UV Decontamination Effectiveness of Esco's CO_2 Incubator

By Dian Susanti

Introduction

Auto *decontamination* using *UV* light is another feature available in *CO_2 incubators.* It has been years since UV light was routinely applied in CO_2 Incubators as a surface disinfection agent, this feature is not an absolute requirement in an incubator system. If necessary, it shall be installed in such a manner that it does not reduce the required performance. Many CO_2 Incubator users insist on equipping their incubator with a UV system to keep their incubator free from contaminants.

Some contamination inside the CO_2 incubator come from water in the humidity pan. Esco CO_2 incubator has one optional feature for disinfection of incubator enclosure, which is by integrating UV light system that is set to glow for 30 minutes. UV lamp is placed on the back bottom of inner chamber of the CO_2 incubator. It allows UV to kill the contaminant in the humidity pan.

In this experiment, the UV system was evaluated for its ability to kill contaminants and those microorganisms were tested in their vegetative and spore form. A series of tests was conducted with several time exposures to UV, i.e., to know the effective time for UV decontamination.

Ultraviolet light properties

Ultraviolet light is the section of the electromagnetic spectrum with a wavelength shorter than visible light and is divided into UVA, UVB, and UVC. UVC and UVB induce changes in the nucleic acids of DNA and RNA (in virus case) altering the molecules to a more reactive state. DNA bases are prone to absorption of UV light at around 260nm and the major biocide role of UV light is disruption of the DNA.

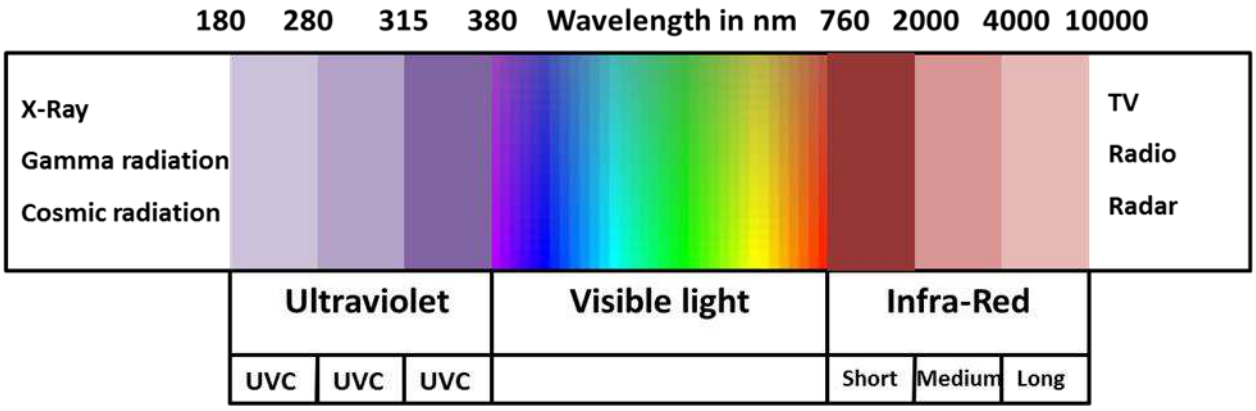


Figure 1. Light Spectrum and Wavelength

Materials and methods

Microorganism. There are 4 types of bacteria, 2 spores, and 3 types of yeast used in this experiment. Microorganisms used all came from Presque Isle Culture collection. The bacteria cultures used are *Staphylococcus epidermidis*; *Enterococcus faecalis*; *Escherichia coli*, and *Serratia marcescens.* Yeasts were *Saccharomyces cerevisiae* and *Candida albicans*. Each of the bacteria and yeast were refrigerated at 4°C in Tryptone soya and saboraud agar slant.

In vegetative cell preparation, bacteria cultures were extracted from their agar plate, and grown aerobically in tryptone soya broth. Yeast cultures in agar slant were also transferred into saboraud dextrose broth and grown aerobically for72 hours at 25°C. All cultures were harvested at their stationary growth phase. Vegetative cells were purified by extracting the cell from growth media via centrifugation process. The harvested cells were diluted in sterile phosphate buffer saline at pH 7.3 and stored in 4°C prior to the UV exposure procedure.

Bacillus subtilis var *globigii,* and *Geobacillus stearothermophilus* spore were obtained as ready-to-use spore suspension. Prior to UV treatment the spore suspension was diluted to the desired concentration in sterile phosphate buffer saline at pH 7.3. Initial concentration of *B.subtilis var globigii* and *G.stearothermophilus* is 1.5×10^{10} and 1.3×10^{8}.

Esco CO_2 Incubator Setting. In this experiment the Esco CO_2 incubator was used, a factory standard UV bulb was attached to the inner chamber of the incubator accordingly and cleaned up prior to the test. The incubator was turned ON and it was ensured that the UV lamp was working by setting on factory menu and then pressing the door switch for a while until the UV lamp is turned ON.

UV Decontamination Procedure. To conduct a quantitative experiment, the number of organisms exposed to UV has to be sampled in the same amount with applied microorganism's prior UV exposure, this way we could retrieve the representative result.

Therefore, each bacterial suspension with concentration 10^{8} cells/ml was prepared by serial dilution. Each 100µl of suspension was poured into 800 ml sterilized DI water in the humidity pan (as standard operation). Run the UV decontamination cycle per time exposure as explained in the method above. After the cycle finishes, 1 ml samples from the humidity pan were used for serial dilution to obtain the quantitative result. Dilution samples were spread into tryptone soya agar and incubated for 48 hr at 25°C.

Result and Discussion

Table 1. UV Decontamination Result

Microorganisms	Initial concentration (cfu/ml)	Final concentration		
		15 min	30 min	60 min
Bacteria vegetative cell				
Staphylococcus epidermidis	4×10^5	4×10^2	0	0
Enterococcus faecalis	3×10^5	4×10^2	0	0
Escherichia coli	8×10^5	5×10^2	0	0
Serratia marcescens	6×10^5	5×10^2	0	0
Spores and mold				
Bacillus subtilis var.globigii	5×10^5	2×10^3	1.2×10^2	3×10^1
Geobacillus stearothermophilus	2×10^5	1.4×10^3	4×10^2	2×10^1
Yeast vegetative cell				
Saccharomyces cerevisiae	5×10^5	4.2×10^2	0	0
Candida albicans	4×10^5	3.5×10^2	0	0

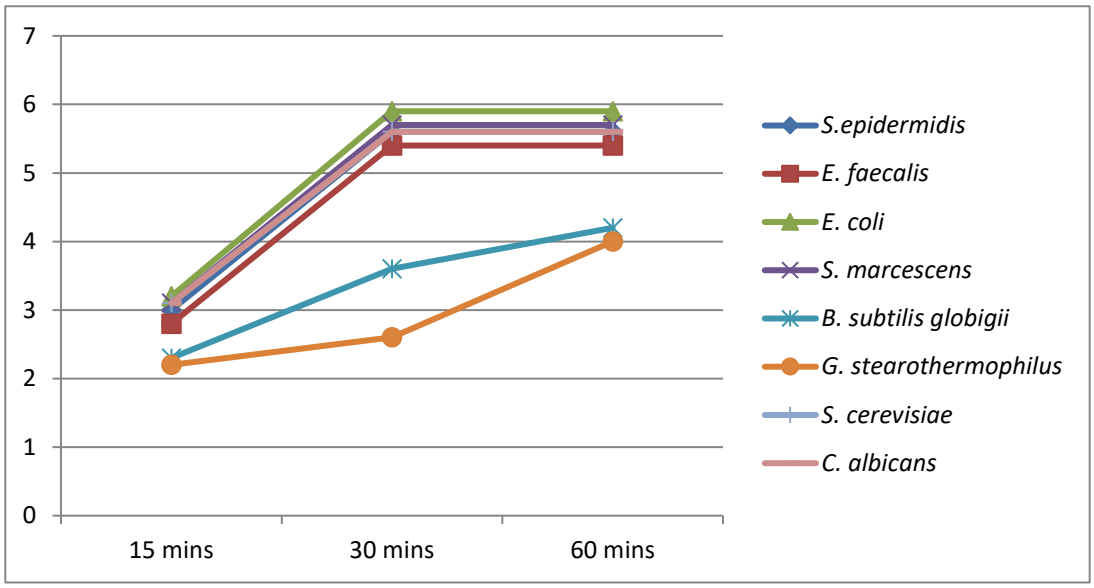


Figure 1. Log reduction for each microorganism against UV exposure time

Observation

1. Table 1 shows that bactericidal effect was determined after 30 minutes and 60 minutes UV exposure. This result indicates UV decontamination is more effective with increasing time.

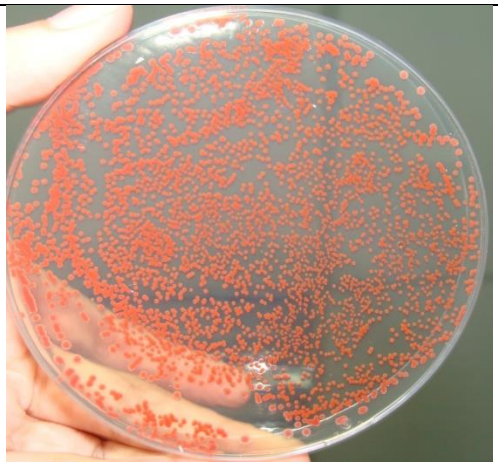
2. With 30 minutes UV light exposure, it is effective to kill all vegetative cells and yeast from the humidity pan. Zero colonies for each microorganism were observed after analysis by plating on agar plates.

3. Figure 1 shows the increasing log reduction in the spores *Bacillus subtilis* var *globigii* and *Geobacillus*

stearothermophilus which means more spores are killed by increasing time exposure to become 60 minutes. Maximal log reductions for both spores are 4.2 and 4 log reduction respectively.

4. From this experiment, it is known that between vegetative and spore form, the second form was more resistant to UV treatment which was observed as low log reduction value. A few bacteria, such as *Bacillus* and *Geobacillus* produce a specialized structure called endospore. They provide resistance to heat, desiccation, radiation and other environmental factors that may threaten the existence of the microorganism. Endospores are also providing a selective advantage for survival and dissemination for some species that produce them.

5. In this experiment, mutation effect prominently observed from *S.marcescens* colony, recovered cell grown in the agar was changing color into yellowish white from previously cultured as in red to pink color. In this case, gene expression for controlling pink colorization of colony appearance was shut down by UV light. No special changing macroscopically were observed from the rest of *E.faecalis*, *S.epidermidis*, *E.coli*, spore derived culture and all of the yeast species colonies. See the picture below:



Conclusion

UV decontamination for Esco CO_2 incubator is effective to kill the vegetative cells and spores with certain concentration. UV effective usage in Esco CO_2incubator will be achieved by setting UV timer to 30 minutes for vegetative cells and yeast and set to 60 minutes for spore decontamination.

References

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