VOL. 10, NO. 11, JUNE 2015 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

THE ROLE OF LASER WAVELENGTH AND PULSE FREQUENCY IN INACTIVATION OF *Escherichia coli* AND *Listeria monocytogenes*

Moses Elisha Kundwal[1, 3], Abd Rahman Tamuri[1] and Mohd Nizam Lani[2]
[1]Department of Physics, Faculty of Science, Universiti Teknologi Malaysia, UTM Johor Bahru, Johor, Malaysia
[2]School of Food Science and Technology, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia
[3]Department of Physics, School of Science, Federal College of Education, Yola, Adamawa State, Nigeria
E-Mail: mekundwal@gmail.com

ABSTRACT

Ultra violet (UV) lasers have been used in food industry, medicine and dentistry to deactivate pathogenic bacteria of various types. The use of laser and other pulsed light systems for phototherapy and other microbial disinfections are based on spectral characteristics and configuration of the light source used. Here we explore the extent to which two pulsed laser parameters (wavelength and pulse frequency) affect deactivation of two pathogenic bacteria; *Escherichia coli* and *Listeria monocytogenes*. Three pulsed laser wavelengths, 1064nm and its 2nd and 3rd harmonics, were used to irradiate samples of *E. coli* at approximately same fluence. A 350nm continuous wave flash lamb, with output configuration close to that of the 3rd harmonic of the laser, was also used for the purpose of comparison. The result indicates that *the log* reductions for the laser wavelengths are higher (almost double) that of continuous wave light. When *E. coli* and *L.monocytogenes* samples were irradiated with the 3rd harmonic wavelength using three different pulse frequencies, the result shows higher deactivation at higher pulse frequency than at lower pulse frequency.

Keywords: pulsed laser, continuous wave light, laser wavelength, pulsed frequency, *Escherichia coli* and *Listeria monocytogenes.*

INTRODUCTION

Traditional methods of pasteurization or sterilization using thermal or chemical treatments to inactivate these bacteria have generally been effective but, nevertheless, they have certain limitations. Pasteurization of food materials and other methods of thermal sterilization results into rise in temperature in the bulk of the material, which may be undesirable. Pasteurization also leads to the emergence of pasteurization-resistant bacteria. There is also the issue of wastage of energy and time in some instances; for example, thermally treated sterile air has to be cooled before being used to aerate pasteurised food for the purpose of packaging. Therefore, there is currently considerable interest in developing alternative methods for the control of microorganisms; methods which will be effective in deactivating the microbes and yet have leave less or minimal damaging effect on the material being processed. Pulsed light system is one such emerging technology which could be an effective alternative to traditional thermal treatment in order to assure the microbial quality and safety of food products.

Both continuous wave and pulsed light systems have been used to inactivate microbes of both the pathogenic and the non-pathogenic type. The choice of which type light source to use depends on several factors such as the type of microbe involved, the medium bearing the microbe and the spectral characteristics and configuration of the light source. For example, both continuous wave and pulsed UV radiations has been reported to be effective in treatment of drinking water [1-3]; partly due to the germicidal effect of the UV light (i.e. spectral characteristics) and also partly due to the high transparency of water. Arguably, there could be some difference in the germicidal effects of the two sources of UV light if a less transparent medium is involved. Generally, disinfection of microbes using continuous wave light has been shown to be effective mostly for superficial treatment of opaque media such as packaging materials [4-6].

Pulsed light systems, particularly pulsed lasers, have potential for microbial disinfection that requires some level of penetration into the bulk of the medium or substrate which houses the microbe. Hence the use of laser for phototherapy in medicine and dentistry has, of recent, featured prominently in various studies [7-10]. Studies in this area are often fashioned out in such a way that most of the resulting applications are tailored towards medicine, surgery or dentistry. Practically all of these applications are for curative purpose. Study of the interaction of lasers with pathogenic microbial organisms could also leads to applications such as food preservation, food safety, decontamination of immediate environment, sterilization of equipment, etc, which are preventive rather than curative. A major advantage in the use of laser is that lasers, particularly, pulsed lasers, can provide narrow-band emissions of high-intensity, with desirable spectral width and penetration depth in samples [11, 12]. Others include the fact that the nature of laser light makes it easier to provide for easy manipulations and automation with lesser waste [13].

In this work, we explore the extent to which two pulsed laser parameters (wavelength and pulse frequency) affect deactivation of *Escherichia coli* and *Listeria monocytogenes*. We also compare the effect of the pulsed laser to that of continuous wave UV light of comparable

output configuration. The two pathogenic bacteria used in this study are notorious for food poisoning. Certain strains of *Escherichia coli* are known to cause diarrhoea, resulting from intake of contaminated water or food. *Listeria monocytogenes,* on the other hand, is the cause of listeriosis, also resulting from eating contaminated food.

MATERIALS AND METHOD

Media and sample preparation

Four liquid media - Nutrient broth (NB), Nutrient agar (NA), Tryptic soy agar (TSA) and Saline (0.85% NaCl) - all manufactured by Merck, Germany, were first prepared in accordance with manufacturer's instruction and autoclaved. About 20 ml of either NA or TSA were poured into 90 mm petri dishes for plating. The NA plates were used for plating *E. coli* samples while the TSA plates were used for *L. monocytogenes* samples. *E. coli* (ATCC 11775) and *L. monocytogenes* (ATCC 7645), both from UNISEL, were used for the study. In preparing each sample, a colony of bacteria sample, previously grown on NA plate for 24 hours at 37°C, was aseptically inoculated into 100 ml of NB in a sterilised universal bottle and this was also incubated for 24 hours and at 37°C. The 100 ml culture was then centrifuged at 10000 rpm and 37°C for 15 min. The pellets of sediment obtained in the centrifuge tubes were washed with saline and centrifuged again under same condition as previously. Then 50 ml of saline was added to the final sediment from each of the tubes and shaken vigorously to form a supernatant. Final sample used for treatment was obtained by diluting 5 ml of the mixed supernatant with 95 ml of saline. Decimal serial dilution and plating were done on the sample to determine the initial bacteria concentration. The initial concentration was of the order of 10^5 or 10^6cfu/ml, similar to those of previous studies conducted with pulsed light [14-17].

Sources of radiation

Three laser wavelengths were used in this study. The fundamental wavelength of 1064 nm of a pulsed infra-red laser (PIRLa), was obtained from a passive Q-switched, flash lamp-pumped Nd:YAG laser (Beijing Anchorfree Model XM100). The second harmonic of the IR laser - a pulsed green laser (PGLa) at 532 nm, and the third harmonic - a pulsed UV-a laser (PUVaLa) at 355 nm were obtained by the process of non-linear optical conversion. Operating input voltage of the laser was fixed at 900V. The output pulse energy, E_p, of the laser was measured with a power meter (MILLES GRIOT) while the maximum beam diameter, d, and pulse duration, τ, were specified by the manufacturer as 6 mm and 6 ns respectively. The specified spot diameter and pulse duration were adopted because focusing lenses were not used. Moreover, spot diameters of the output of the harmonics were confirmed to approximate to the value specified for the fundamental beam, as measured by method of the area of burned photographic paper [18]. Total fluence F_{total} or energy dose delivered to sample was approximated from pulse fluence, F_{pulse}, the pulse spot area, A, pulse energy, E, the pulse repetition rate, R, treatment time, t, using the expression

$$F_{total} = F_{pulse} \times R \times t$$

i.e.

$$F_{total} = \frac{E}{A} \times R \times t \quad (1)$$

In order to make comparison with the laser at the UV domain, a continuous wave UV-A light (CWUV-aLi) from a flash lamp (UV Tools) operating at 350 nm was also used. The power of the lamp was 4W with spot diameter of 13mm.Total fluence F_{total} or energy dose delivered to sample for the lamp was approximated as follows:

$$F_{total} = Fluence\ rate \times t$$

i.e.

$$F_{total} = \frac{P}{A} \times t \quad (2)$$

where P is power of lamb and A is the lamb beam area. The set parameters used for the laser and UV lamp are summarised in Table-1.

Table-1. Laser light and UV lamp parameters used for treatment.

Radiation	**Pulse energy** (mJ)	**Treatment time** (s)	**Approximate energy dose per sample** (J)
PIRLa	396.86	8989	624
		179	1256
		268	1880
		536	3760
PGLa	136.00	260	625
		521	1252
		782	1880
		1564	3760
PUVa-La	118.14	300	627
		600	1253
		900	1880
		1800	3759
CWUVa-Li	*4.00	208	627
		416	1253
		624	1880
		1248	3759

* Value is power (in Watts)

General experimental setup

Three aliquot parts of the prepared sample were poured into three sterilised beakers containing identical magnetic stirrer. One part was used as control while the other two were the treatment samples. All three beakers were wrapped with aluminium foil and placed on three similar stirrer plates, which were set to rotate at same speed. The treatment samples were subjected to laser radiation and continuous wave UV-a light for some appropriate time. The control sample was not irradiated (see Figure-1).

After the treatments, decimal serial dilutions were performed on the samples up to the third decimal. Then 0.1 ml of both control and treated samples were plated in duplicates using an automated plating machine (Interscience easy spiral). The plates were incubated for 24 hours at 37^{o}C after which viable cell counts were performed using a manual colony counter.

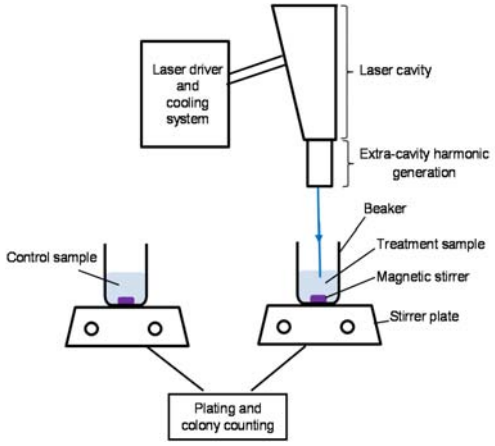

Figure-1. Schematic diagram of experimental setup.

Measure of deactivation for each sample was estimated using the log reduction (*LR*) of population defined as

$$LR = log \frac{(VCD)_0}{(VCD)_{trt}} \quad (3)$$

$$LR = log\ (VCD)_0 - log(VCD)_{trt}$$

Where $(VCD)_o$ is the viable cell density (in cfu/ml) of the original sample and $(VCD)_{trt}$ is the viable cell density (in cfu/ml) of treated sample.

In order to eliminate systematic error arising from factors such as bacteria death due to ambient conditions and the experimental procedure, the actual log reduction of a sample due to a particular radiation was calculated as follows:

$$LR_{rad} = LR_{trt} - LR_{ctr} \quad (4)$$

Where LR_{rad} is the log reduction due to radiation, LR_{trt} is the log reduction of treated sample and LR_{ctr} is the log reduction of control sample.

$$LR_{Rad} = (\log VCD_0 - log\ VCD_{trt}) - (\log VCD_0 - log\ VCD_{ctr})$$

$$LR_{Rad} = log\ VCD_{ctr} - log\ VCD_{trt} \quad (5)$$

Effect of different laser wavelengths

To observe any possible effect of the different laser wavelengths used, 40 ml of was used for both the control and the treatments. The treatment samples were irradiated at approximately same energy dose in sterilised 80 ml beakers, using the pulse infra-red laser and the continuous wave UV-a light. Some other 40 ml each of the *E. coli* samples were also treated with the pulse green laser and pulse UV-A laser at approximately same total energy dose as used for the pulse infra-red laser and the continuous wave UV-a light. All three laser irradiations were done at pulse frequency of 5 Hz. The laser parameters were set such that the three laser wavelengths used deliver approximately same total energy dose (see Table-1). The above procedure was repeated for three other values of total energy dose.

Effect of different pulse frequency

In order to study how pulse repetition rate may affect deactivation, 40 ml of treatment samples of *E. coli* and *L. monocytogens* were both irradiated each for 5 min with the PUVaLa at pulse frequency of 1Hz. The process was repeated for same types and quantity of samples for treatment times of 10 min, 15 min and 30 min. The above procedure was repeated for PUVaLa treatment at pulse frequencies of 3 Hz and 5 Hz.

RESULT

Log reductions for different laser wavelengths at different fluence

The column chart of the log reduction versus fluence for the three laser wavelengths and CWUVaLi is shown in Figure-2. Generally, log reduction achieved was less than 1 for all the types of radiation used, irrespective of the total dose or fluence supplied. But the log reductions for the laser wavelengths is higher (almost double) that of CWUVaLi. However, the log reduction achieved with PUVaLa is slightly higher than for the other two laser wavelengths. The highest value of log reduction was obtained at approximately 2000Jcm^{-2} dose of energy for virtually all the four radiations used, except that the inactivation curve due to PGLa tend to show insignificant variation.

Log reductions for different pulse frequencies

Figure-3 shows the inactivation curves for *E. Coli* and *L. Monocytogenes* using three different pulse frequencies of PUVaLa (355nm). On the whole, higher log reduction was obtained for treatment with UVaLa of higher pulse frequency. Highest log reductions were obtained after about 15 min of treatment for *E. Coli*. The treatment time with the utmost log reduction could be lower than 15 min for L. Monocytogenes. The log reductions values tend converge to a common value after treatment time of about 30 min, especially in the case of *E. Coli*.

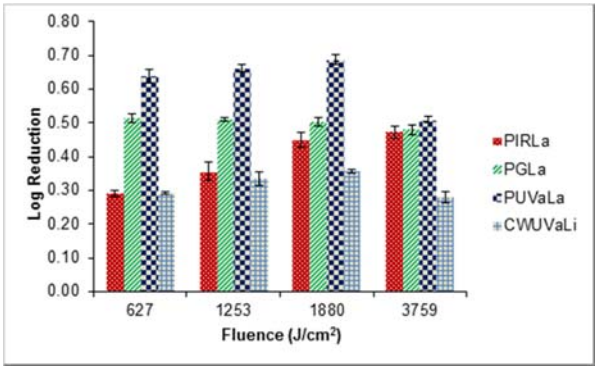

Figure-2. Column chart showing log reduction versus fluence for *E. Coli* using three different laser wavelengths and continuous wave UVa Light. Values are means and standard error of triplicate experiments.

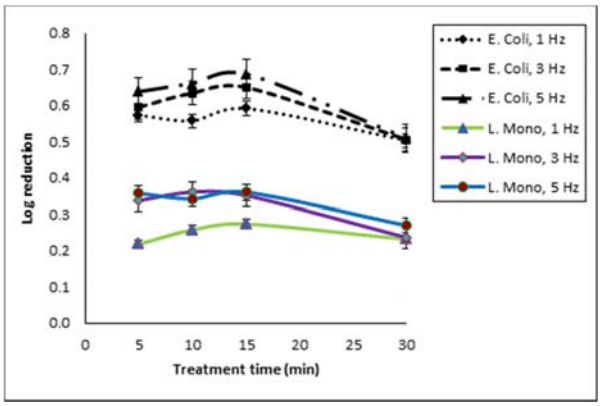

Figure-3. Inactivation curve for *E. Coli* and *L. Monocytogenes* showing log reduction versus treatment time using three different pulsed frequencies of pulsed UVa Laser. Values are means and standard error of triplicate experiments.

DISCUSSIONS

Lethal effect of pulsed light is said to depend on the energy dose (or fluence) incident on the sample in addition to the composition of the emitted light spectrum [19]. Different laser wavelengths have been used to achieve deactivation of bacteria under various conditions. There is therefore the need to study the role played by different pulse laser parameters such as wavelength, pulse width, and pulse power. Before discussing results presented above, it may be worthwhile to mention that for all the experiments conducted during this work, we found that the initial VCD of the prepared sample is always lower that than both the VCD(contol) and VCD(treatment) of same sample. We attributed this to be due to the stirring process. Hence, the adoption of equation (3) above to measure the inactivation effect.

The result from this work indicates that at same dosage, the deactivation of E. coli using PUVaLa is slightly higher than those obtained using PIRLa and PGLa. A similar study for Listeria innocua [20] shows a decrease in the effectiveness of deactivation when the UV region is blocked from full spectrum of pulsed light. Using laser, the spectral width of the portions of the spectrum has been narrowed in order to observe the actual bactericidal effectiveness of these particular regions. While photochemical effect in microbial inactivation with pulsed light is attributed mainly to the UVc region of the spectrum, the photo-thermal effect of same process is said to be due to the entire broad band spectrum (from IR to UV) [21]. However, in this work, no temperature change was observed as to warrant a speculation of the dominance of the photo-thermal effect.

With regards to pulse parameters, a difference in surface morphology of human dentin has been reported when irradiated at different laser pulse duration [22]. Here we looked at the effects of pulse frequency. As can be seen in Figure-3, there is a higher log reduction at higher pulse frequency. This could be due to the inability of the injured bacteria cells to photo-reactivate themselves at higher pulsed repetition. As the pulses are sent in quicker succession, the photo-repair time is shortened, thereby inhibiting any photo-repair process for any photo-repair time longer than the pulse interval. Also, after 30 minutes of treatment time, inactivation curves tend to converge. This could be as a result of shielding effect of the dead bacteria cells. When lethal effect of the irradiation reaches its maximum, dead cells began to shield the living (but injured) ones from the radiation so that further dose of the radiation becomes ineffective on those cells. Hence, there tend to be a higher photo-reactivation activity at this time leading to a reduced killing effect.

CONCLUSIONS

E.coli was irradiated with 1064nm, 532nm and 355nm wavelength of pulsed laser and also 350 nm continuous wave UV light of approximately same profile. The pulsed laser show higher bactericidal effect, with the highest deactivation obtained from 355nm wavelength. Also, for the 355nm pulsed laser, higher deactivation effect was obtained at higher pulse frequency than at lower pulse frequency. Since the health risk associated with radiations from the UVa region is far less than that of the UVc, a careful and well-controlled used of laser wavelengths of the UVa domain could be explored for use in food industries as well as in the dental or medical fields.

ACKNOWLEDGEMENTS

Financial supports for this work were obtained from Universiti Teknologi Malaysia, Universiti Malaysia Terengganu and Education Tax Fund of Federal Republic of Nigeria. The authors wish to acknowledge the support and assistance rendered by the staff of microbiology lab, Universiti Malaysia Terengganu, during this work.

REFERENCES

[1] Sharifi-Yazdi MK,Darghahi H. 2006. Inactivation of pathogenic bacteria using pulsed UV-light and its application in water disinfection and quality control. Acta Medica Iranica. 44: 305-308.

[2] Murphy H, Payne S, Gagnon G. 2008. Sequential UV- and chlorine-based disinfection to mitigate *Escherichia coli* in drinking water biofilms. Water research. 42: 2083-2092.

[3] Hijnen W, Beerendonk E, Medema GJ. 2006. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo) cysts in water: a review. Water research. 40: 3-22.

[4] Mukhopadhyay S, Ukuku DO, Juneja V, Fan X. 2014. Effects of UV-C treatment on inactivation of

Salmonella enterica and *Escherichia coli* O157:H7 on grape tomato surface and stem scars, microbial loads, and quality. Food Control. 44: 110-117.

[5] Luksiene Z, Buchovec I, Paskeviciute E. 2010. Inactivation of several strains of Listeria monocytogenes attached to the surface of packaging material by Na-Chlorophyllin-based photosensitization. Journal of Photochemistry and Photobiology B: Biology. 101: 326-331.

[6] Buchovec I, Paskeviciute E, Luksiene Z. 2010. Photosensitization-based inactivation of food pathogen Listeria monocytogenes in vitro and on the surface of packaging material. Journal of Photochemistry and Photobiology B: Biology. 99: 9-14.

[7] Asnaashari M, Safavi N. 2013. Disinfection of contaminated canals by different laser wavelengths, while performing root canal therapy. Journal of Lasers in Medical Sciences. 4: 8-16.

[8] Guffey JS, Payne W, Jones T, Martin K. 2013. Evidence of resistance development by Staphylococcus aureus to an in vitro, multiple stage application of 405 nm light from a supraluminous diode array. Photomedicine and Laser Surgery. 31: 179-182.

[9] Maden M, Görgül G, Sultan MN, Akça G, Er Ö. 2013. Determination of the effect of Nd:YAG laser irradiation through dentinal tubules on several oral pathogens. Lasers in Medical Science. 28: 281-286.

[10] Schoenly JE, Seka W, Featherstone JDB, Rechmann P. 2012. Near-UV laser treatment of extrinsic dental enamel stains. Lasers in Surgery and Medicine. 44: 339-345.

[11] Azar Daryany MK, Massudi R, Hosseini M. 2008. Photoinactivation of Escherichia coli and Saccharomyces cerevisiae suspended in phosphate-buffered saline-A using 266- and 355-nm pulsed ultraviolet light. Current Microbiology. 56: 423-428.

[12] Falkenstein Z. 2001. Development of an excimer UV light source system for water treatment. Ushio America.

[13] Brygo F, Dutouquet C, Le Guern F, Oltra R, Semerok A, Weulersse JM. 2006. Laser fluence, repetition rate and pulse duration effects on paint ablation. Applied Surface Science. 252: 2131-2138.

[14] Franz CMAP, Specht I, Cho G-S, Graef V, Stahl MR. 2009. UV-C-inactivation of microorganisms in naturally cloudy apple juice using novel inactivation equipment based on Dean Vortex technology. Food Control 20:1103-1107.

[15] Bandla S, Choudhary R, Watson DG, Haddock J. 2012. UV-C treatment of soymilk in coiled tube UV reactors for inactivation of Escherichia coli W1485 and Bacillus cereus endospores. LWT - Food Science and Technology. 46: 71-76.

[16] Gayán E, Monfort S, Álvarez I, Condón S. 2011. UV-C inactivation of Escherichia coli at different temperatures. Innovative Food Science & Emerging Technologies. 12: 531-541.

[17] Maktabi S, Watson I, Parton R. 2011. Synergistic effect of UV, laser and microwave radiation or conventional heating on E. coli and on some spoilage and pathogenic bacteria. Innovative Food Science & Emerging Technologies. 12: 129-134.

[18] Tamuri A, Bidin N, Daud Y. 2008. Quality of the beam produced a pulsed Nd: YAG laser. Laser physics. 18: 18-21.

[19] Artíguez ML, Lasagabaster A, Marañón IM D. 2011. Factors affecting microbial inactivation by Pulsed Light in a continuous flow-through unit for liquid products treatment. Procedia Food Science 1:786-791.

[20] Ramos-Villarroel AY, Aron-Maftei N, Martín-Belloso O, Soliva-Fortuny R. 2012. The role of pulsed light spectral distribution in the inactivation of Escherichia coli and Listeria innocua on fresh-cut mushrooms. Food Control. 24: 206-213.

[21] Muñoz A, Caminiti IM, Palgan I, Pataro G, Noci F, Morgan DJ, Cronin DA, Whyte P, Ferrari G, Lyng JG. 2012. Effects on Escherichia coli inactivation and quality attributes in apple juice treated by combinations of pulsed light and thermosonication. Food Research International. 45: 299-305.

[22] Yamauti M, Senawongse P, Hamakawa T, Otsuki M, Tagami J, Sato S, Sato Y, Eguchi T. 2003. Effect of pulse duration of Er: YAG laser on the dentin surface morphology. International Congress Series. 1248: 139-142.