

ORIGINAL ARTICLE

Solar radiation (UV-A and temperature) in the inactivation of *Vibrio cholerae* in water for human consumption. Factors that condition the efficiency of the process

Uso de la radiación solar (UV-A y temperatura) en la inactivación del *Vibrio cholerae* en agua para consumo humano. Factores que condicionan la eficiencia del proceso

Yezid Solarte¹, Martha Lucía Salas¹, Bernhard Sommer², Carlos Dierolf¹, Martin Wegelin²

1 Instituto de Investigación y Desarrollo de Agua Potable, Saneamiento Básico y Conservación del Recurso Hídrico (CINARA). Universidad del Valle, Cali, Colombia. **2** Swiss Federal Institute for Environmental Science and Technology (EAWAG), Duebendorf, Switzerland.

*yesid.solarte@correounivalle.edu.co



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Vibrio cholerae, Coliformes termotolerantes, Agua potable, Desinfección solar, Radiación solar. Luz ultravioleta, SODIS.

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**Conflicts of interest:**

No conflict of interest

Abstract

Vibrio cholera was inoculated in plastic and glass bottles and plastic bags. The containers were exposed to sunlight during six hours. When temperature raised up to 30° C, 35° C, 40° C, and 45° C, samples of water were taken. The impact of: a) temperature, b) turbidity, c) container and d) initial concentration of vibrio were determined. The bacteria were inactivated with only temperature, when it raised up to 45° C, whereas, with constant temperature of 30° C it was necessary 100 Wh/m² of the radiation and at 50° C, only 10 Wh/m². The turbidity caused interference with SODIS process: total inactivation was achieved with different levels: 105 Wh/m² (40 TNU), 91 Wh/m² (23 TNU) and 36 Wh/m² (5 TNU). The container type and initial different concentration of vibrio did not have influence on the SODIS process. Full correlation among thermotolerant coliforms and *V. cholerae* inactivation was always high (0.86 and 0.99).

Resumen

Se inoculó *Vibrio cholerae* En botellas de vidrio, en botellas de plástico y en bolsas plásticas que contenían agua; se expusieron al sol durante 6 horas y se midió la temperatura y la radiación UV-A. Al alcanzar 30°, 35°, 40° y 45° C se tomaron muestras para determinar el nivel de los vibrios y de los coliformes termotolerantes. Se determinaron los impactos de temperatura, turbiedad, recipiente y concentración de los vibrios. Esta bacteria se logró inactivar al alcanzar gradualmente 45° C y 6 horas de exposición al sol; con temperatura constante de 30° C se necesitaron 100 Wh/m²; y con 50° C sólo 10 Wh/m². La turbiedad es un factor que interfiere con el proceso de SODIS; con 40 UNT se necesitaron 105 Wh/m², con 23 UNT, 91 Wh/m² y con 5 UNT, 36 Wh/m² de UV-A para lograr 100% de mortalidad. No se encontraron diferencias con el tipo de recipiente usado, siempre se logró 100% de inactivación; tampoco se observaron diferencias en la inactivación con diferentes concentraciones del vibrio. Se lograron altas correlaciones entre la inactivación de coliformes termotolerantes y vibrios (0.86 y 0.99).

Introduction

Cholera has taken importance in America since it was established in 1991, at the end of the same year it was extended by 16 countries¹. In Colombia it has caused several epidemics with human and economic losses: the most affected human group is between 15-44 years old².

The cholera, a disease that causes a very severe diarrhea and is considered as one of the most severe intestinal infections^{1,3}, is caused by the *Vibrio cholerae* that have two biotypes: the classic and the E1 TOR, in Colombia it was found that 99.1% of the isolated correspond to the Inaba subtype and the remainder to the Ogawa subtype². The E1 TOR biotype is very much resistant to environmental conditions and survives much longer in the water. Bacteria's can survive in water between 2-20 days at temperatures between 20°-30° C degrees.

Although this depend on the presence of other microorganism and the chemical composition of water. Almost all bacteria's are sensitive to the combined application of radiation and heat due the synergistic effects offered by both measures⁴. Therefore, a combined application of these two processes in the water treatment allows a new alternative to disinfect water, over all in remotes areas with difficult access^{4,5}.

The solar disinfection (SODIS) is based on the use of two or three liters plastic bags that are filled with water and exposed to direct sun light for six hours. With this time of exposure, the thermo-tolerant coliforms bacteria reduction were achieved between 92% and 99%^{6,7}. Therefore, if the level of pathogenic bacteria is considered to be a much lower, a reduction could be achieved to reach insignificant levels for the human health.

In the Instituto de Investigación y Desarrollo de Agua Potable, Saneamiento Básico y Conservación del Recurso Hídrico (CINARA), laboratory and field test have been done, with thermo-tolerant coliforms as indicator of SODIS efficiency. We determined, the mortality rate, levels of solar radiation to inactivate these germs and, the critical temperature to cause a greater mortality of the bacteria's⁷⁻⁹.

With these results, it was decided to investigate some factors that's interfere with the inactivation of *V. cholerae* and determine the potencial use of SODIS in the cholera's control. Since this type of technology is universally available, with low cost and support, and can be used in any site. However, it has some limitations due to atmospheric conditions, the turbidity and the color water and the thickness and the shape of the containers walls¹⁰.

We present the results and potencial uses that SODIS could have in the inactivation of *V. cholera*, under laboratory conditions.

Materials and Methods

The data in this document are part of the research program (SODIS) that is carried out simultaneously in several countries of the world and is coordinated by the Swiss Federal Institute for Environmental Science and Technology (EAWAG/SANDEC), where Cinara participates in the laboratory and in the field evaluations. The experimental part was carried out in the CINARA'S research station during the period from August-1995 to April-1996.

Physical measurements

The waters turbidity was measured with the nephelometric method by means of turbidimeter 2100a, values was given in nephelometric turbidity units (UNT). Suspended solid were measured with the gravimetric method (mg/l) and fiberglass filter with a nominal pore size of 1.2 µm. The intensity of UV-A radiation (watt.hour/m²) was measured every 10 min with a radiometric sensor type macam sd104a-cos, with spectral response of 320-400 nm (ultraviolet light UV-A), this sensor was connected to a data logger and the temperature was measured with a thermocouple type j.

Microbiological test

A strain of the biotype E1 TOR of *V. cholerae* isolated in the microbiology department of Universidad del Valle was used. This strain was kept in nutrient broth at 35.5° C for 24 hours and from this medium 1 ml was inoculated in each bottle, before solar radiation exposure. Before an after sun exposure, 5 test tubes were inoculated. For each sample concentration: 0.1, 1.0 and 10 ml containing alkaline peptone broth, to determinate the most probable number (mpn). These test tubes were incubated during 8 hours at 35.5° C and from test tubes with bacterial growth, a sample was taken with a handle that was inoculated in petri dishes with TCBS agar. These dishes were incubated for 24 hour at 35.5° C. Some typical colonies for *V. cholerae* of each petri dish were

identified with five standard biochemical tests: TSI, LIA, UREA, Motility and Indol^{11,12}. With the results of these biochemical tests was determined the most probable number (MPN) the test where carried out with waters of different turbidity, and in a different containers and environmental conditions. The exposures to solar radiation were made between 9:00 to 17:00 hours.

Temperature impact

A 1.5-liter glass bottle and a 2-liter plastic bottle were painted black to prevent the passage of sunlight and allow for a greater temperature accumulation. To each bottle completely filled with water, 1 ml of *V. cholerae* was added. Which gave a result of 11,000 ml/100 ml. Every 15 min, the water temperature in the containers was measured. When 35.5° C, 40° C, 45° C (the maximum final temperature (47° C) was reached at the end of the test, samples were taken to be sown in tubes with alkaline peptone. The impact of two constant temperatures (with exposure to the sun) on the mortality of the vibrios was also determined. For this 30 ml quartz tubes and a thermostat bath calibrated at 30° C or 50° C were used.

Influence of turbidity

Three tests on different days were carried out using four plastic bottles and four glass bottles. We used different water quality (turbidity level and suspended solids), which resulted in a variable experimental condition for tests with raw water (40 UNT) and pre-filtered water (23 UNT); while for filtered water (5 UNT) only one bottle of each type was used to take the respective sample. All the bottles were painted black in a longitudinal plane. The samples of the first two tests were taken at the beginning (22° C), when the water reached 45° C, at the end of the test and the next day. Once the desired temperature was reached, one bottle of each type was removed. For the last test, the samples were taken at the beginning, upon reaching the water temperature at 30° C, at 40° C, at 45° C, 50° C and at the end of the experiment.

Influence of the type of container

The exposure to solar radiation was made with glass bottles (1.5 liters), plastic bottles (2 liters) and plastic bags in the procedure described above.

Influence of the concentration of *V. cholerae*

Four plastic bottles (2 liters) were used, were added to the bottles water and different starting concentration of *V. cholerae*: 2.5×10^6 , 2.5×10^5 , 2.5×10^4 y 2.5×10^3 MPN/100 ml (the final concentration in the volumen was between 5×10^4 y 5×10^7 MPN/100 ml). The temperatures were measured every 15 min and the samples where taken at the beginning, at 22° C, at 30° C, at 35° C, at 40° C, at 45° C, and finally at the end of the test.

Results

Temperature impact

When was inoculated a 1 ml of culture, the initial concentration that was reached at beginning of evaluation was 16,000 MPN/100 ml of *V. cholerae* (the total concentration in the used volume, by means, is the amount of water that's a person consumes per day was 320,000 MPN for glass bottles and 240,000 MPN for plastic bottles).

Both test showed a slightly different increase in temperature between two types of containers due the different volumes used in the bottles, but the inactivation of *V. cholerae* was similar (Fig. 1). The temperature did a slowly begin and steadily increase until reaching more than 50° C first in the glass bottles (225 minutes) and then the plastics ones (240 minutes). The bacteria reduction was drastic when 49° C (3 Log) was reached in water, after 45° C the bacteria level was <2 MPN/100 ml in both bottles (minimum value that can be given when all the test tubes are negative and according to the numbers of test tubes used in the test).

On Figure 2, we demonstrates again the importance of the temperature in the *V. cholerae* reduction. When we have a constant temperature of 30° C, about 100 Wh/m² are needed to achieve a total inactivation of the vibrio's. in a second test, with 140 Wh/m² of accumulated UV-A, at the end of the experiment were demonstrated a 70 MPN/100 ml of vibrio's (99.5% reduction), when using 50° C, less than 10 Wh/m² was needed to obtain a 100% of inactivation.

Influence of Turbidity

Different turbidity, solar radiation, maximum reached temperature and different initial inoculum

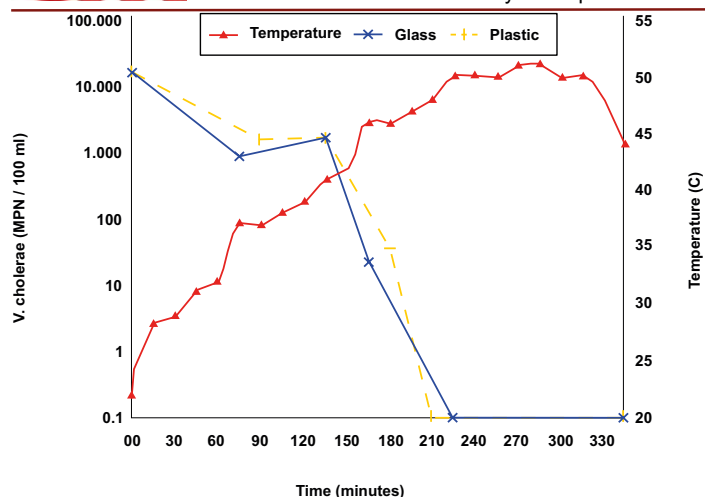


Figure 1. Temperature effect on mortality of *Vibrio cholerae*

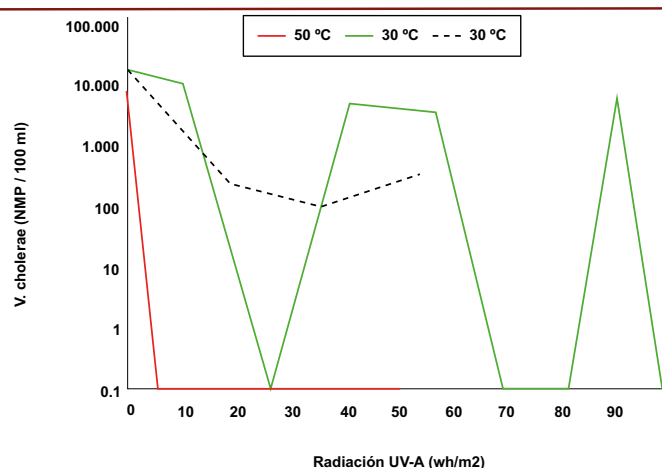


Figure 2. *Vibrio cholerae* mortality when were used two constant temperatures

of *V. cholerae* were obtained due to each test was carried out on different days. The water quality (turbidity and suspended solids) the total UV radiation and the initial concentration of *V. cholerae* are presented in Table 1

The temperature in raw water (40 NTU) and prefiltered (23 NTU) did not reach 50° C, which allowed at the end of the experiment to present a residual concentration of *V. cholerae* (4 MPN/100 ml in plastic and glass bottles). With filtered water (5 NTU) reached the 50° C no vibrio were found in any of the inoculated bottles, after 35° C the tests with raw and prefiltered water were positive for *V. cholerae* at 45° C. There were noticeable differences in radiation and needed time to inactivate the vibrio's in these two water types. In raw water in both plastic and glass bottles: 105 Wh/m² of UV-A radiation were needed to decrease 2.6-2.9 log (from 1,500 MPN to 4 MPN) of vibrio's in 270 min, and with pre-filtered water were needed 46° C and 52 Wh/m² of UV-A radiation for a reduction of 3.2-3.7 log (from 11,000 MPN to 4 MPN) of vibrio's in 130 min.

The water with low turbidity (<5 NTU) requires less solar radiation, temperature and time for the inactivation of these bacteria (Table 2). The inactivation of *V. cholerae* with low turbidity water began at 35° C and 39 Wh/m² of UV-A radiation and the inactivation was total when it reached 40° C and 36 Wh/m² of UV-A radiation (glass bottles) and 44 Wh/m² of UV-A radiation (plastic bottles)

Influence of the type of container

When was comparing the mortality of the *V. cholerae* in the containers can be concluded that the plastic bags reached higher temperature and give to the SODIS process a greater security. At the end of the experiment the results were the same, 100% reduction of the vibrios. Figure 3 shows the differences between the UV-A doses and the temperature necessary to inactivate the vibrio's in the three evaluated containers; these differences are due the level of turbidity in the water samples. The glass and plastic bottles contained water of 9 NTU, while the bags 124 NTU (highest concentration of vibrios) and 14 NTU (lowest concentration of vibrios) (Fig. 3).

Influence of the concentration of *Vibrio cholerae*

When a temperature of 30° C (18 Wh/m² of UV-A radiation) was reached there was a slight reduction in the containers, with lower concentration (2.5 x 10³ NMP/100 ml) reaching 1,600 NMP/100 ml while in the higher concentrations there were no appreciable reductions (2.5x 10⁶, 2.5 x 10⁵, 2.5 x 10⁴ NMP/100 ml).

At 35° C and 28 Wh/m² of UV-A radiation (95 min) there was only a reduction in the two lowest concentrations since they reached 16,000 and 350 NMP/100 ml. When the temperature reached 40° C and UV-A radiation of 36 Wh/m² (125 min) the vibrio's concentration was similar for the

Table 1. Experimental conditions of the tests performed with bottles and three condition of quality water.

	Water quality		
	Raw	Prefiltered	Filtered
Turbidity (NTU)	40	23	5
Suspended solid (mg/l)	75	24	<0.1
UV-A radiation (Wh/m ²)	105	91	96
Vibrio level (MPN)	1,500	11,000	11,000

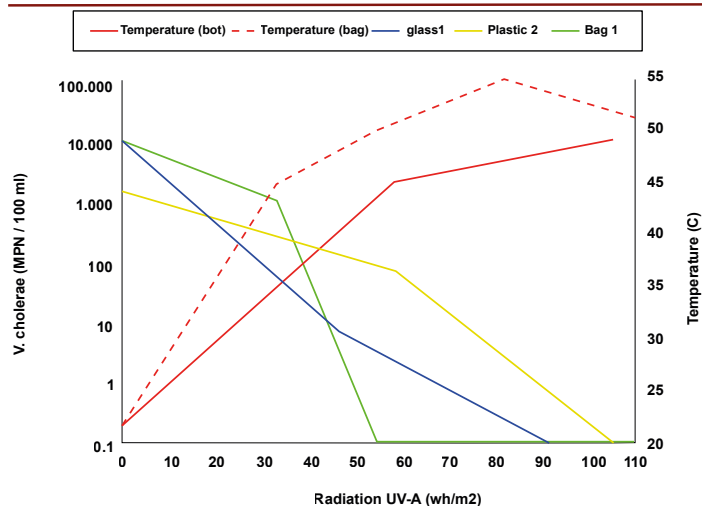


Figure 3. Influence of the container type on the mortality of the *Vibrio cholerae*

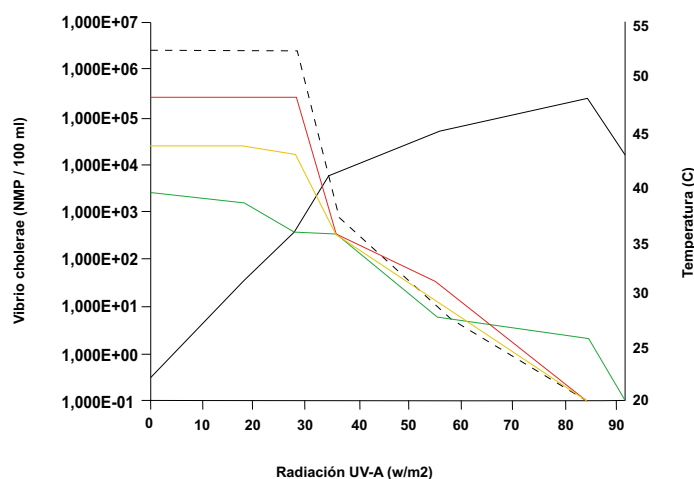


Figure 4. Effect of temperature and UV-A radiation on the mortality of *Vibrio cholerae*, with different initial concentrations

three concentrations, reaching 350 NMP/100 ml with 48° C and 94 Wh/m² of UV-A radiation (305 min) positives tubes for *Vibrio cholerae* were not found (<2 NMP/100 ml) in the different concentrations, with the exception of the lowest in which 2 NMP/100 ml were determined. At the end of the test all the bottles were negative for the vibrio's (345 min) (Fig. 4).

Correlation of Inactivation between thermotolerant coliforms and *Vibrio cholerae*

Nine correlations were established between the inactivation of thermotolerant coliforms and *Vibrio cholerae*. With correlations between 0.82 and 0.99 (Table 3).

Discussion

Different tests showed that *V. cholerae* is sensitive to environmental conditions; this is deduced from the results. The impact of only increasing temperature caused a drastic reductions of the vibrio's (Figs 1 and 2).

This same phenomenon was already observed by Ciocchetti and Metcalf¹³. On the contrary, with the heat-tholerant coliforms, the radiation is much more important than the temperature; these bacteria resist increases up almost to 50° C without causing appreciable mortality¹⁴.

When temperature and solar radiation are combined, the reduction begins at 35° C (less turbidity) at 100 min. When the containers are exposed only to temperature, inactivation is initiated at 40° C and 135 min. In a similar test performed in Nigeria, it took about five hours and approximately 600 w/m² of UV-A radiation for a completed inactivation of *V. cholerae*^{13,15}. MacKenzy et al¹⁶ only achieved 99.9% (3 log) of reduction in vibrio's after six hours of sun exposure, but this exposure started in the middle of the day and the level of temperature reached and the UV-A radiation were not determined.

The increase in temperature causes significant changes in some physical and chemical water parameters. It increases the rate of chemical and biochemical reactions. The solubility of gases decreases and the metabolic rate of microorganisms is increased¹⁷. These changes could be the cause of the destruction of the cellular integrity; while UV-A radiations affects respiration, Transport through the membrane, nucleic acid duplication and protein synthesis¹⁸ and together with dissolved O₂ produces a toxic compound¹⁹. For this reason, the temperature and radiation are synergist

The influence of turbidity in the inactivation of *V. cholerae* was similar to that found in the inactivation of thermotolerant coliforms⁸. Since with higher levels of turbidity in water, higher doses of UV-A and temperature were needed (longer exposure time) to completely inactivate the vibrio's. Alward¹⁴ and Odeyemi¹⁵ demonstrated that bacteria are rapidly inactivated by solar radiation in waters with low turbidity, than in water with high turbidity. The comparison of the obtained results with different turbidity indicates that with raw water (high turbidity) almost twice of the radiation is required than prefiltered water (turbidity media) was needed to cause a small reaction in the level of vibrio. When the test was made with raw water, 50° C temperature was reached and there was a low initial concentration of vibrio's.

Table 2. Comparison of *V. cholerae* survival, time and UV-A radiation needed for Inactivation, with three water quality.

Glass type (°C)	Temperature Raw (45 NTU)			Prefiltered (23 NTU)			Filtered (<5 NTU)			
	UV-A	Time	V. ch	UV-A	Time	V. ch	UV-A	Time	V. ch	
Plastic	22	0	0	1,500	0	0	11,000	0	0	11,000
	30							13	55	11,000
	35							27	100	28
	40							44	150	<2
	45	90	220	75	52	130	2	68	210	<2
	50	105	270	<2				92	210	<2
	end				91	210	4	96	310	<2
OD		<2			4					
Glass	22	0	0	1,500	0	0	11,000	0	0	11,000
	30							13	55	11,000
	35							27	90	150
	40							44	130	<2
	45	58	140	120	46	110	7	68	175	<2
	50							92	270	<2
	END	105	270	4	91	310	<2	96	310	<2
OD			<2			<2				

OD: Recovery calculation 24 hours after exposure. V ch: *Vibrio cholerae*.

Note: As were used 5 tubes per dilution, when all tubes are negative, the value estimated minimum is <2 NMP/100 ml

It was also observed that with prefiltered water it was possible to reach 45° C after (110 min vs 140 min). This suggest that high turbidity (and suspended solids) reduces the efficiency of SODIS; therefore, it is recommended to reduce turbidity before exposing water to solar radiation^{20,21}. Generally, at lower bacteria concentration, the inactivation rate is higher. Odeyemi²² funds high re reductions of bacteria with low concentration. The same author proposes that is due to the easy penetration and inactivation caused by the sun's rays to the bacteria when they are dispersed in the water. The opposite is found in waters with high concentration, since the cells can be found agglomerated, protecting many bacteria's to UV-A radiation

However, in the present investigation with *V. cholerae* the inactivation seems to be not influenced by the concentration of bacteria (Fig. 1); if the UV-A does not reach the bacteria, the temperature would be responsible for activating them. Another important factor for SODIS is the proper type of container^{7,9,10}. The glass of the container serve as UV-A radiation filter. If the contanier causes high reduction it is necessary to increase the time of exposure. As is the case with the plastic bottles used in these experiments, reduces about 30% of the UV-A radiation. On the contrary, the plastic bags reduce about 10% besides, Sommer⁹ has showed that the plastic bags heat up much more than the others one by the ratio area/column of water that turns to be much larger than with the bottles.

When comparisons were made between the inactivation of the thermotolerant coliforms and vibrios, high correlations were found between the inactivation of thermotolerant coliforms and vibrios (between 0.86 and 0.99). The lowest correlation was presented with plastic bottles and the highest correlation with plastic bags. The difference between these containers could be the temperature level that was always higher in the water of the plastics bags. This makes it possible to show the impact of SODIS in glasses is much higher than for thermotolerant coliforms⁹. With these results, the utility of thermotolerant coliforms as a vibrio's mortality indicator is deduced. These results are important due the method used for the isolation and identification of *V. cholera* with respect to those used for thermotolerant coliforms.

Preliminary test shows that there is a potential for SODIS in the contro of this type of infection. These considerations are also based on the results obtained by other researchers in Canada¹⁴, Switzerland⁵ and Lebanon⁴ with the inactivation of thermotolerant coliforms.

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Table 3. Correlations between the inactivation of thermotolerant coliforms and the inactivation of *Vibrio cholerae*

Comparison test	Container type	Test number	Condition	Reduction (%)	UV-A	Time	CC
CT/CT	quartz	2	30° C K	85.7/89.1	74/74	225/225	0.95
				99.9/99.0	99/99	450/450	0.99
CT/Vch	quartz	2	30° C K	97.5/80.0	91/91		
				100/99.4	122/122	263/263	0.86
CT/Vch	quartz	1	50° C K	100/100	12/5	45/23	0.98
CT/Vch	Plastic bottle	2	Variable	79.4/99.98	91/52	210/130	0.82
				97.2/99.86	105/105	270/270	0.99
CT/Vch	Glass bottle	2	Variable	80.0/99.9	91/46	310/110	0.92
				99.6/99.7	105/105	270/270	0.90
CT/Vch	Plastic bag	2	Variable	99.99/99.9	54/54	140/140	0.99
				99.99/99.3	78/78	250/250	0.99

TC: termo-tolerant coliforms

K: constant temperature and solar radiation variable

UV-A: ultraviolet light radiation.

CC: coefficient correlation.

Vch: *Vibrio cholerae*

Variable: temperatura and solar radiation variables

Time: exposure time to achieve maximum inactivation

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