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Original Article

Investigation of Low-Pressure Ultraviolet Radiation on Inactivation of Rhabitidae Nematode from Water

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Abstract

Background: Rhabditidae is a family of free-living nematodes. Free living nematodes due to their active movement and resistance to chlorination, do not remove in conventional water treatment processes thus can be entered to distribution systems and cause adverse health effects. Ultraviolet radiation (UV) can be used as a method of inactivating for these organisms. This cross sectional study was done to investigate the efficiency of ultraviolet lamp in the inactivation of free living nematode in water.

Methods: The effects of radiation time, turbidity, pH and temperature were invistigated in this study. Ultraviolet lamp used in this study was a 11 W lamp and intensity of this lamp was 24 µw / cm².

Results: Radiation time required to achieve 100% efficiency for larvae nematode and adults was 9 and 10 minutes respectively. There was a significant correlation between the increase in radiation time, temperature rise and turbidity reduction with inactivation efficiency of lamp ($P<0.001$). Increase of turbidity up 25 NTU decreased inactivation efficiency of larvae and adult nematodes from 100% to 66% and 100% to 64% respectively. Change in pH range from 6 to 9 did not affect the efficiency of inactivation. With increasing temperature inactivation rate increased. Also the effect of the lamp on inactivation of larvae nematod was mor than adults.

Conclusions: It seems that with requiring the favorable conditions low-pressure ultraviolet radiation systems can be used for disinfection of water containing Rhabitidae nematode.

Keywords: Ultraviolet radiation, Nematode, Rhabditidae, Water

Introduction

In many developing countries, lack of healthy drinking water is one of the most challenging health problems. Undoubtedly, without healthy drinking water supply, human health and welfare will be endangered (1, 2). One of the most common and dangerous pollution of water resources is biological contamination. Water can be contaminated by various types of microorganisms such as bacteria, fungi, viruses and parasites. Among these pollutants, worms' parasitic infection is one of the most prevalent of epidemic water borne diseases around the world (2, 3). Nematodes, which are one of the species of parasitic worms, are a group of organisms in fresh water that are considered food source for invertebrates, vertebrates such as fish and number of fungi. It is estimated that hundreds of millions of nematodes can exist in an area of 4.047 m² in 7.6 cm of drinking water's filter bed (3, 4). Free living nematodes which are usually benthic or residing in wet soils live in natural aerobic places that contain bacterial food. Thus, they can be seen in sandy filters, biological wastewater treatment units and numerously in secondary effluent. Because of their active motion and chlorine resistance, they will not die in common water treatment processes and can enter the water distribution systems. Most of the nematodes in drinking water treatment facilities are caused by soil runoffs and rivers with high flow that floats bed organisms or by sewage effluent (1-4). Fig. 1 shows an adult and a larva nematode.

There are various methods for water disinfection that generally divided into two categories: chemical and physical. Chlorination and the use of ozone gas are common chemical methods, and heat, filtration and radiation are common physical methods (4, 5). The most common disinfection method which is used is chlorination; but with discovery of producing three halomethanes in disinfection with chlorine, the use of ultraviolet lamps has been increased and it causes less use of chlorination in countries with high level of health.

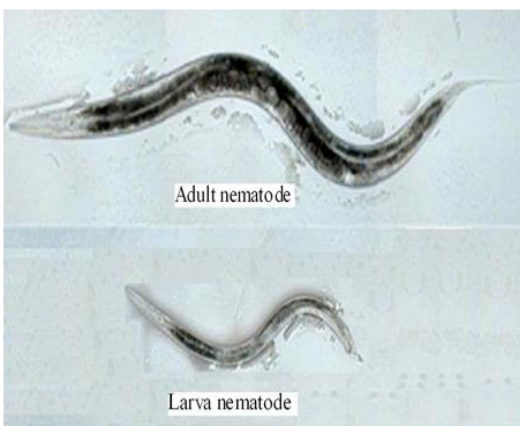

Fig. 1: Adult and a larva Rhabditidae (4)

Using UV lamps in water disinfection is relatively a new technology and has a 40-year background (5, 6). The first mercury-vapor lamp was made in 1986 which was based on electrical discharge in a low-pressure tube containing mercury vapor. Next steps in the application of UV radiation in water disinfection were development of artificial mercury lamps in 1901 and the use of quartz as a UV transmitter in 1906. The first application of water disinfection was performed in Marseille, France in 1910. In 1929, Gates identified the relationship between UV disinfection and UV absorption by nucleic acid (6-10).

In this study, ultraviolet disinfecting lamp efficiency in water treatment containing Rhabitidae nematode was studied and the effects of radiation time, turbidity, pH and temperature on efficiency of UV lamps were assessed.

Materials and Methods

In this descriptive–analytic study, the disinfecting efficiency of ultraviolet lamps in the treatment of water containing Rhabditidae (larva and adult) and the effect of radiation time, turbidity, pH and temperature on the efficiency of ultraviolet light devices were examined. Low-pressure UV lamp that was used was a Polish 11W- Philips-UV light device with 3 cm length and 16 cm width with radiation intensity of 24 **$\mu w/cm^2$**. Because these light devices cannot connect to municipal electricity directly, one transformer was used for the electrical current conversion. A 1000 mL reactor was used in these experiments. The lamp was placed inside the quartz tube and then placed inside the relevant reactor. Before each use, it took 3 to 5 minutes to become warm and achieve steady state. After each examination quartz tube was cleaned to prevent negative effects on lamp performance. Fig. 2 shows components of ultraviolet pilot.

The examined nematode in this study was a kind of free-living nematodes from Rhabditida branch that was cultured in Helminthology Laboratory of Public Health Department in Tehran University of Medical Sciences. Adult and larva nematodes' sizes were 800 and 150 microns, respectively. Thus, light microscope was used for counting. A 0.5 mL sample was taken and number of adult and larva nematodes was counted on the microscope slide. This procedure was repeated three times and finally the average number per volume was specified. Water samples were municipal water. A few drops of sodium thiosulfate were added to the samples to eliminate the effects of chlorine on nematodes.

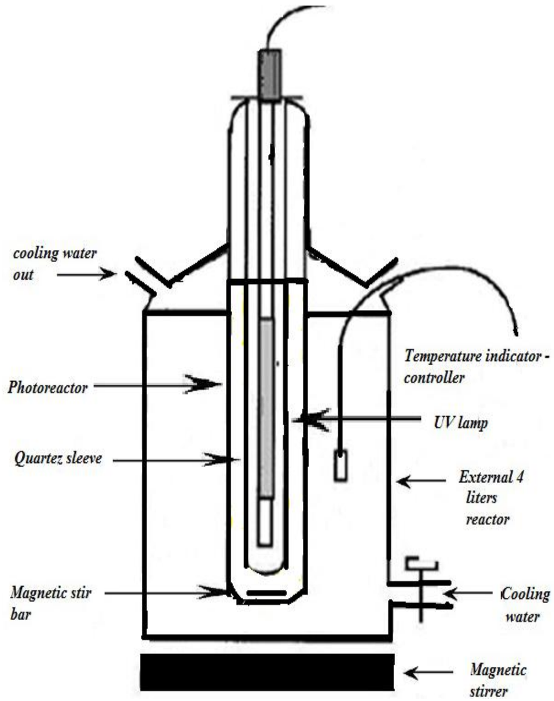

Fig. 2: UV reactor set-up for the inactivation of Rhabditidae

The plates containing cultured nematodes were washed with 20 mL phosphate-buffered saline (PBS) with pH 7.4 to let the nematodes enter the solution and be separated from the cultured area. Fig. 3 show plates containing cultured Rhabitidae.
Then a 200 mL chlorinated water mixture with 20 mL PBS, which had washed the cultured area, was prepared. So, a 220 mL sample was prepared and 110 mL of its volume were poured into a beaker as standard and dead and alive larva's were counted for 5 mL of it and the remaining 110 mL volume was exposed to ultraviolet radiation at different time periods, ranging from 1 to 10 minutes and was analyzed. In this study, retention time was kept constant and equal to 10 minutes and various effects of turbidity on inactivating efficiency of Rhabitidae in UV lamp were evaluated. Turbidity of water samples which were taken from Tehran municipal water were below standard level, mostly were less than 0.5 NTU. Therefore, synthetic turbidity was used in the lab. Clay was used to determine turbidity effects up to 30 NTU on lamp efficiency. Particle sizes of bentonit clay are less than 2 microns. Due to dissolving possibility of clay particles in the pilot area during radiation, mixing should be carried out with a mixer.

Fig. 3: Plates containing cultured Rhabditidae

Also, the effect of temperature in the range of 20 ºC to 45 ºC, turbidity in the range of 5 to 25 NTU and pH in the range of 6 to 9, were assessed. The average distance of sample was 2 cm subject to radiation of the lamp during the experiment and water temperature was kept constant in 20 ºC during the study period except in studying the effect of temperature.

Results

In Tables (1-4) inactivation efficiency of larva and adult nematodes were compared under various experimental conditions. The results (Tables 1 and 2) show that increasing contact time increased lamp efficiency in inactivating the nematodes. Inactivation efficiency of light device reached 100% in the contact time of 9 minutes (3240 dose $\mu w.s/cm^2$) for larva nematode and 10 minutes (3600 dose $\mu w.s/cm^2$) for adult nematode.
As shown in Table (3), during the 10 minutes radiation time, increasing turbidity to 25 NTU decreased inactivation efficiency of larva Rhabitidae from 100% to 66%. For adult Rhabitidae, during the 10 minutes radiation time, inactivation efficiency was constant, up to turbidity of 9 NTU, but with increasing of turbidity to 25 NTU, inactivation efficiency decreased from 100% to 64% (Table 4).

Table 1: Inactivation efficiency of larva Rhabditidae at different radiation times in municipal water

Radiation times (minute)	Radiation dose μw.s/cm²	Survival fraction of larva including the effect of witness	Inactivation efficiency of larva (percentage)
1	360	1	0
2	720	1	12.4
3	1080	0.81	14.2
4	1440	0.81	25
5	1800	0.66	33
6	2160	0.5	57
7	2520	0.3	67
8	2880	0.2	81
9	3240	0.00	100

Table 2: Inactivation efficiency of adult Rhabditidae at different radiation times in municipal water

Radiation times (minute)	Radiation dose μw.s/cm²	Survival fraction of adult including the effect of witness	Inactivation efficiency of adult (percentage)
1	360	0.97	2.9
2	720	0.91	8.83
3	1080	0.87	14.7
4	1440	0.76	23.4
5	1800	0.77	25
6	2160	0.63	36
7	2520	0.41	58
8	2880	0.31	68
9	3240	0.06	93
10	3600	0.00	100

Table 3: Turbidity effect on inactivation efficiency of larva Rhabditidae at 10 minutes radiation time

Turbidity (NTU)	Survival fraction of larva including the effect of witness	Inactivation efficiency of larva (percentage)
6	0.00	100
7	0.00	100
8	0.00	100
9	0.00	100
10	0.00	100
11	0.00	100
12	0.00	100
13	5.2	94
14	4.7	95
15	18	80
20	26	75
25	33	66

For both larva and adult nematodes, changing pH in the range of 6 to 9 did not affect the inactivation efficiency, during the 10 minutes radiation time. During the 10 minutes radiation time, increasing water temperature efficiency increased efficiency of light device in inactivating larva and adult nematodes; this increase in efficiency is from 18.5% at 21 ºC to 89% at 45 ºC and efficiency for adult nematodes increased from 12.5% at 21 ºC to 78.8% at 45 ºC.

Table 4: Turbidity effect on the inactivation efficiency of adult Rhabditidae at 10 minutes radiation time

Turbidity (NTU)	Survival fraction of adult including the effect of witness	Inactivation efficiency of adult (percentage)
6	0.00	100
7	0.00	100
8	0.00	100
9	0.00	100
10	1.80	98
11	2.5	97
12	4.5	95
13	7.6	92
14	10	89
15	20	79
20	29	70
25	36	64

Statistical analyses

Linear regression test was used to determine the relationship between efficiency of inactivating and measured variables. The results show that there was a meaningful relationship between the increase in the radiation time and the increase in efficiency of the light device. There also were meaningful relationships between increasing turbidity and reduction in light device's efficiency and also between increasing temperature and increase in efficiency (P<0.001). Statistical analyses show that there was meaningful relationship between turbidity and inactivation efficiency of nematodes.

Discussion

Effect of radiation time and lamp dose

The results of this study show that the effect of this light device on larva is more than on adult. The minimum dose for inactivating adult Rhabitidae is 3600 μw.s/cm^2, while it is 3240 μw.s/cm^2 for larva nematode, with a 10 minutes radiation time. Vaezi (1980) studied application of ultraviolet radiation for disinfection of wastewater and its impact on Ascaris eggs. The results of this research shows that the minimum dose required for inactivating *Ascaris* eggs is 440 mw.s/cm^2 with a 5 minutes radiation time in 6 cm of lamp (11).

Sarah et al. (2006) studied the inactivation rate of swine *Ascaris* eggs using low-pressure ultraviolet radiation. They concluded that the minimum required value of radiation dose for inactivation is 800 mw.s/cm ^{2} and 50 mw.s/cm ^{2} for intact and peel eggs, respectively (7). Required doses for inactivation in both studies are considerably more than the required dose of the present study; because the sensitivity of larva and adult nematode is more than *Ascaris* worm's egg.

Effect of turbidity

Results of turbidity effect investigation show that the increase in the turbidity will result in the reduction of efficiency of the light device in inactivating the nematodes and this is because of the power of turbidity-causing particles in broadcasting light. The increase in turbidity value makes it hard to achieve an optimal standard treatment by UV lamp and particles, especially large ones, have great effects on level of disinfection efficiency that is compatible with our results (10).

Effect of temperature

In this experiment, the range that was studied for determining the effect of temperature was 21°C to 45 °C. The results showed that the temperature affects the inactivation efficiency and increasing the temperature will increase inactivation efficiency. Effect of the temperature on dose rate response depends on the type of microorganism. For example, related studies have shown that inactivation of bacteriophage MS2, does not depend on temperature (12, 13); but increasing temperature from

5 °C to 35 °C will increase the lamp efficiency about 10%, 10% and 20% in *E. coli* and *Giardia lamblia* and bacteriophage, respectively (14).

Effects of pH

A pH range of 6 to 9 was considered to investigate the effect of this parameter on inactivating the adult and larva nematodes. The results showed that variation of pH in this range has no effect on efficiency of ultraviolet light device in inactivating the nematodes. These results are compatible with the results of Malley (2000) about evaluating ultraviolet systems engineering in drinking water disinfection. Moreover, the present study shows that the variation of pH in range of 6 to 9 has no effect on efficiency of these lamps (15).

Conclusions

It seems that with requiring the favorable conditions, UV radiation systems can be used for disinfection of water and effluent of the sewage treatment plants.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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