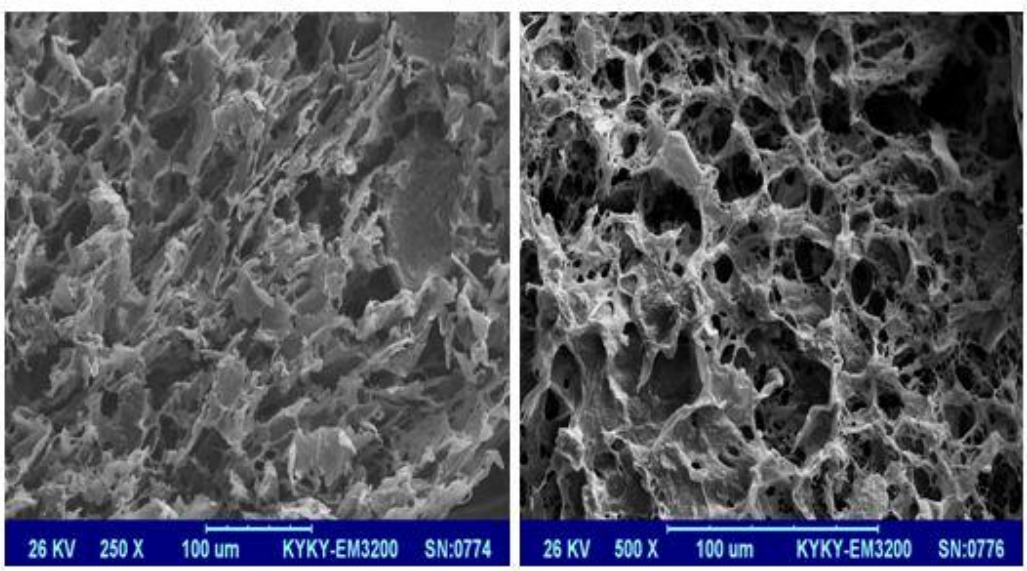


Figure 3: SEM image of polyurethane foam containing silver nanoparticles synthesized with *Verbena officinalis* extract and 2 mM solution of silver nitrate (top and right) and foam control (left).

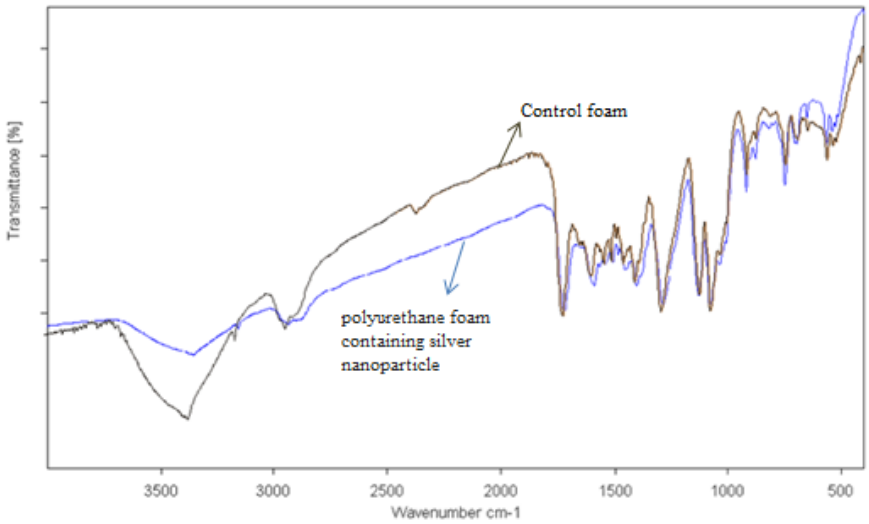


Figure 4: FTIR analysis of foam containing AgNPs obtained from *V. officinalis* leaf extract and control foam.

Tests of inhibition zone diameter of foams containing nanoparticles

Inhibition zone diameter of *Yersinia ruckeri* in contact with foams containing concentrations of 50 and 100mg of silver nanoparticles synthesized is shown in Figures 5 and 6. The analysis of results showed that there was no significant change between mean inhibition zone diameters of *Yersinia ruckeri* created around foam containing silver nanoparticles at concentrations of 100 and 50mg (p> 0.05). The mean of inhibition zone diameters caused by samples of 50 and 100mg silver nanoparticles on *Yersinia ruckeri* were 15.33±1.6 and 14.83±0.76 mm, respectively.

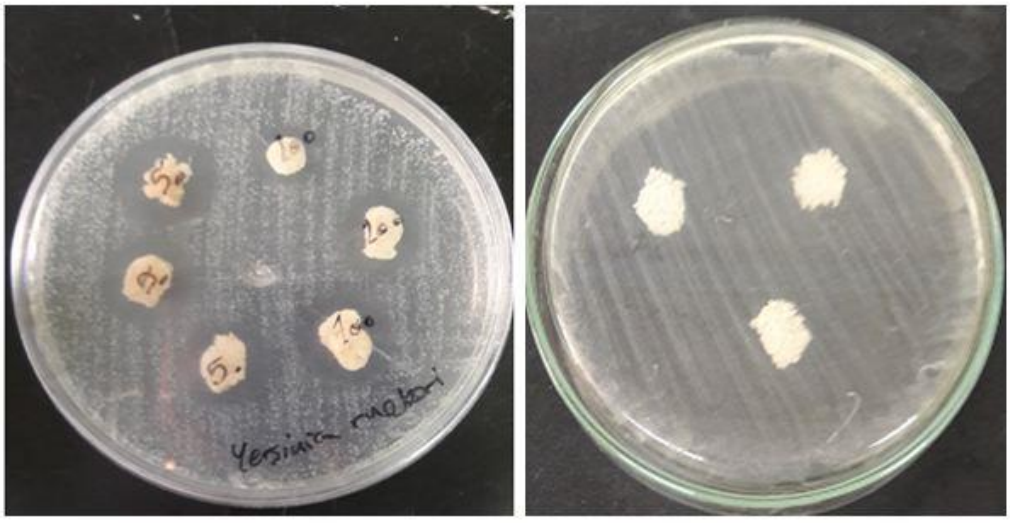


Figure 5: The effect of polyurethane foam containing 100 and 50mg of silver nanoparticles synthesized by biological (left) and control foam (right) on the growth of *Yersinia ruckeri*.

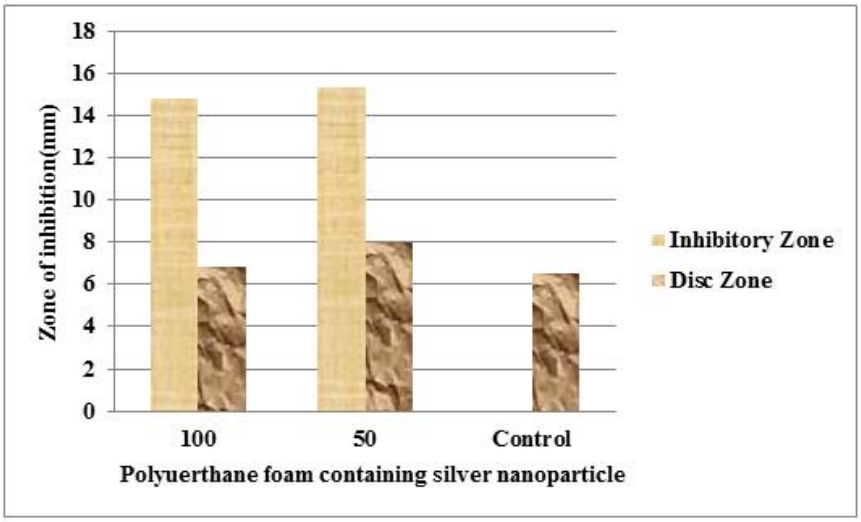


Figure 6: Disk diameter and Inhibition zone diameter of *Yersinia ruckeri* in contact with foams containing concentrations of 50 and 100mg of synthesized silver nanoparticles.

Antibiogram of foam plates on petri dish

Antibiogram of results on petri dish showed no growth of *Yersinia ruckeri* under pieces of foam containing 50 and 100mg of silver nanoparticles and growth of a large number of bacteria in the control foam.

Inhibition of test tube at different times

The density of *Yersinia ruckeri* in distilled water (mL) in contact with the control foam and silver nanoparticles foam at 0, 2 and 24h after inoculation is shown in Table 1.

Table 1: Number of colonies of *Yersinia ruckeri* grown in contact with foam with concentration of 50 and 100mg of silver nanoparticles after 0, 2 and 24h of inoculation.

Type of filter	0	2	24
Control	8×10^3	8.4×10^3	1×10^4
100mg Ag-NPs	1.68×10^3	0	0
50mg Ag-NPs	3.1×10^3	0	0

According to the table, no growth of *Yersinia ruckeri* after 2h with polyurethane foam containing 50 and 100mg concentrations of silver nanoparticles was visible.

Test tube antibiogram experiment

The results of test tube antibiogram experiment showed that after 10min of contact with the water containing bacteria in pieces of foam no colony was observed at concentrations of 50 and 100mg, while in control foam 2×10^3 colonies were observed.

Treatment of contaminated water with bacteria in the absence of fish

Results of antibacterial effects of water filters made of polyurethane foam containing biologically synthesized silver nanoparticles is shown by a chart in Figure 7. As the chart suggests, the filter was able to remove *Yersinia ruckeri* completely after 2h filtration of water in both 50 and 100mg of silver nanoparticles.

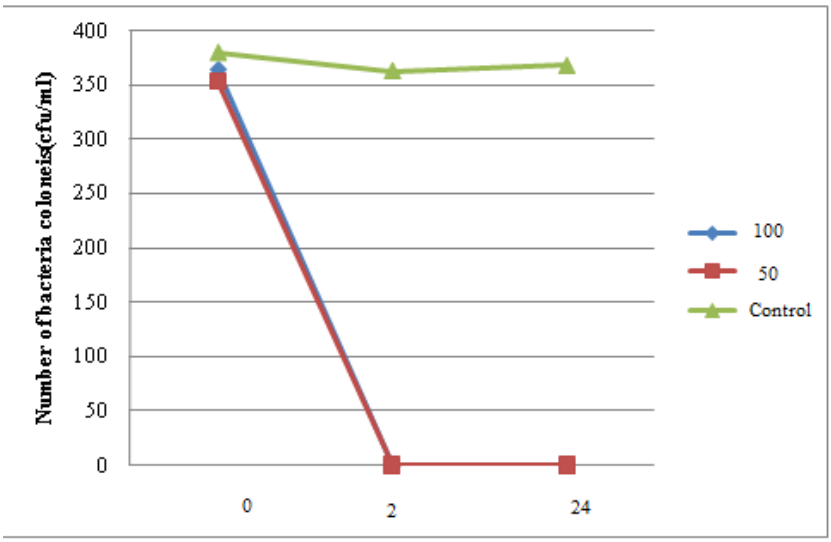


Figure 7: Populations of *Yersinia ruckeri* after infiltration of bacteria contaminated water from polyurethane foam containing 50 and 100mg synthesized silver nanoparticles at 0, 2 and 24h after contact.

Treatment of contaminated water with bacteria in the presence of fish

Bacterial density changes of *Yersinia ruckeri* after being passed through various filters (0, 50 and 100mg of nanoparticles) during different times (2, 24, 48 and 96 h) is shown in Table 2. Bacterial density at the beginning of the experiment (0h) was the same and no significant difference was observed between the treatments (p<0.05). No significant difference was observed between the treatments after 2h of water passing through the filter. However, after 24h there was a significant difference between treatments of 50 and 100mg of nanoparticles and control group. After 48 and 96h there were significant differences among all treatments, in such a way that the filter containing 100mg could reduce the bacterial density to 10^1 CFU/mL after 48h and this trend remained constant up to 96h and could not reach 0 CFU/mL. Whereas treatment of the filter containing 50mg after 48h could reduce bacterial density to 10^2 CFU/mL, and this process remained constant for 96h and could not reach 0 CFU/mL.

Table 2: Bacterial density changes of *Yersinia ruckeri* after being passed through various filters (0, 50 and 100mg of nanoparticles) during times of 2, 24, 48 and 96h. Different superscripts in the same column indicate significant differences (p<0.05).

Type of filter	2	24	48	96
Control	$1.43\times10^3\pm4.16\times10^{2\,a}$	$1.26\times10^3\pm3.66\times10^{2b}$	$1.04\times10^3\pm3.13\times10^{2c}$	$1.19\times10^3\pm1.76\times10^{2c}$
100mg Ag-NPs	$1.30\times10^3\pm7.11\times10^{2a}$	$7.43\times10^2\pm1.91\times10^{2a}$	$7.53\times10^1\pm5.7\times10^{1a}$	$1.23\times10^1\pm0.32\times10^{1a}$
50mg Ag-NPs	$1.27\times10^3\pm3.74\times10^{2a}$	$8\times10^2\pm4.35\times10^{2a}$	$7.90\times10^2\pm2.88\times10^{2b}$	$3.16\times10^2\pm1.15\times10^{2b}$

Discussion

Polymer nanocomposites attracted a great deal of attention due to their unique optical, electrical, catalytic and antimicrobial properties (Rothschild *et al.*, 2003). This enables them to be used in novel applications such as sensors, catalysts and antibacterial materials (Hyeyoung and Jyongsik, 2008). Inhibition zone diameter of foams containing 50 and 100mg silver nanoparticles were 15.33±1.6 and 14.83±0.76 mm respectively, which is almost the same as the results of Sarkhil *et al.* (2015) that mean diameter of inhibition zone of *Vibrio* sp. Persian 1(KC505639.2) bacteria in contact with stabilized nanoparticles on silica particles was 13.19-15.07 mm. Also Lv *et al.* (2009) showed a 10mm inhibition zone diameter against *E. coli* for silver nanoparticles stabilized on porous ceramic. In the study of Duan *et al.* (2016), mean inhibition zone diameter of polyurethane foam with various percentages (0.2, 0.4, 0.6, 0.8 and 10%) of 1, 3, 5- (TNO), for *E. coli*, *Staphylococcus aureus* and *Bacillus subtilis* was obtained as 14-23, 10-22 and 10-17 respectively. It seems that the type of bacteria (gram positive and gram negative), the type of filter and the amount of nanoparticles of silver contained in filters play a role in their antibacterial properties.

In the present study, *Yersinia ruckeri was* completely removed after 10min contact with foams of 50 and 100mg AgNPs using test tube test which is in accordance with the results of Jain and Pradeep (2005) that showed that after polyurethane foam was exposed to a colloidal solution of silver nitrate for one day, *E. coli* was completely eliminated after 10min of contact, while Mulongo *et al.* (2011) reported the percentage of viable *E. coli* and *Bacillus subtilis* after 10min of contact with polyurethane foam coated with nanoparticles tube to be 9% and 5%. In in vitro conditions in test tubes, foams containing silver nanoparticles at both concentrations of 50 and 100mg were able to eliminate *Yersinia ruckeri* completely, the cause of which can be not flowing of the water containing bacteria in in vitro conditions, high volume of filter, and more contact of infected water with the bacteria in foam (Shahim *et al.*, 2015).

In the present study, water purifying filter made from polyurethane foam containing silver nanoparticles was able to remove *Yersinia ruckeri* completely after 2h of contact with silver nanoparticles contained in the filter at both 50 and 100mg nanoparticles of silver. The results of Phong *et al.* (2009) showed a 100% bactericidal efficacy of filters made of polyurethane foam containing silver colloids that were used to filter *Bacillus subtilis* and *E. coli* infected water after 5h of contact. Karnib *et al.* (2013) reported a 100% reduction in number of *E. coli* 1h after contact with various concentrations of silver nanoparticles stabilized on activated carbon. In Nadafan *et al.* (2016) study, the growth curve of *E. coli* showed that, optical density of the bacterium in the control group increased from 0 to 2.2, but the final OD after 28h of contact with polyurethane foam coated with silver

nanoparticles reached 0.23. Silver Nanoparticles use different mechanisms to perform antibacterial activity (Jin *et al.*, 2010). Stabilized silver nanoparticles may apply their antibacterial activity through release of silver ions and direct contact (Sotiriou and Pratsinis, 2010). The same antibacterial activity in two concentrations of 50 and 100mg of silver nanoparticles on *Yersinia ruckeri* bacteria was found in the present study. It can be concluded that direct contact is associated with destruction of bacteria. In all forms of silver, including silver chloride, silver zeolite, silver titanium dioxide composite, silver activated carbon, silver-polymer nanoparticle composites their antimicrobial activity is partially due to release of silver ions. It is shown that silver nanoparticles impose additional antimicrobial activity in comparison to accumulation state of silver ion (Chen and Schluesener, 2008) *Yersinia ruckeri* was removed after being filtered by foams containing silver nanoparticles in the aquarium for 2h. In the present study, *Yersinia ruckeri* density was reduced in contact with the filter containing 100 and 50mg nanoparticles to 10^1 and 10^2 after 48h and was not completely eliminated, which is in line with the results of Shahim *et al.* (2015) in which the density of *Streptococcus iniae* decreased to 10^2 after 96h contact with polyurethane foam containing silver zeolite in presence of *Oncorhynchus mykiss*. One of the reasons for not eliminating bacteria in in vivo conditions (Treatment of contaminated water with bacteria with the presence of fish) is due to the presence of fish and excretion of organic matter including stool, mucus from its body that increase dissolved organic carbon (DOC) in the water. DOC is regarded as a nutrient and suitable for bacterial growth and also can reduce silver antimicrobial properties through bonding with it which as a result increases the amount of bacteria (Brett, 2006). The mechanism of the effect of silver nanoparticles synthesized from leaf extract of *Verbena officinalis* on *Yersinia ruckeri* can be either antibacterial or bacteriostatic. Or the bacteria were directly killed by silver ions released from polyurethane foam, it is argued that the positive ion of silver is bound to the negative charge of the cell wall of bacteria and can cause cell lysis and bacterial death (Jeon *et al.*, 2003). Or silver ion can interfere with the mechanism of DNA replication of cell membrane and the outer layer of sensitive cells, antibacterial effects (Lin *et al.*, 1996; Siddhartha *et al.*, 2007). Silver ions interfere with bacterial growth signaling pathways that produce peptides for cell survival and cell division, bacteriostatic effect (Lee and Meisel, 1982). The bacterial growth rate was significantly decreased in NA (nutrient agar) medium, therefore disappearance of bacteria may be due to antibacterial or bacteriostatic effects of silver (Nadafan *et al.*, 2016).

In all of the above studies, polyurethane foam was prepared and immersed in the solution for 8 to 10h so that nanoparticles be coated on it, but in the present study, as a new method, of silver

nanoparticles were biologically synthesized by material of polyurethane foam and polyurethane foam containing silver nanoparticles. Therefore, it is introduced as a new highly effective method for removal and control of pathogenic bacteria.

References

Asharani, P.V., Wu, Y.L., Gong, Z. and Valiyaveettil, S., 2008. Toxicity of silver nanoparticles in zebrafish models. *Nanotechnology*, 19, 1-8. DOI:10.1088/0957-4484/19/25/255102.

Bilberg, K., Hovgaard, M.B., Besenbacher, F. and Baatrup, E., 2012. In vivo toxicity of silver nanoparticles and silver ions in zebrafish (*Danio rerio*). *Journal of Toxicology*, 1, 1-9. DOI:10.1155/2012/293784.

Brett, D.W., 2006. A discussion of silver as an antimicrobial agent: alleviating the confusion. *Ostomy Wound Manage*, 52, 34-41. PMID: 16464989**.**

Callegari, A., Tonti, D. and Chergui, M., 2003. Photochemically grown silver nanoparticles with wavelength-controlled size and shape. *Nano Letter*, 3, 1565–1568. DOI : 10.1021/nl034757a.

Chae, Y.J., Pham, C.H., Lee, J., Bae, E., Yi, J. and Gu, M.B., 2009. Evaluation of the toxic impact of silver nanoparticles on Japanese medaka (*Oryzias latipes*). *Aquatic Toxicology*, 94, 320-327. DOI: 10.1016/j.aquatox.2009.07.019.

Chen, X. and Schluesener, H.J., 2008. Nanosilver: a nanoproduct in medical application. *Toxicology Letter*, 176(**1**), 1-12. DOI: 10.1016/j.toxlet.2007.10.004.

Dimitrijevic, N.M., Bartels, D.M., Jonah, C.D., Takahashi, K. and Rajh, T., 2001. Radiolytically induced formation and optical absorption spectra of colloidal silver nanoparticles in supercritical ethane. *Journal of Physiology and Biochemistry*, 105, 954-959.

Duan, G.H., Yin, F. and Jiang, G.C., 2016. Synthesis and simultaneous self-assembly of novel antibacterial polyurethanes. Global Conference on Polymer and Composite, *IOP Coference Materials, Science and Engineering*, 137(**1**), 012028. DOI:10.1088/1757-899X/137/1/012028.

Gooch, J.W., 2007. Encyclopedic dictionary of polymers. Springer Verlag, New York, USA.

Hu, S.J. and Li, Y.B., 2014. Two-step sequential liquefaction of lignocellulosic biomass by crude glycerol for the production of polyols and polyurethane foams. Bioresource Technology, 161, 410-415. PMID: 24727702.

Hyeyoung, K. and Jyongsik, J., 2008. Antibacterial properties of novel poly(methyl methacrylate) nanofiber containing silver nanoparticles. *Langmuir*, 24, 2051-2056. DOI: 10.1021/la703085e.

Jain, P. and Pradeep, T., 2005. Potential of silver nanoparticle-coated polyurethane foam as an

antibacterial water filter. *Biotechnology and Bioengineering*, 90, 59-63. DOI: 10.1002/bit.20368.

Jeon, H.J., Yi, S.C. and Oh, S.G., 2003. Preparation and antibacterial effects of Ag–SiO2 thin films by sol-gel method. *Biomaterials*, 24, 4921-4928. DOI:10.1016/s0142-9612(03)00415-0.

Jin, X.L.I.M., Wang, J.W., Marambio-Jones, C., Peng, F., Hung, X., Damoiseaux, R. and Hoek, E.V., 2010. High-throughput screening of silver nanoparticle stability and bacterial inactivation in aquatic media: influence of specific ions. *Environmental Science and Technology*, 44, 7321-7328. DOI: 10.1021/es100854g.

Karnib, M., Holail, H., Olama, Z., Kabbani, A. and Hines, M., 2013. The antibacterial activity of activated carbon, silver, silver impregnated activated carbon and silica sand nanoparticles against pathogenic *E. coli* BL21. *International Journal of Current Microbiology and Applied Sciences*, 2(**4**), 20-30.

Khalil, M.M.H., Ismail, E.H., El-Baghdady, K.Z. and Mohamed, D., 2014. Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. *Arabian Journal of Chemistry*, 7(**6**), 1131–1139. DOI: 10.1016/j.arabjc.2013.04.007.

Kumarasamyraja, D. and Jeganathan, N.S., 2013. Green synthesis of silver nanoparticle using aqueous extract of *Acalypha indica* and its antimicrobial activity. *International Journal of Pharma and Bio Sciences*, 4(**3**), 469-476. DOI: 10.1.1.422.7047

Lee, P.C. and Meisel, D., 1982. Adsorption and surface-enhanced Raman of dyes on silver and gold sols. *Journal of Physical and Chemical*, 86, 3391-3395. DOI: 10.1021/j100214a025.

Liau, S.Y., Read, D.C., Pugh, W.J., Furr, J.R. and Russell, A.D., 1997. Interaction of silver nitrate with readily identifiable groups: relationship to the antibacterial action of silver ions. *Letters in Applied Microbiology*, 25(**4**), 279-283. DOI: 10.1046/j.1472-765x.1997.00219.x.

Lin, Y.S.E., Vidic, R.D., Stout, J.E. and Yu, V.L., 1996. Individual and combined effects of copper and silver ions on inactivation of *Legionella pneumophila*. *Water Res*earch, 30, 1905-1913. DOI: 10.1016/0043-1354(96)00077-2**.**

Lv, Y., Liu, H., Wang, Z., Liu, S., Hao, L., Sang, Y., Liu, D. Wang, J. and Boughton, R.I., 2009. Silver nanoparticle-decorated porous ceramic composite for water treatment. *Journal of Membrance Science*, 331(**1-2**), 50-56. DOI: 10.1016/j.memsci.2009.01.007.

Monfared, A.L. and Soltani, S., 2013. Effects of silver nanoparticles administration on the liver of rainbow trout (*Oncorhynchus mykiss*), histological and biochemical studies. *European Journal of Experimental Biology*, 3, 285-289.

Mulongo, G., Mbabazi, J., Nnamuyomba, P. and Song, H.C.,

2011. Water bactericidal properties of nanosilver polyurethane composites. *Nanoscience and Nanotechnology*, 1(**2**), 40-42. DOI: 10.5923/j.nn.20110102.07.

Nadafan, M., Malekfar, R., Izadi-Darbandi, A. and Dehghani, Z., 2016. Microstructural and antibacterial properties of silver nanoparticle-decorated porous polyurethane surface for water purification. *Journal of Desalination and Water Treatment*, 57(**45**). Published online. DOI: 10.1080/19443994.2015.1123194.

Naik, R.R., Stringer, S.J., Agarwal, G., Jones, S., and Stone, M.O., 2002. Biomimetic synthesis and patterning of silver nanoparticles. *Nature Material*, 1, 169-172. DOI: 10.1038/nmat758.

Nomiya, K., Yoshizawa, A., Tsukagoshi, K., Kasuga, N.C., Hirakawa, S. and Watanabe, J., 2004. Synthesis and structural characterization of silver (I), aluminium (III) and cobalt (II) complexes with 4-isopropyltropolone (hinokitiol) showing noteworthy biological activities. Action of silver (I)-oxygen bonding complexes on the antimicrobial activities. *Journal of Inorganic Biochemistry*, 98(**1**), 46-60.DOI: 10.1016/j.jinorgbio.2003.07.002.

Palanivelu, J., Kunjumon, M.M., Anushree Suresh, A. and Ashwini Nair, A., 2015. Green synthesis of silver nanoparticles from *Dracaena mahatma* leaf extract and its antimicrobial activity. *Journal of Pharmaceutical Science and Research*, 7(**9**), 690-695.

Phong, N.T.P., Thanh, N.V.K. and Phuong, P.H., 2009. Fabrication of antibacterial water filter by coating silver nanoparticles on flexible polyurethane foams. *Journal of Physics: Conference Series*, 187(**1**), 012079. DOI: 10.1088/1742-6596/187/1/012079.

Rai, M., Yada, A. and Gade, A., 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnological Advanced*, 27, 76-83. DOI: 10.1016/j.biotechadv.2008.09.002.

Ramezani Kakroodi, A., Khazabi, M., Maynard, K., Mohini, S. and Kwon, O.S., 2015. Soy-based polyurethane spray foam insulations for light weight wall panels and their performances under monotonic and static cyclic shear forces. *Industrial Crops and Products*, 74, 1-8. DOI: 10.1016/j.indcrop.2015.03.092

Ross, A.J., Rucker, R.R. and Ewing, W.H., 1966. Description of a bacterium associated with redmouth disease of rainbow trout. *Journal of Microbiology*, 12, 763-770. DOI: 10.1139/m66-103.

Rothschild, A., Komem, Y., Levakov, A., Ashkenasi, N., Shapiro, Y., 2003. Electronic and transport properties of reduced and oxidized nanocrystalline TiO2 films. *Applied Physical Letters*, 82, 574-576. DOI: 10.1063/1.1539556.

Shahim, A., Kalbasi, M.R., Soltani, M., Johari, A., 2015. Application of polyurethane foam containing silver

zeolite in filtration system of water rainbow trout for controlling of infections caused by *Streptococcus iniae*. *Journal of Veterinary Research*, 70(**1**), 63-71, in Persian.

Siddhartha, S., Tanmay, B., Arnab, R., Gajendra, S., Ramachandrarao, P. and Debabrata, D., 2007. Characterization of enhanced antibacterial effects of novel silver nanoparticles. *Nanotechnology*, 18, 225103-225111. DOI: 10.1088/0957-4484/18/22/225103.

Sotiriou, G.A. and Pratsinis, S.E., 2010. Antibacterial activity of nanosilver ions and particles. *Environmental Science Technology*, 44, 5649-5654. DOI: 10.1021/es101072s

Sun, Y., Yin, Y., Mayers, B.T., Herricks, T. and Xia, Y., 2002. Uniform form silver nanowires synthesis by reducing AgNO3 with ethylene glycol in presence of seeds and poly (vinyl pyrrolidone). *Chemistry of Materials*, 14, 4736-4745. DOI: 10.1021/cm020587b.

Veerasamy, R., Xin, T.Z., Gunasagaran, S., Xiang, T.F.W., Yang, E.F.C., Jeyakumar, N. and Dhanaraj, S.A., 2011. Biosynthesis of silver nanoparticles using mangosteen leaf extract and evaluation of their antimicrobial activities. *Journal of Saudi Chemical Society*, 15(**2**), 113-120. DOI: 10.1016/j.jscs.2010.06.004.

Yin, B., Ma, H., Wang, S. and Chen, S., 2003. Electrochemical synthesis of silver nanoparticles under protection of poly (N-vinylpyrrolidone). *Journal of Physiology and Biochemistry*, 107, 8898-8904. DOI: 10.1021/jp0349031.

Zhang, L., Shen, Y.H., Xie, A.J., Li, S.K., Jin, B.K. and Zhang, Q.F., 2006. One-step synthesis of monodisperse silver nanoparticles beneath vitamin E Langmuir monolayers. *Journal of Physiology and Biochemistry*, 110, 6615-6620. DOI:10.1021/jp0570216.