SOLAR WATER PURIFICATION: STORAGE TEST FOR THE DETERMINATION OF BACTERIA REGROWTH AND TASTE TEST IN TIO_2 COATED PET BOTTLES

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ABSTRACT

The exposure to sunlight of polyethylene (PET) bottles coated with photocatalyst titanium dioxide has been shown to be an effective technique for the disinfection of contaminated water as well as the degradation of pesticides and herbicides. However, e. coli bacteria have self-repair mechanisms which allow them to potentially regrow during storage after disinfection. It is hypothesized that because photocatalytic oxidation physically breaks apart the structures of the bacteria, that bacteria cannot reactivate after treatment. A storage experiment showed the effectiveness of the method to resist any regrowth of bacteria for 190 days of storage. An experiment with 15 tasters to differentiate spring water from spring water disinfected using the method showed no distaste resulting from the procedure.

1. INTRODUCTION

Water born illness is a tremendous health issue in the developing world. Over 1 billion people lack access to clean water. Children are particularly susceptible to mortality from water born illness. About 400 children below age 5 die per hour in the developing world from water born diarrheal diseases (World Health Organization, 1996). The Solar Disinfection (SODIS) method was developed to provide a simple and resource minimal way to disinfect water. Individuals expose clear plastic PET bottles filled with contaminated water to full sunlight for 8 hours. The UV radiation in the solar spectrum disinfects water by inactivating bacteria DNA. However, bacteria have a self-defense mechanism, the ability to perform enzymatic DNA repair, which can result in bacterial reactivation. Therefore, while bacteria count directly after disinfection may reveal very low bacteria concentration, the bacteria with time may return in large numbers.

The photocatalytic property of TiO_2 is well documented. Ultraviolet light present in the solar spectrum strikes the TiO_2 and bumps an electron from its valence band to its conduction band creating a hole (h^+) and a conduction band electron (e_{cb}^-). Oxidizing species such as $OH^\bullet$, O_2^-, and H_2O_2 are formed. These highly reactive radicals break down organic molecules including those composing bacteria cell membranes (Sunada et al. 2003). Exposure to sunlight of plastic PET bottles coated with titanium dioxide, TiO_2, offers a method for disinfecting water inexpensively and effectively even in remote corners of the globe. Water treated by the SODIS method is safe to consume only shortly after disinfection, while water stored for a long period may harbor reactivated bacteria. It is desired to learn whether water disinfected using this method can be stored following disinfection and then consumed at a later time.

Taste of the disinfected water is important to the local acceptance of the TiO_2 water purification method. Often local people have very particular sensitivity to the taste of water, and it is crucial that the disinfected water has either no effect on the taste or a positive, pleasing effect. Unfortunately, if disinfected water has a displeasing taste, despite being safe to drink, it will be far more difficult to gain local acceptance of the method.

2. DISINFECTION PROCEDURE

The process to coat bottles with TiO_2 originates in Litter et al. (2005) and is modified by Heredia (2006). The original procedure calls for a dilute solution of perchloric acid ($HClO_4$) at 2.5 pH with 2% w/v Degussa P-25 TiO_2 (Heredia, 2006). Morgan (2008) has found that sodium acetate is an effective substitute for the more hazardous perchloric acid. Additionally, the original procedure called for applying two coatings of 2% solution to the bottles. However, it has been found that one application of a 4% solution works equally well. Dubro (2007) developed a dye indicator pill which slowly releases indigo carmine dye into the bottles during disinfection. The dye gives the water a blue hue. Because the oxidation of the dye molecule causes decolorisation, clear water signifies that the water has been fully disinfected. Figure 1 shows a bottle with the dye pill. Figure 2 shows use of the method in the field.

Fig. 1. Coated PET bottle with dye pill (Williams, 2009).

Fig. 2 Field Testing of the Solar Purification Method (Williams, 2009)

3. BACTERA REGROWTH

The ability to store water after it has been disinfected is important because it allows people to consume the water a long time after disinfection. If bacteria regrow in the bottles while in storage, people could become ill when they consume it. Because oxidation physically breaks apart the structures of the bacteria, it is reasonable to hypothesize that bacteria cannot reactivate after photocatalytic oxidation. Gelover et al. (2006) found no regrowth for seven days of their samples disinfected by TiO_2 films. Following disinfection, Rincon et al. (2004) found no regrowth after 60 hours of incubation in the dark under optimal growth conditions. Bottled water is considered to have an indefinite safe shelf life, if it is produced in accordance with current good manufacturing processes (CGMP) and quality standard regulations and is stored in an unopened, properly sealed container (Raj, 2005). Therefore, it is reasonable to hypothesize that bottles disinfected by photocatalytic oxidation can be safely stored indefinitely as well.

4. STORAGE EXPERIMENT

4.1 Experimental Procedure

Plastic 500mL bottles coated with TiO_2 were filled with distilled water. The bottles were inoculated with e.coli to a concentration of 10,000 cfu per mL. The bottles were transferred to the roof of the engineering building and exposed to sunlight. Later, the bottles were removed and stored in darkness inside boxes. The matrix of tests that was performed is displayed in Table 1.

Table 1: Storage Test Matrix

Set	TiO_2 Coating	Dye Indicator Pill	Air space in bottle	Sun exposure	Re-growth anticipated
A	Y	Y	N	Full	N
B	Y	Y	N	Low – cloudy	N
C	Y	N	N	Full	N
D	N	N	N	Full (SODIS)	N < 3 days Y > 3 days
E	Y	Y	Y	Full	Y
F	Y	N	N	None	Y
G	N	N	N	None	Y
H	Y	Y	N	N	Y

Periodically, a bottle from each set was removed and tested for the presence of e.coli bacteria using the coliscan membrane filtration method. Then 100mL was withdrawn from a sample bottle and pulled through a membrane filter. The filter was placed in a petri dish with a sterile pad containing 2mL of Coliscan MF broth medium. The dishes were placed in an incubator for 24-48 hours. After incubation, e. coli bacteria produce purple or blue colonies on the dish and are easily identifiable.

4.2. Storage Test Results

The storage test results are displayed in Table 2.

Table 2: Storage Test Results

	E. Coli Concentration (cfu/100mL)						
Storage Period (days)	1	4	11	40	110	190	360
A	5	0	0	0	0	0	NA°
B	0	0	0	0	0	0	NA
C	9	0	0	0	0	0	NA
D	4	0	0	0	0	0	NA
E	7	0	0	0	0	0	NA
F	TMC*	TMC	TMC	TMC	0	0	NA
G	TMC	TMC	TMC	TMC	0	3	NA
H	TMC	TMC	TMC	TMC	0	0	NA

*TMC = Too Many to Count °NA = Data Not Available

Generally, the results were as expected. The three controls all tested highly positive for E. coli, and the disinfected sets all showed no growth after 4 days. However, there were two oddities in the results. The first is that Set D, the SODIS method, showed no regrowth of bacteria after 3 days of storage. Apparently, the bacteria was not able to reactivate itself in this test. While this is somewhat curious, it was not a critical feature of this research to analyze the SODIS method in great detail. The second oddity in the results is that the test for e.coli after a storage of one day was positive for the bacteria, albeit in extraordinarily small quantities. Even Set C, which had the highest quantity at 9 cfu/100mL, had an extremely small quantity present. Despite the tiny quantity of bacteria persisting, the fact that some bacteria appear to have survived through the disinfection process, is a reason for concern. The stated public health goal of the EPA is zero coliforms and it was hoped that the method could adhere to this goal.

4.3 Second Storage Test

To research the matter of these few persistent bacteria, a second storage test experiment was performed. This time, water was purified and sampled after 0,1,2,3, and 4 days, to determine if the bacteria persist for greater than one day, and to test the repeatability of the initial results. Also, the additional storage test was performed to determine if the persistent bacteria are present directly following disinfection, or if they repair themselves during storage only to die off again later.

4.4 Second Storage Test Results

The second storage test results are displayed in Table 3.

Table 3: Second Storage Test Results

	E. Coli Concentration (cfu/100mL)				
Storage Period (days)	0	1	2	3	4
A	12	0	0	0	0
D	0	0	0	0	0

Again, in this test, a few bacteria remain present after disinfection. But it appears that bacteria do not reappear at least on the time scale of one day.

5. TASTE TEST

5.1 Experimental Procedure

Participants sampled from three different containers. One container contained disinfected water. However, for reasons of safety, the water was not inoculated with e. coli before disinfection, and contained simply spring water put through the disinfection procedure. The other two containers contained spring water. The samplers chose the sample which they believed was different from the other two and listed the characteristics which led them to believe that the sample was different.

5.2 Statistical Analysis

The null hypothesis was that the tasters could not tell the difference between the samples. So the probability of selecting the different sample would be 1/3. The number of successes which would imply a false null hypothesis, signifying that tasters could identify the different sample, can be calculated. The probability that random guessing would produce greater than or equal to a certain number of successes is calculated with the cumulative binomial distribution formula:

$$\sum_{y}^{n} \frac{n!}{y!(n-y)!} p^{y}(1-p)^{n-y} \qquad (1)$$

Where n is the number of samples, y is the number of successes, and p is the probability. The number of successes signifies the number of tasters who identified the disinfected sample. Values calculated for different success numbers using the cumulative binomial distribution and the binomial distribution are presented in Table 4.

Table 4: Values for different success numbers

# of	cumulative	binomial

successes	binomial distribution	distribution
0	1.000000	0.002284
1	0.997716	0.017127
2	0.980589	0.059946
3	0.920643	0.129883
4	0.790760	0.194825
5	0.595935	0.214307
6	0.381628	0.178589
7	0.203039	0.114807
8	0.088232	0.057404
9	0.030828	0.022324
10	0.008504	0.006697
11	0.001807	0.001522
12	0.000285	0.000254
13	0.000031	0.000029
14	0.000002	0.000002
15	0.000000	0.000000

A conventional margin of statistical significance is 5%. There is a 3.1% chance that 9 or greater successes would occur through chance alone. Therefore, 9 or more successes can be considered statistically significant and would imply a false null hypothesis.

5.3 Taste Test Results

Sample A contained the spring water put through the disinfection procedure. Eight tasters selected sample A, five tasters selected sample B, and two tasters selected sample C. While the results suggest that the disinfected water is distinguishable from regular spring water, a statistically significant level was not reached. Even more encouraging, those tasters who chose sample A noted that the taste of the disinfected water was not unpleasing, and three tasters preferred it to the spring water.

6. CONCLUSIONS

The storage test demonstrates that bacteria do not regrow during storage. However, the results indicate that a tiny amount of bacteria were not killed during disinfection and persist after 1 day in storage. Therefore, it is recommended to users that bottles be stored for two days prior to consuming the water in order to ensure that there are no bacteria. It is recommended to researchers to study whether the dye quantity should be increased to become a more conservative indicator of readiness or whether the dye binder becomes a substrate for bacteria growth. The taste test indicates that the disinfection procedure does not significantly alter the taste of the water. More details of this study are available in Williams (2009).

7. ACKNOWLEDGMENTS

Partial support from NCIIA (National Collegiate Inventors and Innovators Alliance) is gratefully acknowledged. Thank you to Prof. Hines and Gail Skinner-Brasard of the UML Biology Department for their assistance with bacteria culturing and concentration measurement and for generous use of their lab space.

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