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Testing the efficacy of UV light in disinfecting bacteria

1. Objective

The objective of this laboratory work is to:
Test the UV lamps against cultured E.Coli bacteria in water to check if they are effective in disinfecting the water.

2. Theory

2.1 Bacteria in Water

Bacteria are one of the major pathogens in water that can transmit diseases. Among them coliform bacterias are a grouping of bacteria that includes many strains.These bacterias can live in soil, water, groundwater, as well as in the gastrointestinal tracts of animals. There are many which are harmless but also few which can cause diseases.Studies have shown that one can become immune to bacteria in their own water, but someone else not used to the water may suffer from diarrhea or other form of stomach bugs.[1] Coliform bacteria are a group of bacteria consisting of various strains of bacteria. *Escherichia coli* are one of the types of coliform which are commonly found in the intestines of animals and humans, The presence of these bacteria in water therefore indicate presence of sewage and animal waste in water,this means possible presence of other pathogens that can feed off the waste in water and cause other diseases like cholera, dysentery and so on.[2]

Microbiological parameters

Parameter	Parametric value (number/100 ml)
Escherichia coli (E. coli)	0
Enterococci	0

The following applies to water offered for sale in bottles or containers:

Parameter	Parametric value
Escherichia coli (E. coli)	0/250 ml
Enterococci	0/250 ml
Pseudomonas aeruginosa	0/250 ml
Colony count 22 °C	100/ml
Colony count 37 °C	20/ml

[1] "Microorganisms, Bacteria, and Viruses in ... - Extoxnet." 2004. 5 Apr. 2016 <http://extoxnet.orst.edu/faqs/safedrink/microorg.htm>
[2] "E coli bacteria - Drinking Water Contaminants, Facts ..." 2003. 5 Apr. 2016 <http://www.freedrinkingwater.com/water-contamination/ecoli-bacteria-removal-water.htm>

Figure.1 : Microbiological parameters determined by EU.[3]("EUR-Lex - 31998L0083 - EN - EUR-Lex - Europa.eu." 2015. 5 Apr. 2016)
As per European Union 'COUNCIL DIRECTIVE 98/83/EC of 3 November 1998 on the quality of water intended for human consumption', the given sample of water should not contain any colony per 100 ml of sample taken.

2.2 Ultraviolet light disinfection

The wavelength of Ultraviolet light (UV light) lie just below the visible light range. There are 3 subtypes of UV light. UV-A and UV-B are the radiations that are cause sunburns and their wavelength range from 100-280 nm while wavelength of UV-C range from 200-280 nm. These radiations are blocked by the Ozone layer in our atmosphere. UV lamps that are generally used to disinfect water also emit UV-C light, have wavelength peaking at 265 nm. 85% of total electricity is converted to Uv light while 15 % is consumed as heat. [4]
Ultraviolet disinfection has close relationship with dosage time therefore it is crucial that dosage time be considered along with the intensity of the light.

$$Duv = It \qquad (1)$$

Duv is the dosage of ultraviolet radiation [μW*s/cm^2], I is the fluence [μW*s/cm^2], t is the exposure time[s]

The survival rate of microorganisms is dependent on the fact that some microorganisms require more or less irradiance than other species. It is also dependent on the dimensions of the UVIR. The survival fraction of a microbial population exposed to UV germicidal irradiation is an exponential function of dose:[5]

$$S = e - kDuv \qquad (2)$$

Where S is the fraction of a microbial population that survives UVGI, k is a species dependent deactivation rate constant [cm^2/μJ]

[3] "EUR-Lex - 31998L0083 - EN - EUR-Lex - Europa.eu." 2015. 5 Apr. 2016 <http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A31998L0083>
[4] "FAQs - Zontec Ozone, Inc." 5 Apr. 2016 <http://www.zontecozone.com/faqs/>
[5] Ryan, RM. "Effect of enhanced ultraviolet germicidal ... - Steril-Aire." 2011. <http://steril-aire.com/images/wchob.pdf>

3. Apparatus and Equipments

- Bucket
- UV light
- 12 V Pump
- Inlet and outlet hose
- Incubator
- Laminar flow booth
- Autoclave
- Sterilized water
- Graduated measuring cylinder
- RAPID 'E.coli 2 Agar
- Peptone
- Beef extract
- Lab cultured E. coli bacteria
- Petri dish
- Test tubes (10 ml)
- Pipettes (100-1000 µl)
- Sodium chloride
- Erlenmeyer flask

4. Methodology

The experimental set is divided into four main steps:

1) Sample preparation
2) Running the water through UV radiation tube
3) Bacteria Count for before and after samples

4.1 Sample preparation

The first part of the sample preparation involved preparing the bacteria in broth instead of mixing the bacteria from the test tube directly to the water sample.

- Two 500 ml erlenmeyer flasks are taken to prepare broth (See appendix 1.) and inoculated with the E.coli bacteria sample and left in the mixer for 24 hours.
- After 24 hours in the shaker the 100 ml broth is then mixed into the 15 l tap-water is filled in the 'Bucket 1' and stirred vigorously for 5 minutes.
- 15 ml sample from the 'Bucket 1' is taken and marked as '1B'
- Another identical bucket, 'Bucket 2' is taken with 15 l of tap water and 100 ml of the broth is then mixed to the water and mixed vigorously for 5 minutes.
- 15 ml sample from 'Bucket 2' is taken and marked as '2B'

4.2 UV treatment

The UV treatment system was turned on for 3 minutes with the pump running on full speed with tap water before the actual test was performed.

- The outflow rate when the valve was fully opened was measured to be 14 L /min and when partially opened was 11 L /min
- The pump is then immersed into the 'Bucket 1' and then run at full speed and valve fully opened, the outflow is collected in 'Bucket 1 A'
- 15 ml sample from Bucket 1 A is marked as 1A
- Another outflow rate was measured to be 11 L/min when the valve was partially opened
- The pump is then immersed into the 'Bucket 2' and then run at full speed , the outflow is collected in Bucket 2A
- 15 ml of sample from Bucket 2A is marked as 2A

4.3 Bacteria Count

The bacteria is counted using the pour plate technique. 8 dilutions for each samples 1B, 2B, 1A, 2A and replicates for last 4 dilutions for all of the four samples.A standard zero sample dilution was also done.The agar plates were prepared and diluted samples were poured and incubated for 48 hours.(See appendix 2)

5. Results

The bacteria count from the plates are shown in the table below

Table 1: Bacteria count in the plates

	(C.F.U)							
Samples	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1B1	N.A	N.A	112	10	-	-	-	-
1B2					-	-	-	-
2B1	N.A	N.A	140	28	-	-	-	-
2B2					-	-	-	-
1A1	-	-	-	-	-	-	-	-
1A2	-	--	-	-	-	-	-	-
2A1	-	-	-	-	-	-	-	-
2A2	-	-	-	-	-	-	-	-

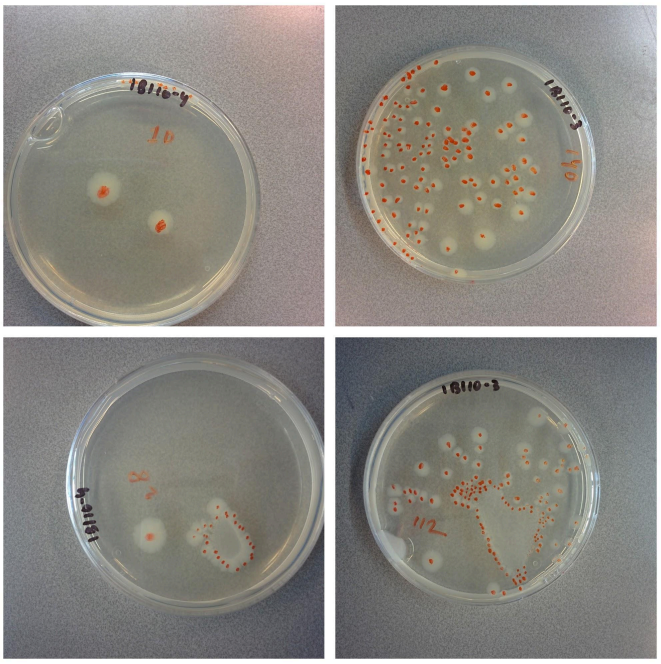

Figure 2: Plates showing CFU formed in the Before samples

Calculations

Initially 0.5 ml of sample was diluted to 4.5ml sterilized saline water.
Dilution factor = 5/0.5 = 10

For sample 1B1

For 10^{-3} dilution, Final dilution factor = $10 \times 100 \times 100 \times 100 = 10^7$

CFU/ml =$(112 \times 10^7) \times 0.1 = 112 \times 10^8 \, CFU/ml$

For 10^{-4} dilution, final dilution factor =$10 \times 100 \times 100 \times 100 \times 100 = 10$

CFU/ml =$(10 \times 10^9) \times 0.1 = 10 \times 10^{10} \, CFU/ml$

For sample 2B1

For 10^{-3} dilution, final dilution factor = $10 \times 100 \times 100 \times 100 = 10^7$

CFU/ml =$(140 \times 10^7) \times 0.1 = 1 \times 10^8 \, CFU/ml$

For 10^{-4} dilution, final dilution factor =$10 \times 100 \times 100 \times 100 \times 100 = 10$

CFU/ml =$(28 \times 10^{9}) \times 0.1$ = $28 \times 10^{10}\ CFU/ml$

The number and colonies for 10^{-1} and 10^{-2} samples for both 1B1 and 2B1 were either too many or too erratic to count while the replicates were done for 10^{-5} – 10^{-8} samples and no CFU were observed.

There were no CFU observed in any of the After samples for all dilutions.

6. Conclusion

The results of the experiment quite clearly shows that the UV light is effective in disinfecting the water from the bacteria. Calculations in the before sample show that there was substantial number of CFU of bacteria in the water but after the corresponding batch of the water was passed through the UV system at 14 L/min and 11 L/min, the water was disinfected. Although the test was done at only two flow rates the disinfection system should perform even better when the flow rate is decreased.

Appendix 1 : Broth Preparation

Broth preparation

1. Two 500 ml Erlenmeyer flasks are taken
2. 0.5 g peptone , 0.3 g Beef extract, and 0.8 g sodium chloride in each flasks along with 100ml sterilized water
3. The solution is shaken well and allowed to soak for 5 minutes
4. A test tube with E. Coli is taken and innoculated to the solution made
5. The innoculated solution is then taken to incubation shaker and left for 24 hrs at 37 C temperature

Appendix 2 : Plates Preparation

Preparing plates

1. Two 1000 ml erlenmeyer flasks are taken
2. 14 g RAPID 'E.coli 2 Agar is added to each of the flasks
3. To each flask 500 ml sterilized water is added
4. In order to make the dilutions, 32 sterilized test tubes are taken
5. The saline solution is prepared by adding 1.8 g sodium chloride to 200 ml sterilzed water
6. The flasks, both agar solution and saline are left in the autoclave for 20 minutes to reach 120 C temperature and allowed to cool for 40 minutes
7. When the agar reaches about 40 C temperature it is poured onto the petri dishes in laminar flow booth
8. Altogether 56 petri dishes are used to pour the agar and left it to cool and solidify
9. Then subsequent serial dilution are made for 1B, 2B, 1A, 2A and 0 samples.
10. 4.5 ml of sterilized saline solution is taken for each sample
    and then 0.1 ml of the water sample 1B1 added to that test tube and mixed
11. 0.1 ml from the previous test tube is pipetted and mixed with the test tube with 4.5 ml solution
12. This step is repeated for next 7 times to create 8 dilutions
13. Step 10-12 is repeated for 2B,1A,2A and zero sample
14. The dilutions are ready to be poured onto the petri dishes now
15. 0.1 ml from each test tube marked with the dilutions is pippetted onto the corresponding petri dish and spread well in laminar flow booth
16. After all the dilution are pipetted and flipped and placed in the incubator at 37 C temperature