Visible Spectrum Role in Bacterial Inasctivation through Continuous and pulse wavelength

Devender Arora, Sneh Anand, Anjana Sharma

Abstract— In the current research scenario UV light is the most prominent source used widely to create bactericidal effect. But UV light associates many harmful effect with it and it needs more safety precautions while disinfectant the bacteria. E.coli is a widely studied bacterium with both virulent and non-virulent strains. Due to the virulent nature this bacterium leads to major outbreaks to society by causing serious illness and thus highlighted in the last few year. In the current investigation we enumerate the effect of stress of three different source of visible light with blue light (peak at 470 nm), Green light (peak at 525nm) and Red Light (peak at 700nm) on electromagnetic spectrum, considering two different approaches one pulse light and another with continuous approach on these bacteria in MaCConkey Agar media and evaluate out their effect at different growth pattern when condition of exposure change with change in wavelength.

Index Terms- Pulse light, continuous light, Stress, Electromagnetic spectrum, Exposure.

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1 INTRODUCTION

E.coli mainly found in intestine of warm blooded animalranges up to 2µm (Tchobanoglous & Schroeder1987). They are facultative anaerobic (grow aerobically and anaerobically) and oxidase negative. One finds enterobacteriaceae ever where (ubiquitous) and the port of entry into the human body is usually orally. Although only a few strains are pathogenic to humans, these serotypes, such as E. coli O157:H7, have caused frequent outbreaks associated with food and drinking water (Betts, 2000; Kuhnert et al., 2000; Rasmussen and Casey, 2001; Swerdlow et al., 1992). Infirmity caused by the ingestion of tainted food is major health problems throughout the world. (MEAD et al., 1999). Pulsed light is an alternative method proposed to continuous light and more effective in inhibiting bacteria, fungi, viruses and protozoa, and its efficiency is much greater and in a much shorter time than with continuous treatment (Dunn et al., 1995; Huffman et al., 2000; Roberts & Hope, 2003; Takeshita Et Al., 2003; Feuilloleyet al., 2006).

There is an increasing use of Ultraviolet light (UV)radiation in Sterilization of food packages, water purifier, in biological lab.(Decho, 2000; Wilson, 1994).Visible light lasers have been used as bactericidal agents to remove bacterial biofilms (Nanda kumar et al., 2006).

As Visible light also shown Bactericidal effect it is worthy to know which wavelength is lethal for the cause. As pulse light approach shows more lethal effect over continuous, our aim of this study was to investigate the efficiency of pulsed light and continuous light in bacterial inactivation of E.coli on MaC-Conkey Agar media.

Material and methods

E.Coli strain ATCC-25922 obtained from American type cell culture. This bacterium grown aerobically on Trypticase soy agar at 37°C and it is a non-diarrheagenic clinical strain used as a standard E. coli strain for several microbiological tests. Pulse Light Equipment

An Integrated circuit IC-555 used to create pulse of desire ranges of second working on a formula.1 one minute on and off to create disturbance in the metabolic activity when the wavelength source linked with the IC

0		
t=1/f = 0.6	$59(R_1+2R_2) \times C$	(1)

The HIGH and LOW times of each pulse can be calculated from:

HIGH time =
$$0.69(R_1+2R_2) \times C$$
 (2)
LOW time = $0.69(R_1+2R_2) \times C$ (3)

We considered $R_1=1 \ k\Omega$ this helps to give the output pulses a duty cycle close to 50%, that is, the HIGH and LOW times of the pulses are approximately equal.

Bacterial growth and treatment with continuous and pulse light.

The bacterial cells to be treated by pulse and continuous light were prepared as broth medium; a 0.1ml sample of the cell suspension from each inoculum was surface-seeded onto solid MacConkey Agar media Surface-inoculated solid media plates were treated with pulse, continuous exposure and the control and treated plates were incubated for 24hrs. After the incubation period the growth were enumerated for all the plates. Systematic representation of the pulse light treatment system is shown in fig. 1. Experiment driven in duplicate so

Devender Arora is currently pursuing masters degree program inBiotechnology Engineering from Uttarakhand Technical University, India, PH-+919868967868. E-mail: devarora@hotmail.com

[•] Co-Author Anjana Sharma is a Senior ScientistRP Centre AIIMS, Delhi, E-mail: <u>anjanasharma@rediffmail.com</u>

Co-Author 2: Prof. Sneh Anand is the Head of department of Biomedical in Indian Institute of technology, Delhi E-mail: snehanand@rediffmail.com

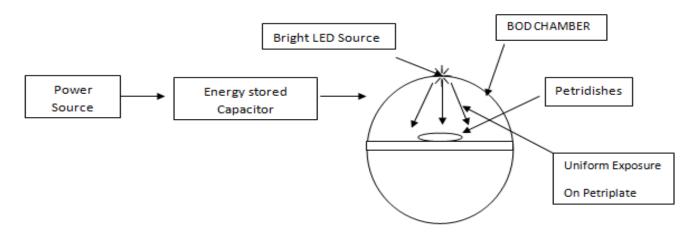


Fig. 1.Schematic diagram of pulsed light treatment system

error if any can be evaluated but both the times with same dilution the result was same.

Result and Discussion

Effect of pulse and continuous Visible Light on E. coli: Data produced during the experiments performed on E.coli provides vital information regarding the behavior of bacteria at different wavelength of light. We observed different growth rate with change in morphological characteristics of E.coli.

E.coli growth at 470nm and 535nm of light was found to be bactericidal. When the continuous light approach compared with control we observed bacterial colonies reduced to half of the normal growth at 470nm and 525nm show little effect on it. Similarly when we compared control with pulse light the observation found most lethal in first case as colonies reduced more than half of the original growth (Table1 & Table 2), and as shown in figure2.

Beside these two wavelengths which shows bactericidal effect when we slant to extreme right of visible spectrum and carry the same procedure at 700nm peak of light we found this range helps E.coli to proliferate when the light fall continuously and it adapts the pulse light condition and grown at the same rate as in control (table3). Graphical representation shows the overall impact of visible light on E.coli Bacterial colonies.

Conclusion:

The study conducted by us tells that even small changes in condition of visible light for a short period of time have an effect on these microorganisms. Various studies on this aspect however did not conclude the overall effect of visible light. As visible part of electromagnetic spectrum ranges from 400nm to 700nm. In our study we demonstrate that

Classification	Sham Exposed	3o minute	Pulse exposure		
		Exposure	overnight		
Colony Number.	144	76	61		
Elevation	Slightly Raised	Slightly Raised	Flattened		
Pigmentation	Pinkish	Pinkish	Light Pinkish		
			Spread		
Size	2-3mm	2-3mm	2-3.5mm		
Shiny	++	++	+		
Table: 1 Effect of Plue light (neals at 470 pm) on E. coli					

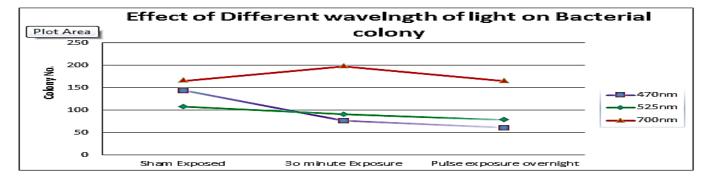
Table: 1 Effect of Blue light (peak at 470 nm) on E. coli

wavelength ranging at 470nm causes the highest of bactericidal effect and it changes to proliferation effect at 700nm

Classification	Sham Exposed	3o minute	Pulse exposure
		Exposure	overnight
Colony Number.	108	91	79
Elevation	Slightly Raised	Slightly Raised	Slightly Raised
Pigmentation	Dark Pinkish	Dark Pinkish	Light Pink
Size	2-3mm	2-3mm	2-3.3mm
Shiny	++	++	+

Table: 2 Effect of Green light (peak at 525 nm) on E.coli

even when it compares with pulse light at 470nm and 525nm is considered lethal but with 700nm the bacteria adapt to the condition of exposure and shows similar growth behavior as control observed.



Graph: Represent the overall impact of visible light on E.coli Bacterial colonies.

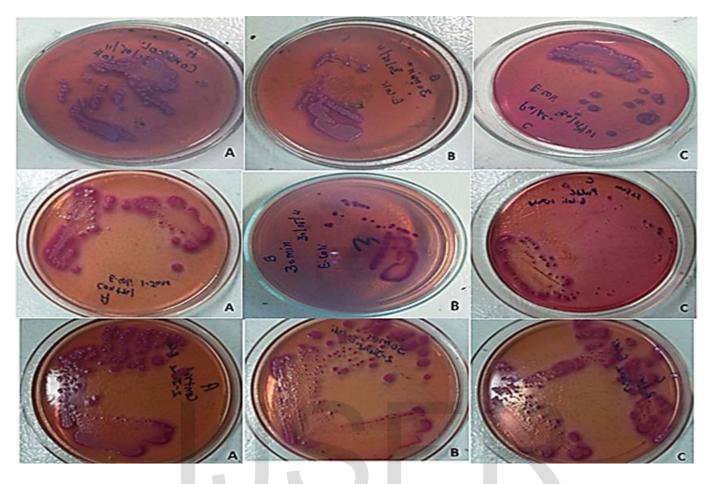


Fig: 2 Representing 1: affect at 470nm, 2: affect at 525nm, 3: affect at 700nm A: Sham Exposed, B: 30 min Exposure, C: Pulse light for 24hrs

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