INACTIVATION OF *Escherichia coli* AND *Listeria monocytogenes* WITH Nd:YAG LASER AND ITS HARMONICS

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To my wife, Dorcas Moses, and daughter, Bwalasom Moses, who endured a lot of inconveniences during the period of this study

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ABSTRACT

Ultraviolet (UV) lasers have been employed for many applications such as in the food industry, medicine and dentistry. 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 used. The objective of this study was to use a flash lamp-pumped passive Q-switched Nd:YAG laser for inactivation two pathogenic bacteria, namely *Escherichia coli* and *Listeria monocytogenes*. A 1064 nm pulsed infrared (IR) laser and its 2^{nd} and 3^{rd} harmonics were used to irradiate *Escherichia coli* at various values of fluence. A 350 nm continuous wave (CW) UV lamp was also used in the inactivation process for the purpose of comparison with the 3^{rd} harmonic (355 nm) pulsed laser. The result indicates that there is a statistical significant difference between mean $\log_{10}$ reductions for the three laser wavelengths. The mean $\log_{10}$ reductions for the 355 nm pulsed (UV) laser are higher than the corresponding mean $\log_{10}$ reductions for the CW UV light. A *t-test* conducted on the mean $\log_{10}$ reductions obtained for the pulsed UV laser and the CW UV light indicates that there is a significant difference between the two sets of mean $\log_{10}$ reductions. When *E. coli* and *L. monocytogenes* samples were irradiated with the pulsed UV laser at three different pulse frequencies, the result shows higher inactivation effect at higher pulse frequency than at lower pulse frequency. Statistical analysis, using two-way ANOVA, shows that the mean $\log_{10}$ reductions for the three pulsed frequencies were significantly different. However, no statistical significant difference was observed between mean $\log_{10}$ reductions obtained for treatment with the pulsed UV laser and the CW UV light on three different sample volumes of *E. coli* and *L. monocytogenes.*

ABSTRAK

Laser lampau ungu (UV) telah digunakan untuk pelbagai aplikasi seperti dalam industri makanan, perubatan dan pergigian. Penggunaan laser dan sistem denyutan cahaya lain untuk fototerapi dan disinfeksi mikrob lain adalah berdasarkan ciri-ciri spektrum dan konfigurasi cahaya yang digunakan. Objektif kajian ini adalah untuk menggunakan laser Nd:YAG suis-Q pasif dipam lampu kilat bagi menyahaktifkan dua bakteria patogenik, iaitu *Escherichia coli* dan *Listeria monocytogenes*. Laser denyutan inframerah (IR) 1064 nm dan harmoniknya yong ke-2 dan ke-3 telah digunakan untuk menyinari *Escherichia coli* pada pelbagai nilai dos tenaga. Lampu UV gelombang selanjar (CW) 350 nm juga digunakan dalam proses menyahaktif bagi tujuan perbandingan dengan denyutan laser harmonik ke-3 (355 nm). Keputusan menunjukkan bahawa terdapat perbezaan statistik yang signifikan antara pengurangan $\log_{10}$ purata bagi tiga panjang gelombang laser. Pengurangan $\log_{10}$ untuk 355 nm laser denyut (UV) adalah lebih tinggi daripada pengurangan $\log_{10}$ purata sepadan untuk gelombang cahaya UV CW. *Ujian-t* telah dijalankan ke atas pengurangan $\log_{10}$ purata diperoleh bagi laser denyut UV dan cahaya UV CW menunjukkan bahawa terdapat perbezaan yang signifikan antara kedua-dua set pengurangan $\log_{10}$ purata. Apabila sampel *E. coli* dan *L. monocytogenes* telah disinarkan dengan laser denyut UV pada tiga frekuensi denyutan yang berbeza, keputusan menunjukkan kesan menyahaktif lebih tinggi pada frekuensi denyut tinggi daripada frekuensi denyut lebih rendah. Analisis statistik, menggunakan dua-cara ANOVA, menunjukkan bahawa pengurangan $\log_{10}$ purata bagi tiga frekuensi denyutan adalah jauh berbeza. Walau bagaimanapun, tiada perbezaan statistik yang signifikan telah diperhatikan antara pengurangan $\log_{10}$ purata diperoleh bagi rawatan dengan laser denyut UV dan cahaya UV CW pada tiga jilid sampel yang berbeza *E. coli* dan *L. monocytogenes*.

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LIST OF ABBREVIATIONS

Abbreviation		Meaning
AC	-	Alternating Current
ANOVA	-	Analysis of Variance
CFU	-	Colony Forming Units
CW	-	Continuous Wave
CWUVaLi	-	Continuous Wave UV-A Light
DC	-	Direct Current
df	-	Degree of Freedom
DFG	-	Difference-Frequency Generation
DNA	-	Deoxyribonucleic Acid
DVD	-	Digital Versatile Disc
FWHM	-	Full-Width Half-Maximum
IPL	-	Intense Pulsed Light
IR	-	Infrared
LR	-	Log_{10} Reduction
M	-	Mean
MS	-	Mean Squared
NA	-	Nutrient Agar
NB	-	Nutrient Broth
PGLa	-	Pulsed Green Laser
PIRLa	-	Pulsed Infrared Laser
PK	-	Percentage Kill

PLS	-	Pulsed Light System
PUVaLa	-	Pulsed UV-A Laser
SE	-	Standard Error
SF	-	Survival Fraction
SFG	-	Sum-Frequency Generation
SHG	-	Second Harmonic Generation
SPC	-	Standard Plate Count
SS	-	Sum of Squared
STA	-	Spectrophotometric Turbidimetric Analysis
THG	-	Third Harmonic Generation
TPC	-	Total Plate Count
TSA	-	Tryptic Soy Agar
TVC	-	Total Viable Count
USFDA	-	United States Food and Drug Administration
UV	-	Ultraviolet
VPC	-	Viable Plate Count

LIST OF SYMBOLS

Symbol		Meaning
A	-	Area
c	-	Speed of light
C	-	Capacitance
D	-	Duty cycle
d_{eff}	-	Effective nonlinear coefficient
D_{F1}	-	Decimal reduction fluence
$\boldsymbol{E}$	-	Electric field
E_p	-	Pulse energy
F	-	Fluence
f, ν	-	Frequency
h	-	Planck constant
I	-	Intensity
L	-	Length
n	-	Refractive index
N	-	Number of the pulses
P	-	Power
P_{NL}	-	Non-linear polarization
R	-	Repetition rate
T	-	Time
V	-	Voltage
x_R	-	Rayleigh range
δ	-	Dephasing factor
Δk	-	Phase mismatch

η	-	Conversion efficiency
λ	-	Wavelength
ρ	-	Walk-off angle
τ	-	Pulse duration
χ^2	-	Second order susceptibility
ω	-	Angular frequency

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CHAPTER 1

INTRODUCTION

1.1 Background

Ever since the first laser was constructed by Theodore Maiman in 1960 (Koechner, 2006), studies aimed at exploring the nature and expanding the applicability of lasers has been continued by scientists across the globe. The ruby (or Maiman) laser was of the red visible light radiation (694.3 nm) with pulse-duration of the order of milliseconds, pulse energy of about 1 J and an average power of the order of kilowatts (Abramczyk, 2005). Progressively, other types of laser were developed and today we have gas lasers, liquid lasers, solid state lasers and semiconductor lasers, among others. Laser lights are now being produced for virtually the entire visible range of the electromagnetic spectrum (Figure 1.1) and even beyond (up to the x-ray region), with pulse-duration reaching the order of attosecond (10^{-18} s) and an attainable average peak power of zetawatts (10^{21} W) (Koechner, 2006; Milonni and Eberly, 2010).

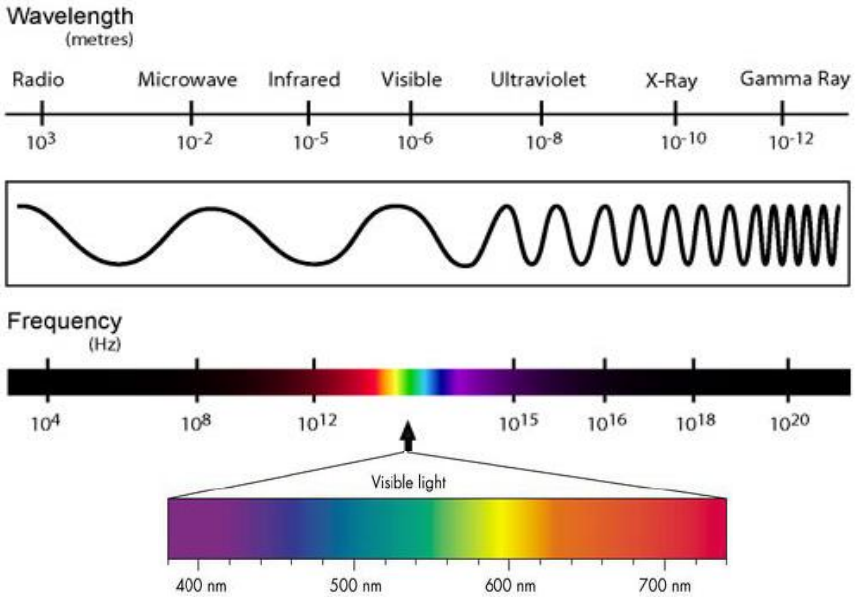

Figure 1.1 The electromagnetic spectrum (modified from Shapley (2015))

The original energy or power of laser lights obtained from an oscillator is usually too low for most applications. Therefore, it needs further amplification for it to be useful for such applications. Output pulse energies from femtosecond lasers typically do not exceed a few nanojoules, and peak powers of megawatts (Abramczyk, 2005). Hence, laser or pulse amplifiers are employed to raise output power to useful levels required for various applications. In seeking methods to shorten pulses and to increase peak powers and peak intensities on intended targets, researchers often trade-off various design and input parameters in order to obtain an optimize state of operation for a particular application. With pre-determined design parameters, the next option for optimization is for a researcher to manipulate the input parameters.

Applications of lasers vary as widely as their range of the wavelengths, pulse-width or peak power. Applications are found in industries (e.g. laser machining, laser

cutting or drilling), medicine (e.g. dentistry, neural network, dermatology, and surgery), home or office appliances (e.g. laser printer, laser scanner and laser pointer), research laboratories (e.g. spectrometry and diffraction experiments), military (e.g. laser guided smart bombs and laser blinders), communication (e.g. laser fibers), etc (Diels and Arissian, 2011). Lasers are also important parts of common devices such as bar-code scanners used in supermarkets, compact disc systems, and digital versatile disc (DVD) players.

In the health sector, laser irradiation has been adopted as a technique for curing or controlling various bacteria-related diseases in medicine and dentistry (Andrade *et al.*, 2008; Franzen *et al.*, 2011; Nandakumar *et al.*, 2003; Schoenly *et al.*, 2012). The use of lasers in these and other health-related fields depends on the absorption ability of interacting targets or media. The power and wavelength of beam, in addition to the absorption properties of the interacting biological tissue determine the depth of penetration of a laser beam (Abramczyk, 2005; Diels and Arissian, 2011). For bacteria and similar microbes, absorption of radiation is said to depend on the wavelength of the incident radiation, with the ultraviolet (UV) region providing the most effective absorption when compared to other regions of the electromagnetic spectrum.

The UV laser finds applications in diverse fields such as in material processing (Ya *et al.*, 2009b; Zhai *et al.*, 2013), lithography (Ya, *et al.*, 2009b; Yang *et al.*, 2009), various spectroscopic techniques (Franzke *et al.*, 1998; Suzuki *et al.*, 2008; Ya, *et al.*, 2009b; Yang, *et al.*, 2009), medicine (Ya, *et al.*, 2009b), and others. One area in which lasers are put into applications that are more closely related to human systems is in the interaction of lasers with bio-samples. 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.

The UV spectrum is the portion of the electromagnetic spectrum between visible light and X-rays, with wavelength ranging from about 10 nm to about 400

nm. Depending on its spectral properties and applications, the UV spectrum is further subdivided into four regions as shown in Figure 1.2. Another classification mode of the UV spectrum refers to the longer wavelengths greater than 200 nm as near-UV while those below 200 nm are called far-UV.

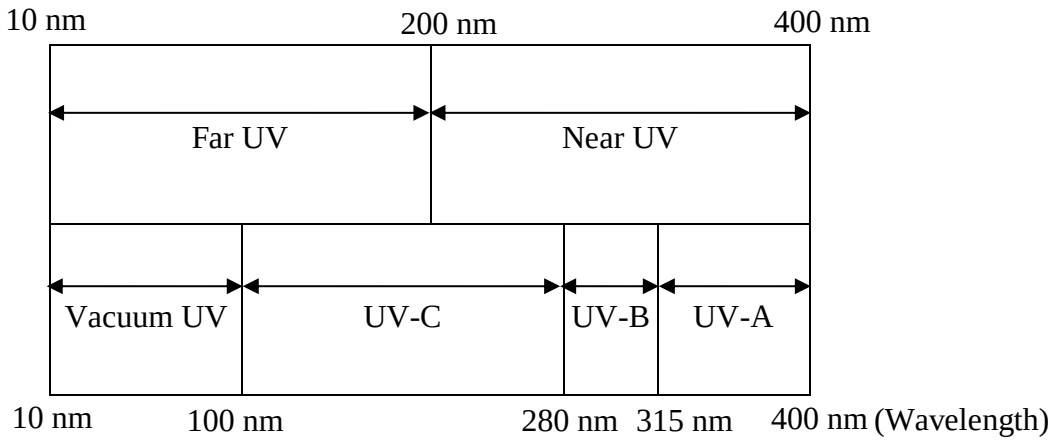

Figure 1.2 Divisions of the UV portion of the electromagnetic spectrum showing wavelengths in nm

Although the broad spectrum from infrared to UV can be used for microbial inactivation, most of the germicidal effects have been attributed to the UV region, particularly the UV-C. Several documented studies (Daryany *et al.*, 2009; Bohrerova *et al.*, 2008; Farrell *et al.*, 2011; Marquenie *et al.*, 2003; Matafonova and Batoev, 2012; Villarroel *et al.*, 2012a) indicate that UV light can be used for various decontamination and sterilization processes. Specifically, pulsed UV light has been reported to be more efficient in inactivation of bacteria and other microbial organisms than continuous wave UV light (Bohrerova, *et al.*, 2008; Cheigh *et al.*, 2012; Farrell, *et al.*, 2011; Hierro *et al.*, 2011). The transmission electron micrographs of intense pulsed light (IPL) and UV-C-induced cell damage observed by Cheigh, *et al.* (2012) indicate that bacterial cell structures were destroyed by IPL treatment but not by UV-C treatment. Lethal effects of pulsed light on microbes is said to also depend on the fluence incident on the sample, the composition of the

emitted light spectrum and the distance of the sample from the light source (Artíguez *et al.*, 2011). Other factors that affect microbial inactivation in liquid samples are thickness, colour, opacity, viscosity, product flow conditions and presence of particulate material (Pataro *et al.*, 2011).

By the nature of pulsed laser, it should have more power than an ordinary continuous wave light because of its coherence and directionality. Hence, it is premised that the interaction of pulsed UV laser with bacteria should have more lethal effect than continuous wave UV light of similar output configuration. However, it was stated (López *et al.*, 2007) that there are no independent experimental reports to confirm some claims of pulse light having more penetrating power than continuous wave light. Studying the effects of parameters of pulsed laser, such as, fluence, spectral distribution and pulse frequency on inactivation of bacteria in comparison to result obtained for continuous wave UV light could verify the veracity of this supposition. So far, most of the studies conducted using laser for either direct (Dinu *et al.*, 2002; Nandakumar, *et al.*, 2003) or indirect (Andrade, *et al.*, 2008; Baudelet *et al.*, 2009; Franzen, *et al.*, 2011; Schoenly, *et al.*, 2012) microbial inactivation were aimed at applications in dentistry and medicine, with emphasis on roles played by laser wavelength, pulse power and pulse width.

Apart from wavelength, other parameters of pulsed laser, like pulse width and pulse frequency, could as well play some roles in bacteria inactivation process for a particular wavelength of a pulsed laser. This may have some implications for optimization of lasers or other pulsed light systems to maximise performance. This study is designed to further explore the potential of pulsed UV laser light as a means of inactivation of bacteria in liquid medium by looking into roles played by laser fluence and pulse frequency. The two pathogenic bacteria chosen for this study have been known to cause either food poisoning or food spoilage. Certain strains of *Escherichia coli* are known to cause diarrhoea, resulting from intake of contaminated water or food. In addition, *Listeria monocytogenes* is the cause of listeriosis, also resulting from eating contaminated food. The food poisoning resulting from the actions of these bacteria could lead to illnesses of epidemic proportion. The result of

food spoilage could lead to economic losses from both production companies and retailers.

1.2 Statement of problem

Non-thermal methods of inactivating bacteria are being sought because such methods leave less damaging effects on processed materials such as food and surgical materials. Heat treatment of foods at high temperature can affect texture, flavour and appearance of the product whereas less severe thermal treatment may result to inadequate decontamination (Maktabi *et al.*, 2011). Pasteurization of food materials and other methods of thermal sterilization also results into rise in temperature in the bulk of the material, which may be undesirable. Using chemical disinfection is effective, but it may also leave behind residual by-products, which may be toxic or be mutagenic (Daryany *et al.*, 2008). Previous studies conducted indicate the potentiality of pulsed light, especially of the UV type (Bohrerova, *et al.*, 2008; Cheigh, *et al.*, 2012; Farrell, *et al.*, 2011; Hierro, *et al.*, 2011), for inactivation of bacteria. However, a basic limitation in the use of pulsed light is in the depth of penetration of light in the materials being processed, especially coloured liquids. Depth of penetration is usually limited by the power of the pulsed light in addition to the level of transparency of the sample material being processed. Therefore, pulsed UV lasers, which are narrow-banded and provide high-intensity emissions, with desirable penetration depth in water and other fluids may be potentially more effective (Daryany, *et al.*, 2008). This study was designed to look into the effectiveness of pulsed UV laser in decontamination and sterilization, especially with regards to any possible roles by the laser wavelength, pulse frequency and sample depth, in liquid sample. Determination of prime values for laser parameters, at which inactivation is optimal, may lead to efficient use of laser systems for the purpose of bacteria inactivation in liquid food items.

1.3 Objectives

The main objective of the study was to use a flash lamp-pumped passive Q-switched Nd:YAG laser for inactivation of selected pathogenic bacteria. This main objective was pursued through the following sub-objectives:

(i) To modulate the output of the Nd:YAG laser from IR to its 3rd harmonic in the UV region by using non-linear crystals.

(ii) To examine the effectiveness of the 3rd harmonic of the Nd:YAG laser in inactivation of two selected pathogenic bacteria (*Escherichia coli* and *Listeria monocytogenes*).

(iii) To observe the effect of pulse frequency (or pulse repetition rate) of the 3rd harmonic of the Nd:YAG laser in the process of the bacteria inactivation.

(iv) To observe the effect of sample volume in the process of inactivation of bacteria with the 3rd harmonic of the Nd:YAG laser.

1.4 Scope of study

A flash lamp-pump Q-switched Nd:YAG laser with pulse duration of 6 ns was employed for the study. The pulse energy of the laser can be varied in the range 0 – 1000 mJ. The pulse repetition is in the range of 1 – 5 Hz, in steps 1 Hz. The laser wavelengths used for the study are 1064 nm, 532 nm and 355 nm. A 350 nm continuous wave UV lamp, rated 4 W, was also used for the study for the purpose of

comparison. Two species of pathogenic bacteria, *Escherichia coli* (ATCC 11775) and *Listeria monocytogenes* (ATCC 7645), obtained from Institute of Bio-Information Technology, Universiti Selangor, were the samples used for the study. The bacteria samples used are common pathogenic bacteria associated with food poisoning. All experiments were conducted under ambient conditions of room temperature and atmospheric pressure.

1.5 Significance of study

Study of the interaction of lasers with microbial organisms (particularly the pathogenic types) leads to applications such as food preservation, food safety, decontamination of immediate environment, sterilization of equipment, etc; which are preventive rather than curative. Hence, possible application from this study is in the development of efficient point-of-use UV laser devices for decontamination and sterilization for use at homes, hospitals, industries and work places.

1.6 Original contributions of this study

The following are some of the academic contributions derivable from this study:

- The study compares the effects of three pulsed laser wavelengths in inactivation of *Escherichia coli* and it was found that 355 nm pulsed laser was more efficient in the inactivation process than 532 nm and 1064 nm pulsed lasers.

- The effectiveness of inactivation of the 355 nm pulsed laser was compared with that of a 350 nm continuous wave UV light of close output configuration and it was found that the 355 nm laser was more efficient in the inactivation of *Escherichia coli*.

- The study also reveals that inactivation of both *Escherichia coli* and *Listeria monocytogenes* is more efficient at higher laser pulse frequency than at lower frequency.

1.7 Thesis structure and organization

This thesis is composed of five chapters. Chapter 1 gives a general background of the study followed by problem statement, objectives of study, scope of study, significance of study and contributions of study, in that order. In chapter 2, literature related to the study was reviewed. This includes review of some past studies done on harmonic generation using non-linear optics and microbial inactivation with pulsed light. Relevant formulae for the study are also highlighted. Chapter 3 describes the methodology employed for the study which includes list of materials used, a brief description of experimental procedures and mode of data analysis. Details of the experimental results obtained as well as analysis of results for harmonic generation by non-linear optical conversion and interaction of laser with samples studied are presented in chapter 4. Chapter 5 is the conclusion and recommendations.

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