

### 1. Objective

The targeted goal of this experiment is to compare the recovery of microorganisms (see Table I) after contact times of 10, 20 and 30 seconds with tap water and with ozonized tap water in order to observe the microbicidal activity of ozone.

### 2. Protocol

The ozone water generator was set by the client to a medium level and a maximal water flow rate was used throughout the experiment. An inoculum of between  $1 \times 10^7$  and  $1 \times 10^8$  microorganisms was added in sterile 250 mL bottles. After the water had been running for 15 seconds, 200 mL of ozonized water was added to the bottles containing the microorganisms. After 10, 20 and 30 seconds of contact with the ozonized water, three portions of 1 mL were plated with Tryptic Soy Agar (TSA). The plates were incubated at 30-35°C for 3 days. (in anaerobic conditions for *Clostridium difficile*)

The preceding steps were repeated with plain tap water and inoculum controls were made to ensure that the targeted inoculum was reached. Since no bactericidal activity was expected with tap water, a dilution was added to the protocol in order to obtain a plate count of around 30-300 colonies per plate. Therefore, *Clostridium difficile*, *Escherichia coli* and *Salmonella thyphimurium* were diluted to  $10^{-3}$  and *Staphylococcus aureus* diluted to  $10^{-4}$ .

### 3. Raw data

**Table I: Bacterial inoculum controls**

Strain (ATCC#)*	Inoculum per mL of water
<i>Clostridium difficile</i> (43593)	350 000 CFU**
<i>Escherichia coli</i> (8739)	212 500 CFU
<i>Salmonella thyphimurium</i> (14028)	110 000 CFU
<i>Staphylococcus aureus</i> (6538)	325 000 CFU

\*American Type Culture Collection

\*\* Colony forming units

**Table II: Results for *Clostridium difficile***

Contact time	Tap water (mean)*	Ozonized tap water (mean)
10 seconds	157-203-104 (155 000)	240-440-329 (336)
20 seconds	105-249-97 (150 000)	252-135-227 (205)
30 seconds	97-257-247 (200 000)	81-40-32 (51)

\* Tap water samples were diluted. Numbers shown are observed results for the pour plate count and therefore have to be multiplied by  $1 \times 10^3$ . The mean has been multiplied by this factor.

**Table III:** Results for *Escherichia coli*

Contact time	Tap water* (mean)	Ozonized tap water (mean)
10 seconds	270-298-204 (257 000)	0-0-0 (<1)
20 seconds	218-277-267 (254 000)	0-0-0 (<1)
30 seconds	229-231-247 (236 000)	0-0-0 (<1)

\* Tap water samples were diluted. Numbers shown are observed results for the pour plate count and therefore have to be multiplied by  $1 \times 10^3$ . The mean has been multiplied by this factor.

**Table IV:** Results for *Salmonella thyphimurium*

Contact time	Tap water* (mean)	Ozonized tap water (mean)
10 seconds	102-107-127 (112 000)	2-2-1 (2)
20 seconds	98-68-130 (99 000)	0-0-0 (<1)
30 seconds	134-142-87 (121 000)	0-0-0 (<1)

\* Tap water samples were diluted. Numbers shown are observed results for the pour plate count and therefore have to be multiplied by  $1 \times 10^3$ . The mean has been multiplied by this factor.

**Table V:** Results for *Staphylococcus aureus*

Contact time	Tap water* (mean)	Ozonized tap water (mean)
10 seconds	40-31-22 (310 000)	5-5-6 (5)
20 seconds	31-29-35 (320 000)	1-0-0 (<1)
30 seconds	20-35-39 (310 000)	0-0-0 (<1)

\* Tap water samples were diluted. Numbers shown are observed results for the pour plate count and therefore have to be multiplied by  $1 \times 10^4$ . The mean has been multiplied by this factor.

#### 4. Results

**Table VI:** Reduction percentage and logarithms for strains in contact with ozonized water and tap water.

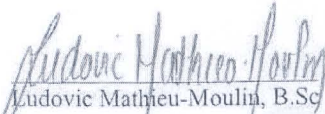
	Reduction percentage (ozone)	Reduction logarithms (ozone)	Reduction percentage (tap)	Reduction logarithms (tap)
<i>Clostridium difficile</i>				
10 seconds	99.9040	3.0177	55.7142	0.3537
20 seconds	99.9414	3.2323	57.1429	0.3680
30 seconds	99.9854	3.8365	42.8571	0.2430
<i>Escherichia coli</i>				
10 seconds	99.9995	5.3274	NA	NA
20 seconds	99.9995	5.3274	NA	NA
30 seconds	99.9995	5.3274	NA	NA
<i>Salmonella thyphimurium</i>				
10 seconds	99.9982	4.7403	NA	NA
20 seconds	99.9991	5.0414	10.0000	0.0458
30 seconds	99.9991	5.0414	NA	NA
<i>Staphylococcus aureus</i>				
10 seconds	99.9985	4.8129	4.6154	0.0205
20 seconds	99.9997	5.5188	1.5385	0.0067
30 seconds	99.9997	5.5188	4.6154	0.0205




## 5. Conclusion

Results shown in table VI clearly demonstrates the antibacterial efficiency of ozone when added to tap water. The lowest reduction percentage for *Clostridium difficile* might come from the fact that this microorganism is sporulated and therefore more difficult to eliminate. For all other strains, the number of microorganisms recovered in tap water is close to the inoculum control. This is not true for *Clostridium difficile* since this organism grows in anaerobic condition and the time span in which the experiment was done might have exposed this strain to oxygenated air. This contact with oxygen might have reduced the strain's viability for both sets of results; tap water and ozonized tap water.

- The ozonized water has shown a microbial contamination reduction percentage of more than 99.99% after 10 seconds for *Salmonella thyphimurium* and *Staphylococcus aureus*. After 20 seconds, the reduction reached 5 logs.
- For *Escherichia coli*, the 5 logs reduction was observed after 10 seconds
- *Clostridium difficile*'s reduction reached 3.0177 logs after 10 seconds

  
Ludovic Mathieu-Moulin, B.Sc  
Microbiologist

20070713  
date

  
Sylvie Martin, M.Sc  
Microbiology Laboratories Manager

20070713  
date