

EVALUATION OF INJURED E. COLI IN CHLORINATION AND ULTRAVIOLET IRRADIATION OF WATER

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1. Introduction

Bacterial injury is a subject of interest because of its importance in assuring the safety of water. Injury is the physiological result of exposure to stresses. Under proper conditions, the injured bacteria can recover and become undistinguishable from normal bacteria. In order to evaluate the injury of E. coli in disinfection processes, chlorination, uv irradiation and combination of the both were applied to detect the presence of and the degree of injury. Additionally, ozonation was used.

2. Materials and methods

Experiments were performed using sodium hypochlorite solution, uv light generated by a mercury lamp and ozone solution. Chlorine demand free water, adjusted pH by phosphate buffered water to 7.2, was used. Water temperature was $20 \pm 2^\circ\text{C}$ for chlorination and uv irradiation, and $13 \pm 2^\circ\text{C}$ for ozonation. E. coli - ATCC 11775 - was used. Initial density of E. coli was in the order of 10^7 /ml for both chlorination and uv irradiation and 10^5 /ml for ozonation. Initial chlorine concentration ranged from 0.15 to 1.96 mg/l. The intensity of uv light ranged from 115 to $420 \mu\text{W}/\text{cm}^2$. Ozone concentration ranged from 0.21 to 2.02 mg/l.

E. coli was dosed into stirred water containing chlorine or ozone in a beaker. After selected contact times, residual chlorine or ozone was neutralized by 0.1 N sodium thiosulphate solution. Residual chlorine was detected by DPD method (SHIMADZU UV-160A spectrophotometer or HACH DR/3000 spectrophotometer) and residual ozone by indigo method (HACH DR/3000 spectrophotometer). For uv irradiation, stirred thin layer water containing E. coli in a large petri dish was exposed to uv light. Water sample were taken away after selected contact time. Uv light intensity was measured by uv radiometer (TOPCON UVR-254).

Three media were used; nutrient agar, deoxycholate agar and m-T7 medium (the newly designed medium for recovery of the injured coliforms). Pour plate method was used for nutrient and deoxycholate agars and membrane filtration method for m-T7 medium. Incubation was done at 37°C for 24 ± 2 hours.

3. Results and Discussion

Fig.1 and Fig.2 show the effects of chlorination and uv irradiation on survival of E. coli. There are large differences in decrease due to chlorine concentration and uv light intensity. The results of nutrient agar and m-T7 medium were very close. But there is a certain difference between the results of the both and that of deoxycholate agar. For this reason, in the experiments of simultaneous application of both chlorination and uv irradiation, and ozonation, only m-T7 medium and deoxycholate agar were used.

Fig. 3 shows the effects of simultaneous application of chlorination and uv irradiation together with the results of chlorination alone and uv irradiation alone. The effect of different uv intensity are summarized in Fig.4. From these results, the accelerated effect of

combined use of chlorination and uv irradiation is clear and the increased uv intensity resulted in the faster reduction of *E. coli* after a certain contact time.

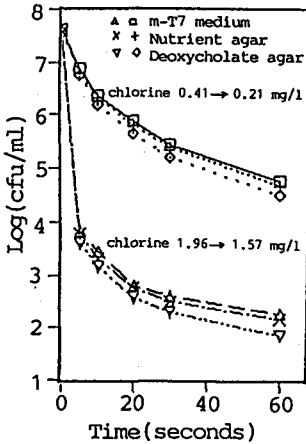


Fig.1 Effect of chlorine on survival of *E. coli*

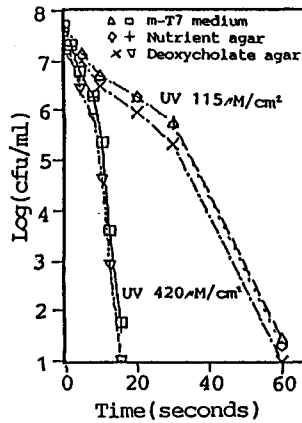


Fig.2 Effect of uv irradiation on survival of *E. coli*

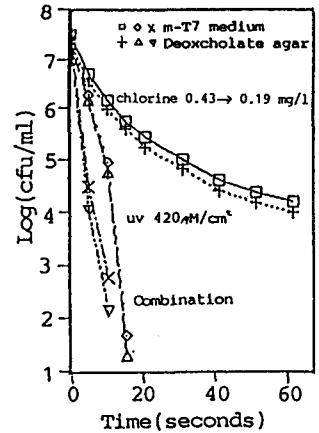


Fig.3 Effect of combined chlorine and uv on survival of *E. coli*

The disinfection effects of ozone was relatively small even at high ozone concentration as shown in Fig.5, because of a sharpe decay of ozone as shown in Fig.6. This decay might be caused by high organic compound in the suspension of *E. coli*. At these experiments, many times of swabbing were required because of insufficient growth of *E. coli* on the agar slope.

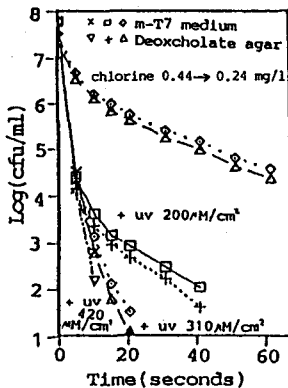


Fig.4 Comparison between effects of various intensity of uv light with same concentration of chlorine on *E. coli*

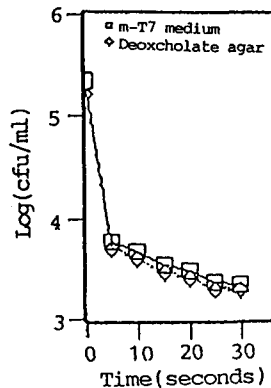


Fig.5 Effect of ozone on survival of *E. coli*

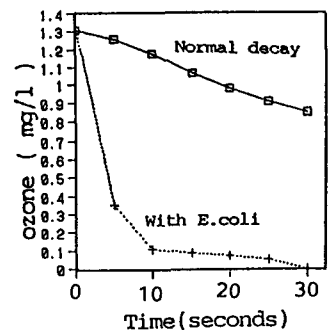


Fig.6 Comparison between decay of ozone

In all cases, the number of colonies on m-T7 medium were larger than that of deoxycholate agar. However, the difference was small and less than one order at each contact time. It leads to the conclusion that the occurrence of *E. coli* injury in any disinfection methods is obvious, but the degree is not so large.

Fig.7 summarizes the ratio of the number of colonies on m-T7 medium to that on deoxycholate agar. It increases according to the increase of dose and uv light intensity. In chlorination, this ratio was 2 to 2.5 even after one minute while in uv irradiation, it was 3 at $115 \mu\text{W}/\text{cm}^2$ after one minute and increased to 6 at $420 \mu\text{W}/\text{cm}^2$ after 15 seconds. In simultaneous application of chlorine and uv light, the ratio increases by increasing uv intensity level, but it still similar to that of uv irradiation alone.

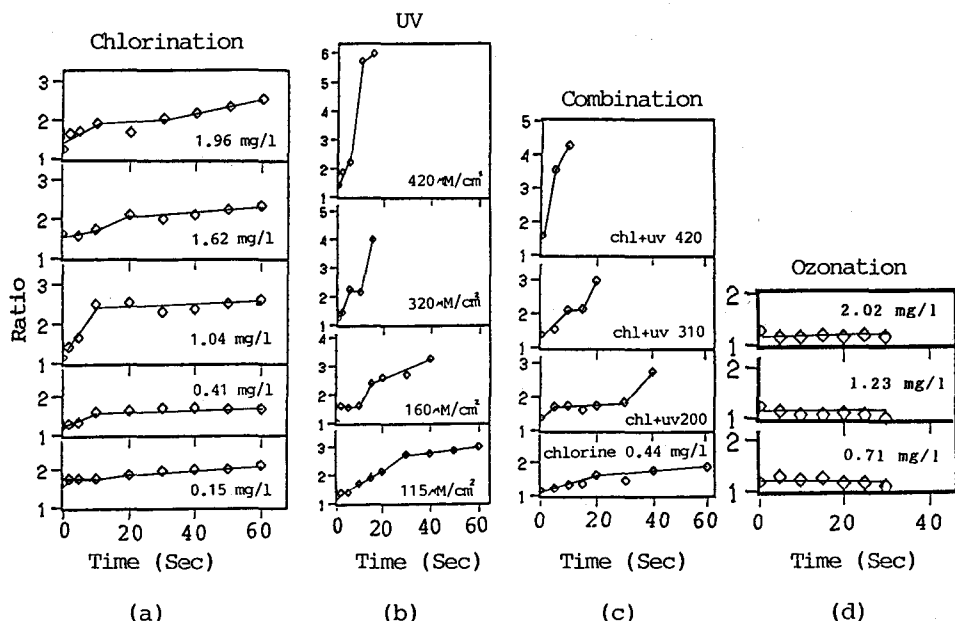


Fig.7 Ratio between numbers of colonies of *E. coli* on m-T7 medium and deoxycholate agar. (a) By chlorination (b) uv irradiation (c) Combination of chlorination and uv irradiation (d) Ozonation

Chlorine's killing effects on *E. coli* is almost complete and not produce a large portion of injured *E. coli*. However, uv light does not bring complete damage on *E. coli* at low *E. coli* concentration and this cause a larger ratio. As for ozone, more data should be accumulated to discuss this matter, but the effects might be thought to be similar to chlorine because of low ratio even at high ozone concentration.

4. Conclusion

Injury of indicator bacteria can occur by all disinfectant methods. But the degree of injury is different from one method to another. Ultraviolet irradiation causes the larger injury than chlorination. Injured coliforms are of public health importance and may help in the early detection of treatment deficiencies or contamination of water system.

References

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- 2- Gordon A. McFeter, Ajaib Singh and Joyce S. Kippin. Health significance and occurrence of injured bacteria in drinking water Wat.Sci. Tec. 1986