

EFFECTS OF PULSED ULTRAVIOLET LIGHT ON MICROFLORA AND
TEMPERATURE OF PROPYLENE GLYCOL COOLING MEDIUM

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF
FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE
OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2013

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To my family and friends

ACKNOWLEDGEMENTS

I would like to gratefully acknowledge my major advisor Dr. Wade Yang for providing me with this opportunity to be a part of UF FSHN family, his mentorship with patience and advice in my master's degree. I'm also thankful to Dr. Anita Wright and Dr. Melanie J. Correll for serving on my master's degree committee and taking their precious time to advise me when I met with them.

I would like to say that a great advantage of being at University of Florida is to have met my great lab group members, Akshay K. Anugu, Bhaskar Janve, Braulio Macias, Dr. Cheryl R. Rock, Kelsey Guo, Senem Guner, Tara Faidhalla and Xingyu Zhao. I thank you all for your splendid friendship and mental support. I also would like to thank Dr. Hale Z. Toklu for her contribution in the statistical analysis of my data.

Last but not least, I would like to express my sincere appreciation to my family for their love, encouragement, and support. They always believed in me. I would also like to thank my great friend Busra Koksall, Caglar Doguer and my uncles Ahmet Kada and Hakan Kada for their mental support in my good or bad times.

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LIST OF ABBREVIATIONS

AB	Aerobic Bacteria
ADP	Adenosine Di-phosphate
AOAC	Association of Official Analytical Chemists
APC	Aerobic Plate Count
APHA	American Public Health Association
ATP	Adenosine Tri-phosphate
CFU	Colony Forming Unit
DOW	The Dow Chemical Company
DPG	Di-propylene Glycol
FDA	Food Drug Administration
GC	Guanine and Cytosine
LAB	Lactic Acid Bacteria
MRS	De Man, Rogosa and Sharpe
MPG	Mono-propylene Glycol
PG	Propylene Glycol
PO	Propylene Oxide
PL	Pulsed Ultraviolet Light
RNA	Ribonucleic Acid
TPG	Tri-propylene Glycol
UV	Ultraviolet

Abstract of Thesis Presented to the Graduate School of the University of Florida in
Partial Fulfillment of the Requirements for the Degree of Master of Science

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August 2013

Chair: Wade Yang

Major: Food Science and Human Nutrition

As a nonthermal inactivation technology, PL has gained significant interest in the food industry. Due to its decontamination effect on different food products, PL may offer a potential disinfection tool for the microflora control of PG, a refrigeration medium widely used in the food industry. The objective of this study was to examine the effect of PL on inactivating the microflora present in PG and determine the sample temperature profile during PL illumination. Propylene Glycol samples of 5, 10, and 15 mL in aluminum dishes were treated in a Xenon PL processor (Model RC847) at a distance of 6 cm from the quartz window for 5, 10, 15, 20, and 25 s. Thickness of the sample was 2.5, 5.0 and 7.6 mm for 5, 10, and 15 mL of PG, respectively. The initial total and lactic acid bacteria were in the concentration of 6.48-log_{10} and 3.88-log_{10} , respectively. PL illumination for 15 s for 5 mL PG and 20 s for 10 mL glycol resulted in complete sterilization, whereas 20 s illumination for 15 mL glycol resulted in 5.30-log_{10} reduction in APC. Complete sterilization, 5.70-log_{10} and 4.11-log_{10} reduction in APC was obtained for 5, 10, and 15 mL samples, respectively, with 15 s illuminations. Lactic acid bacteria were totally inactivated (detection limit <10 CFU/mL) for all the samples treated for 10 s. The initial temperature was 30.6°C and an increase of 22.3, 16.5, and 14.8°C was

observed respectively for 5, 10, and 15 mL of PG sample treated for 20 s at 6 cm distance from quartz window. Sample temperature and reduction in microflora decreased with increased sample thickness, as the penetration depth of the sample was a limiting factor for PL illumination. This study confirmed that the efficacy of PL in inactivating microflora in PG. Hence, PL could be used as a novice technology for the food industry in inactivating the microflora present in PG.

CHAPTER 1 BACKGROUND

1.1 Introduction

Ground source heat pump systems, which can utilize the energy of the earth's shallow geothermal sources for heating and cooling, has been significantly used in industry, along with different types of organic anti-freeze substances such as ethylene glycol (EG), propylene glycol (PG) and betaine in the system as a coolant (Klotzbucher and others 2007). Additionally, PG ($C_3H_8O_2$) is increasingly used for therapeutic, cosmetic purposes and food applications in the industry. Propylene glycol may exist in the following forms: mono- (MPG), di- (DPG), and tri-propylene glycols (TPG) (DOW 2005). The glycols are obtained from the hydrolysis of propylene oxide (PO)(C_3H_6O), in which MPG is the most popular with the common commercial name of 1,2-propanediol. Furthermore, PG is a colorless, nearly odorless and tasteless viscous liquid (Shigeno and Nakahara 1991). It may be used as an effective humectant, preservative and stabilizer in diverse food and cosmetic products such as pet food, bakery goods, food flavorings, salad dressing and shaving cream (DOW 2012). The Food and Drug Administration (FDA) allows PG to be used in foods in the following percentages: 2.5% in frozen dairy products, 5% in alcoholic beverages, 5% nuts and nut products, 24% in confections and frostings, 97% in seasonings and flavorings (FDA 2012).

Moreover, United States Pharmacopeia (USP) recommended the use of PG in chiller systems and also approved it for food applications and for high quality food processing.

There are several problems related to different sources for heating and cooling systems. The most major one is groundwater, which is a potential contaminant for

PG. As previously alluded to, addition of other solvents such as water to PG during circulation in the chilling and freezing systems may cause contamination problems (Dentinger and others 1995). Moreover, improper handling and management practices could also result in the contamination of PG. Consequently, these practices may reduce the microbial safety, overall quality and also cause biodegradation of PG (Aas and others 1993). Although PG possesses antibacterial and antifungal properties (Kinnunen and other 1991), it may lose these properties because of oxidation due to chemical and microbial action. That is why food preservation techniques can play a critical role for modern mass food production and distribution besides handling systems. As such, different preservation technologies have been developed and adopted successfully in food industry.

There are several technologies such as, power ultrasound (PU), irradiation, microwave, pulsed electric field (PEF), magnetic field (MF), high pressure processing (HPP), and ohmic heating (OH) treatments available for using in industry to inactivate microorganisms (pathogenic or spoilage) (FDA 2012c). However, there are many limitations affiliated with the aforementioned technologies and consequently, there is continual need to develop novel preservation techniques to improve efficiency, minimize cost, and achieve minimal quality changes to the product. Such limitations may included the following: Attenuation of ultrasonic intensity based on the medium, inactivation of thermo-resistant microorganisms in HPP (cold sterilization) and contamination of the medium with metal deposits from the degradation and corrosion of electrodes used in OH systems.

By the beginning of nineteenth century, ultraviolet (UV) light has been used as a novel technology for bactericidal inactivation (Demirci and others 2008). However, pulsed UV light has been proven to be more efficient than other commercial techniques such as steam, microwave on the inactivation of pathogens and decontamination of food surfaces and packing material. The use of PL has achieved high levels of microbial inactivation on relatively simple surfaces, while resulting in only 1-3 log₁₀ reductions on complex surfaces such as meats (Demirci and others 2008). Other applications of PL include the pasteurization of milk and mitigation of food allergens (Demirci and others 2008). The mechanism of PL light produces three major effects, which are the following: Photo-chemical, photo-thermal and photo-physical (Krishnamurthy and others 2010). The main mechanism of the PL treatment on microbial inactivation is attributed to the photochemical effect, in which the structural changes in DNA of most of microorganisms are observed. DNAs are converted into thymine dimers (Krishnamurthy and others 2008), thus inhibiting the microorganism from DNA transcription and replication and leading to cell death (Miller and others 1999). The degree of microbial inactivation exerted by PL depends also on food composition attributed to macromolecules such as: carbohydrates, water, protein and fats. Krishnamurthy and others (2004) reported that PL is more suitable for solids surfaces than liquids (e.g., wine, fresh juices) in which the process differs as affected by solids concentration closely related to absorbance.

1.2 Justification of Study

As previously mentioned, PL is a non-thermal technology gaining commercial interest. It has been proven to be effective in inactivating microorganisms in several food products and liquids, and hence is expected to exhibit similar effect on microflora present in PG. The significance and applications of PG in the food industry provides

premise to evaluate the effects of PL on inactivation of microorganism. Additionally, there is no published data regarding the effect of PL on the microflora in PG as well as its effects on quality. It is hypothesized in this study that PL exposure may have an equally synergistic effect on inactivation of bacteria and microflora of PG.

1.3 Overall Objective

The overall objective of this study is to investigate the efficacy of PL on the microflora (lactic acid bacteria and aerobic plate count) of PG.

1.4 Specific Objectives

Listed below are the specific objectives of this study, which will subsequently appear in their respective chapters.

The specific objectives of this research are to:

1. Determine the temperature profile of the PG during PL illumination at 6 and 9 cm distance from quartz window
2. Determine thermal and/or non-thermal effect of PL on inactivation of APC in PG at the same sample thickness with different distances from quartz window
3. Determine the efficiency of PL treatment to inactivate LAB in microflora of PG

CHAPTER 2 REVIEW OF LITERATURE

2.1 Propylene Glycol (PG)

Propylene glycol (PG) is an organic compound with formula $C_3H_8O_2$, which is present in numerous forms including mono- (MPG), di- (DPG), and tripropylene glycols (TPG) (Charles and John 2002). The most popular PG is referred to as 1,2-propanediol and also known as 1,2-propylene glycol, 1,2-dihydroxypropane, methylene glycol, and methyl glycol. The chemical structure of PG is simple, in which two-hydroxyl groups characterize it as a glycol (Harris 1992). With both a primary and a secondary hydroxyl, the 1,2-propanediol (MPG) is a difunctional alcohol (Parker and Issaacs 1959). Therefore, the solubility characteristics and other properties of glycols tend to be between simple alcohols and glycerin (Ruddick 1972). Table 2-1 shows some physical properties of PG.

2.1.1 Production of Propylene Glycol

In the industry, PG is synthesized from the hydrolysis of PO (C_3H_6O , PO). There are two different hydrolysis methods for PG. First occurs in the presence of ion exchange resin, and the other occurs in the presence of a small amount of sulfuric acid. Both reactions are non-catalytic under high pressure in the range of 1200 psi to 1600 psi and high temperature at 200 or 220°C or with a catalytic reaction at lower temperature such as 150 to 180°C (DOW 2012). After the production process, PG consists of 20 % 1,2-propanediol, 1,5 DPG and a minute amounts of other PG. Moreover, PG can be obtained from hydrolyses of glycerol and biodiesel byproduct (Shigeno and Nakahara 1991). Additionally, the subsequent formation of dipropylene

(DPG) and tripropylene (TPG) glycol is obtained by the same process involving the production of PG.

2.1.2 Applications of Propylene Glycol

Propylene glycol can be used as an effective humectant, preservative and stabilizer, especially in or food products (ice cream, wine, pet foods) or personal care products such as shampoo, conditioner and soap (DOW 2012). Additionally, PG is listed as a direct additive for particular foods in the regulation, and sorted as generally recognized as safe (GRAS) by Food and Drug Administration (FDA). The PG can be used for direct and indirect food additive applications such as antioxidant and emulsifier to give food some physical and technical attributes (DOW 2012).

2.1.3 Propylene Glycol as a Coolant

A coolant is a fluid, which prevents overheating and provides the transfer of heat between product and heating source. Coolants can be found in different forms such as liquid, gas or solids (Martin and Murphy 1994). Propylene glycol also acts as a coolant that keeps products from melting in heat and/or freezing when it is cold to enhance penetration, as previously introduced in Chapter 1 (DOW 2005). Because of some reasons such as consequently cooling of liquids, there may be either crystal or ice formation causing the fluid to become viscous and decreasing the flow rate. Propylene glycol does not have any sharp freezing points. Because of that, it can be mixed with other liquids foods such as wine and beer to prevent super-cooling effects, which may initiate the formation of ice and crystals (DOW 2012).

2.1.4 Toxicology of Propylene Glycol

All glycols have a lower degree of toxicity for human health and desirable formulation properties; thus, it has been a significant ingredient for different application

areas such as food industry and cosmetics (DOW 2012). Additionally, it is considered to be biodegradable, and hence it will not remain as a chemical remnant, and it can be used for aerobic and anaerobic conditions as a source of carbon (DOW 2012).

Moreover, an animal study showed that a mixture of PG and stearic acid enhanced the in- vitro permeability of nimodipine through rat skin. Nimodipine is a calcium channel blocker with vasodilating properties, which may be used as an anti-hypertensive drug (FDA 2012). Also, it has been shown that a mixture containing PG enhanced the absorption of verapamil, which is another calcium channel blocker (Breslin and others 1996).

2.1.5 Biodegradation of Glycols Under Oxidic Conditions

The PG and EG are widely used as a carbon and energy source among aerobic microorganisms. Some bacteria groups have ability of degrading PG and EG under oxidic conditions (Klotzbucher and others 2007). Willetts (1979) reported that the degradation of PG is based on metabolic pathways and proceeds via lactaldehyde and pyruvate. In the next steps, pyruvate is metabolized to acetyl-CoA, which is oxidized to CO₂ in the tricarboxylic acid cycle. As regards to this information, degradation of PG may occur without any accumulation of toxic and persistent organic intermediates under oxidic conditions (Klotzbucher and others 2007).

There is not sufficient information about the kinetics of PG for aerobic degradation in groundwater. However, rapid aerobic biodegradation of PG was investigated in studies conducted with sewage-sludge and soil samples. For instance, Klecka (1993) investigated that degradation of PG in soil at concentration of 6000 ppm was changed at different conditions like an average rate of ~2 ppm/day at 2°C, ~27 ppm/day at 8°C and ~93 ppm/day at 25°C. The rate of degradation of PG and EG in

aquifers are lower than soils owing to microbial population densities like in oligotrophic systems. Further studies still need to be quantified such degradation rates.

2.1.6 Propylene Glycol Degradation by Pure Cultures of Anaerobic Bacteria

Diverse pure bacterial strains and enrichment cultures are capable of degrading PG anaerobically. These types of microorganism were isolated from various habitats, high nutrient capacity of sewage sludge, wastewater (Dwyer and Tiedje 1983; Obradors 1988), also sediments of an oligotrophic lake (Sass and Cypionka 2004). Anaerobic biodegradation of PG takes place in most anoxic environments. Gaston and Stadtman (1963); and Toraya (1979) reported that fermenting bacteria were able to degrade EG and PG to acetate and propionate, and ethanol and propanol (Figure 2-1).

Eichler and Schink (1985) investigated that EG and PG were degraded to their acids like acetate as sole organic products via production of CH_4 or H_2 . Thus, evidence of the corresponding aldehydes such as acetaldehyde and propionaldehyde were investigated as degradation products (Toraya 1979; Eichler and Schink 1985). In addition to fermenting, EG and PG were degraded by sulfate-reducing bacteria (SRB) (Klotzbucher and others 2007). There is not known data about the complete oxidation of the glycols to CO_2 by SBR. Sass and Cypionka (2004) reported that most SBR have capability to use other electron acceptors, thus, anaerobic degradation of glycols may involve reduction of nitrate, iron (III) or manganese (IV) as potential electron acceptors.

2.1.7 Anaerobic Degradation of EG and PG by Mixed Microbial Populations

Klotzbucher and others (2007) demonstrated that under anoxic conditions in sewage sludge and in soil, PG maybe degraded by mixed microbial populations. Dwyer and Tiedje (1983), and Veltman (1998) reported that EG and PG were initially degraded by fermentation in sludge to the same amount of acids and alcohols. In further

degradation methane and CO₂ were obtained. During the microbial degradation of PG in soil columns, Fe³⁺ and Mn⁴⁺ oxides were poorly used (Jaesche and others 2006). The reduction of iron and manganese is based on two sequential processes, which are directly oxidation of PG and oxidation of fermentation product (propanol and propionate) of PG. Therefore, it is likely PG degradation can depend on aerobic microorganism reactions.

2.2 Pulsed UV Light

Pulsed UV light (PL) has been used as an emerging processing technology in many different areas of the food industry, most commonly to decontaminate food products and surfaces. Pulsed UV light treatment involves the use of radiation, which comes from the ultraviolet region of the electromagnetic spectrum, which aids in product disinfection. Additionally, PL, which is generated by lamps, has a wide spectrum ranging from UV (10 nm - 390 nm) to the infrared (750nm-1mm) (Demirci and others 2008). During the generation of PL light, electrical energy is stored in a capacitor, and the energy is released as short pulses in several times per second with pulse lasting between 100 ns and 2 ms (Demirci and others 2008). Thus, the PL light has higher energy than continuous ultraviolet light (UV) system (Xenon 2003). Pulsed UV light has intensity 20,000 times more than the sunlight and the germicidal effect of PL seems below 400 nm (Dunn and others 1995). There are three effects exhibited by PL light. These are photo-chemical, photo-thermal and photo-physical, and contribute to the inactivation mechanisms of PL light and are described in more detail in the sections below.

2.2.1 Photo-Chemical Mechanism

Any chemical reaction is caused by absorption of light (including visible, ultraviolet, and infrared). The light excites atoms and molecules (shifts some of their electrons to a higher energy level) and thus makes them more reactive. In comparison to ordinary reactions using thermal energy alone, photochemical reactions can follow different routes and are more likely to produce free radicals, which can trigger and sustain chain reactions. The germicidal effect of PL light includes photochemical damage to deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) of microorganisms. In the wavelength of 240 to 280 nm, the most important absorbers of light are known microorganism nucleic acids (Krishnamurthy and others 2010). Any damage to either of these substances (DNA and RNA) can sterilize the organisms because they carry necessary genetic information for reproduction. Pulsed ultraviolet (PL) does not inactivate microorganisms by chemical reaction but radiation of light can cause a photochemical reaction in the organisms DNA and RNA (Murov 1973).

The mechanism is based on changing the structure of DNA and performing chemical modifications. The photochemical transformation of pyrimidine, which is based on DNA of bacteria, viruses, and other pathogens, underlies the germicidal effects of UV light (Giese and Darby 2000). Such modifications to DNA bonds cause unzipping implicated in replication, and hence the organisms are unable to proliferate. This is as a result of mutations; abnormal gene transcriptions and impaired replications occur, and then cause death (Krishnamurthy and others 2010). Some experiments showed that after PL treatment there is not any enzymatic repair of DNA. It may be considered that the same effect can inactivate the DNA repair system (Dunn and others 1995; McDonald and others 2002; Smith and others 2002).

Although the UV light shows germicidal properties between 100-280 nm, according to some previous studies the shorter wavelengths are more efficient on the inactivation of microorganism than longer wavelengths because of their higher energy levels (Rowan and others 1999). UV light part of the lamp can provide almost 6-9 log₁₀ reduction effects on the microorganism, and UV-C is considerable for 50% of all effects. Furthermore, using a flash lamp for UV-C fluxes can provide a sufficient inactivation for all microorganisms (Wekhof 2000). When light pulses combine with UV-C light there is a synergistic inactivation of conidia of *Botrytis cinerea* and *Monilia frugigena* fungi (Marquenie and others 2003b). Pulsed ultraviolet (PL) light has a germicidal effect between 230-300 nm on *Escherichia coli*. Although PL shows a maximum effect at 270 nm there is no inactivation, which can be observed above 300 nm (Krishnamurthy and others 2010).

After PL treatment there are some DNA damage such as the formation of single strand breaks and pyrimidine dimers, which have been induced in, yeast cells. But, the inactivation effect of continuous UV light at 254 nm is slightly greater than PL does although killing level of treated yeast cells is almost the same in both cases (Takeshita and others 2003).

2.2.2 Photo-Thermal Mechanism

Photo-thermal effect is a phenomenon associated with electromagnetic radiation. It is produced by photo excitation resulting in the production of thermal energy (heat). This mechanism is based on the differences in the heating rates of bacteria and the surrounding media. It can cause damage to bacterial cells because of absorption of energy of light by bacteria cells. The intensity in destruction of microbes via pulsed could be partly due to the photo-thermal effect. It was proposed that energy exceeding

0.5J-cm⁻² could enhance disinfection by bacterial disruption during short time overheating resulting from absorption of all UV light from a flash lamp (Wekhof 2000). In this process, the water content of bacteria vaporizes and leads to bacterial disruption. Thus ultimate inactivation occurs (Takeshita and others 2003). With *Aspergillus niger* spores, the overheating due to internal explosion resulted in “evacuation” of the cell contents during the light pulse. During the PL treatment, photo-thermal effects are enhanced via biocidal action. Since proteins are heat sensitive, it can be supposed that high doses would result in cell death (Cover and others 2001).

2.2.3 Photo-Physical Mechanism

Photo-physical mechanism of PL is based on the structural damage to bacterial cells because of disturbances of intermittent high-energy pulses. There is much research, which illustrates this mechanism. For example, damage to proteins and cell membranes during exposure to PL are definitely correlated with destruction of nucleic acid. In a study for inactivation of *Saccharomyces cerevisiae* cells by pulsed light and classic UV was observed (Takeshita and others 2003). It was found that concentration of eluted protein from yeast cells after treatment with PL was higher than under UV treatment. This indicates that there was potential cell membrane damage. The electron micrograph of yeast cells revealed changes in cell structure including large vacuoles, cell membrane distortion, and change to circular shape (Wang and others 2005). On the contrary, the UV treated cells were almost similar to the non-treated cells (Takeshita and others 2003.). Electron micrograph of treated *A. niger* spores showed ruptured in the top surface of the spore. In the treatment of light pulse, there were collapsed and deformed spores with deep craters observed (Wekhof and others 2001). In UV

treatment of most *Bacillus subtilis* spores had become disintegrated or lost its shape (Wekhof and others 2001).

2.2.4 Microbial Inactivation by Pulsed UV Light

Although PL obtains energy from UV, the visible and infrared regions contribute to the inactivation mechanism with UV being the predominant cause. However, UV light of the wide spectrum is known as the predominate cause of inactivation. Moreover, PL may cause different damage to cells such as cell wall breakage, cellular membrane structure, and also induce leakage of the cell content in *S. aureus* (Krishnamurthy and others 2010). On the other hand, a small increase of 2-3 °C in the temperature was seen as negligible during the treatment, and hence PL can induce some shocking effect on the cell wall of bacteria (Krishnamurthy 2006). In addition to shocking effect there is a thermal stress on the bacteria cell because of exposure to PL, especially at higher density of light (0.5 J/cm^2), and it might lead to cell rupture. Absorption of energy from light depends on the characteristic of the bacteria and the surrounding medium; therefore, bacteria may also be overheated in different amounts (Fine and Gervais 2004). There might be some changes, which may lead to membrane destruction in the cells such as steam flow and vaporization. There are a lot of researchers, who have proven these approaches. For instance, according to Gomez-Lopez (2005), different microorganisms were inoculated on agar media to represent high decontamination effects, ranging from 1.2 to 5.9 \log_{10} of intense pulsed light with a pulse duration of 30 μs and pulse intensity of 7 J (Gomez-Lopez and others 2005a). Another study chose *E. coli* and *S. enteritidis* to study inactivation effects of pulsed light. 5 to 100 pulses with a range of 200-530 nm were applied to bacterial suspension contained in petri dishes. The results show a 9- \log_{10} order reduction after treatment with 100 pulses of 9 J for *E.*

coli, and 100 pulses of 4.5 J for each pulse produced a 7- \log_{10} reduction, and merely 0.5 \log_{10} reduction were observed after 5 pulses. Results indicate that high energy per pulse is heavily responsible for this mechanism. Low reduction rate in the beginning of the first few pulses is possibly due to high cell population, that is approximately $1.3 \times 10^9 \text{CFU}\cdot\text{mL}^{-1}$, which causes the intensity to shift off through 3.28 mm depth of the sample (Ghasemi and others 2003).

Results show a 6 \log_{10} reduction in *Listeria monocytogenes*, *E. coli* O157:H7, *S. enteritidis*, *P. aeruginosa*, *B. cereus*, and *S. aureus*. These pathogens were inoculated on agar plates using 200 pulses of high-UV light. When low UV-light was used, there was only 1-2- \log_{10} reduction. Pulsed light source was also less effective than higher UV intensity, which makes the UV part of spectrum to be solely responsible for microbial deactivation (Rowan and others 1999). Other results show that 64 light pulses (spectral range of 200-530 nm) of 1 μs duration and 3J intensity are required to inactivate and reduce *E. coli* O157:H7 and *L. monocytogenes* populations by 2-4- \log_{10} reductions. This reduction is increased to 7 and 6 by increasing the pulse rate of 512 (Macgregor and others 1998). 7-8 \log_{10} reduction can be achieved for *S. aureus* in both suspended cultures and agar seeded cells, which were treated for 5 s by pulsed UV light at 5.6 $\text{J}\cdot\text{cm}^{-2}$ with a pulse duration of 360 μs (Krishnamurthy and others 2004). Another recent study showed that a single light pulse at a dose of 1 $\text{J}\cdot\text{cm}^{-2}$ is adequate for reduction of bacterial population of *P. aeruginosa*, about $10^6 \text{CFU}\cdot\text{mL}^{-1}$ was suspended in solution for injection (Feuilloley and others 2006). A different study showed that 20 pulses with 1 $\text{J}\cdot\text{cm}^{-2}$ of 0.3 s duration can have a reduction of more than 6 logs for *Bacillus pumilus* spores in aqueous suspension in a polyethylene container (Dunn and others 1997).

Similar study showed 6-8- \log_{10} reductions for spores of *Bacillus subtilis*, *Bacillus pumilus*, and *Bacillus stearothermophilus*, which are utterly inactivated by 3 pulses. Roughly 3.7 and greater than 5.9- \log_{10} reduction was reported in *Bacillus circulans* and *Bacillus cereus*, with approximately 50 pulses with each pulse having 7 J when this bacterium were treated on agar surface (Gomez-Lopez and others 2005a).

2.2.5 Applications of Pulsed UV Light

There are developing new applications areas, which use PL treatment to disinfect food products or packaging materials. Recently, some commercial companies have started to use it on packaging materials in the final steps of disinfection in the lack of chemical preservatives and disinfectants. Moreover, PL is used on the where surface contamination is seen as a concern for microbial contamination such as food with smooth surfaces, for example, whole fruit, vegetables, meat and cheese.

Additionally, it can be used on eggs or mushrooms to enhance the content of vitamin D. Due to the potential sunlight effect of PL, researchers have already changed 7-dehydroxycholesterol to natural vitamin D₃ (cholecalciferol) in the wavelength of 280-310 nm. On the other hand, it is used to fortify edible mushrooms rich in vitamin D₂. Furthermore, while using the UV light combinations some researchers ranged the content of vitamin D₂ from 22.9 \pm 2.7 to 184.0 \pm 5.7 for various mushrooms (Krishnamurthy and others 2010).

Another study showed that it has inactivation effect on the toxins, and there was a sufficient reduction of 3-100 % aflatoxin M₁ in milk when 2-60 min of PL treatment was applied (Krishnamurthy and others 2010). Additionally, the authors noticed that it could be used to inactivate allergens in soybean and peanuts. They reported that after PL treatment there was a remarkable reduction rate of two important peanut allergens,

which are Ara h1 and h3, in the liquid peanut. Hereby, this study opens new avenues to reduce content of allergens in other food products.

2.2.6 Effects of Pulsed UV Light on Food Components and Quality

There are some kind of effects of PL on the food quality and components although it can be used as a useful technology. One of them is that UV light can depolymerize starch in the presence of air, and metal oxides components (ZnO) enhance this process (Tomasik 2004). Moreover, UV light can increase other oxidation mechanism such as free radicals, and catalyze other steps of the process. Additionally, Kolakowska (2003) reported that UV light has an effect to form some components such as lipid radicals, superoxide radicals (SOR), and H_2O_2 . Some functions of components such as carbohydrate crosslinking, protein crosslinking, protein fragmentation, and peroxidation of unsaturated fatty acid can be induced by SOR under UV light. On the other hand, UV light can expose textural changes in milk because of denaturation of proteins, enzymes, and amino acids. Absorption of UV photons by water leads to production of OH^- and H^+ radicals; therefore, high dose of UV light may affect product quality under high doses applications besides just changing chemistry of food components. But they seem mostly useful changes because it is detrimental to microbial growth. Thus, for maintaining the quality of food products and to ensure their safety the optimal properties of disinfections steps are necessary. Moreover, under UV light treatment the fat-soluble vitamins like vitamin A and colored compounds like vitamin B2 can be affected by photo-degradation and peroxides produced, and these changes in food components may cause changes in nutritional quality of food. Additionally, long time treatment with UV-light and high doses of UV radiation may

cause temperature increase of food products, which can lead to changes in food quality because of changes in flavor, color and enzymatic browning.

Under normal conditions the inactivation of microorganisms takes a few seconds to a minute but the time depends on the opacity of the food products, microorganism type, and doses of UV radiation. Furthermore, UV light may induce flavor of products, which is caused because of activation of riboflavin, which is responsible for the conversion of methionine from methanol which leads to a burnt- protein like, burnt- feathers like, or medicinal-like flavor.

Generally, there is not any adverse effect after UV-light treatment of food if it is applied under optimal conditions such as moderate amounts, which are needed to provide required inactivation of microorganisms or increase temperature. To have successful implementation of the process in some foods, modification and optimization of the UV-light treatment might be necessary.

2.2.7 Inactivation Studies by Continuous and Pulsed UV Light

There was a comparison between effectiveness of a continuous UV-light source and a PL source to decontaminate surfaces. It showed almost the same level of inactivation of *B. subtilis* with $4 \times 10^{-3} \text{ J/cm}^2$ of PL source and $8 \times 10^{-3} \text{ J/cm}^2$ continuous UV-light source (McDonald and others 2000). Also PL is used to inactivate *E. coli*, *S. Typhi*, *Shigellasonnei*, *S. faecalis*, and *S. aureus* (Chang and others 1985). It was investigated that the bacteria were grown at 35°C for 20 to 24 h in a nutrient broth. The prepared culture was filtered with a 0.45- μm filter and washed with sterile buffer water. Re-suspended and aggregated bacteria in sterile buffer water were removed while using a nucleopore polycarbonate membrane (1.0 μm). After the filtering treatment with UV-light, bacteria colonies were grown in nutrient agar and counted. Although *E. coli*, *S.*

aureus, *S. sonnei*, and *S. typhi* displayed similar resistance to the UV-light and approximately $7 \times 10^{-3} \text{ J/cm}^2$ energy caused 3- \log_{10} reductions, *S. faecalis* needed a 1.4 times higher dose to get 3- \log_{10} reduction of inactivation.

Rowan and others (1999) investigated the efficiency of UV-light emission with low or high UV content on inactivation of bacteria such as *L. monocytogenes*, *E. coli*, *S. enteritidis*, *Psudeomonas aeruginosa*, *B. cerus*, and *S. aureus*. Seeded bacteria on the surface of Tryptone soya-yeast extract agar were treated with a PL source with low and high UV content. After PL treatment with 200 pulsed while pulsed duration is 100 ns, 2 and 6 \log_{10} reductions were obtained for low and high content, respectively. While using diluted samples concentration of 1×10^9 (Sample A), 1×10^8 (Sample B), or 1×10^7 (Sample C) with sterile deionized water, the effect of high-intensity UV light on inactivation of *B. subtilis* spores was investigated by Sonenshein (2003). The three types of positions were used and a 50 μL sample from each concentration were placed on them. The first position was on the lamp axis and at the midpoint of the lamp, second one was 1 cm above the lamp axis and at the midpoint of the lamp and last one was 1 cm above the lamp axis and 172 mm to the right of the midpoint of the lamp. When samples placed at the lamp axis and at the midpoint of the lamp, more than 6.5 \log_{10} CFU/mL for sample B and 5.5- \log_{10} CFU/mL for sample C reduction was investigated after UV-light treatment (three pulses for 1 s).

Yaun and others (2004) investigated that efficiency of continuous ultraviolet energy for inhibition of the pathogens on fresh product. After inoculation of the surfaces of red delicious apples, leaf lettuce, and tomatoes with *Salmonella* spp. or *E. coli* O157:H7, the samples were treated with UV-C light at a wavelength of 253.7 nm with

range of 1.5 to 24×10^{-3} W/cm². Different reduction amounts of *E. coli* O157:H7 were obtained for different samples like $3.3 \log_{10}$ CFU/apple at 24×10^{-3} W/cm² but $2.19 \log_{10}$ CFU/ tomato slices at the same dose. Similar results were obtained for *Salmonella* spp. and *E. coli* O157:H7 on inoculated lettuce as 2.65 and 2.79 -log₁₀ CFU/lettuce reductions, respectively.

The mechanisms of damage of yeast cells induced by PL and continuous UV-light were investigated by Takeshita and others (2003). According to this research, the DNA damage induced by continuous UV-light was slightly higher than that of PL. Moreover, efficiency of PL on protein elution was higher than that of continuous UV-light. The inactivation mechanism of PL was based on investigations of both the germicidal action of UV-C light and rapture of microorganisms due to thermal stress caused by the UV component (Wekhof and other 2000). Jun and others (2003) showed inactivation efficiency of PL on *Aspergillus niger* spores in corn meal. After 100 s treatment of PL when the sample distance from quartz window was at 8 cm, a 4.95 -log₁₀ reduction of *A. niger* on inoculated corn meal was investigated (Jun and others 2003). Moreover, PL inactivated *E. coli* O157:H7 on alfalfa seeds with reduction of 0.09 to $4.89 \log_{10}$ CFU/g for various thickness and treatment time (Sharma and Demirci 2003). As the thickness was adjusted at 1.02 mm, the completely inactivation (4.80 -log₁₀ CFU/g) of *E. coli* O157:H7 was obtained after 30 s treatment of PL. An increase in treatment time caused higher reduction for all thickness. In another study, Hillegas and Demirci (2003) showed that PL is an effective treatment method to inactivate *Clostridium sporogenes* in honey. In this study, almost 88% of reduction was obtained for *Clostridium sporogenes* for 45 s treatment of PL (initial inoculum level was 6.24 -

\log_{10} CFU/g) as a 2 mm depth of honey sample distance from quartz window was kept at 8 cm, although 180 s treatments was required to reach 89.4% reduction at 20 cm distance from quartz window.

The bulk tank of bovine milk was exposed to PL created by pulsed laser excimer at 284 nm. After treated with PL (25 J/cm² energy for 114 s exposure) there were not any obtained growing cultures of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella dublin*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Aeromonus hydrophillia*, and *Serratia marcescens* in bovine milk. The reduction amount obtained was more than 2 \log_{10} CFU/mL (Smith and others 2002). Furthermore, PL can also be effective to degrade toxins. 2 to 60 min treatment of PL achieved very important reduction range 3.6 to 100% of alfatoxin M1 in milk (Yousef and Marth 1985). These inactivation effects of PL are based on several factors such as, transmission of the food product, geometry of the waveguide, lamp power, and exposed wavelength range.

2.2.8 Economical Impact of UV-Light Disinfection System

Pulsed UV light disinfection technology is cheaper than other available systems. According to researchers, the estimated treatment cost at 4 J/cm² with the PureBright® PL treatment system is 0.1¢/ft² of treated area (Dunn 1997). The estimated cost comprises of conservative estimate of electricity, maintenance, and equipment amortization. Also with cost of investment in a hooded high intensity lamp and power unit, the cost of treatment with the PureBright® system is estimated as 0.1¢/ft² by Lander (1996).

For the apple cider industry, it is approximated to be cheaper to utilize PL pasteurization (Choi and other 2005), since its cost is approximately \$15,000 (Higgins 2001). The cost of annual maintenance like power consumption and lamp replacement

is based on lowest dosage of 30,000 MW.s/cm² for multi-lamp and single lamp disinfection source of continuous UV-light as \$2,465 and \$3,060 for an 8,000 h run time (Anonymous 1989). Taghipour (2004) reported that the costs for 4 log₁₀ reduction of *E. coli* in primary waste water by UV-light, electron beam, and gamma irradiation were 0.4¢/m³, 1.25¢/m³, and 25¢/m³, respectively.

The treatment costs of UV-light, electron beam, and gamma irradiation to inactivate *E. coli* (4 log₁₀) in primary wastewater were reported as 0.4¢/m³, 1.25¢/m³, and 25¢/m³, respectively (Taghipour 2004). It is clear to indicate that the UV-light treatment to inactivate pathogenic microorganisms is cost-effective.

2.3 Lactic Acid Bacteria (LAB)

Lactic acid bacteria are gram-positive rods, non-spore forming cocci or coccobacilli with a DNA base composition of high GC (Guanine and Cytosine content). They produce lactic acid as a major fermentation product or lactic acid, CO₂ and ethanol simultaneously (Jay and others 1986). They are generally non-respiratory and lack catalase, however, they have the superoxide dismutase and alternative means for detoxifying peroxide radicals. All members of this group obtain energy only from by substrate level phosphorylation because of not carrying out electron transport phosphorylation (Hardie and Whiley 1995). They can grow in the presence of O₂ as well as in its absence, and hence they are known as aerotolerant anaerobes (Holdeman and others 1975).

Lactic Acid Bacteria (LAB) has been classified according to their cell morphology, DNA composition, and also type of fermentative metabolism. Members of this group, which are *Leuconostoc*, *Pediococcus*, *Lactococcus* and *Streptococcus*, have almost familiar DNA composition, maybe there is a little difference from strain to strain. Most of

LAB gain energy from metabolism of sugar; thus, they are usually restricted to environments in which sugars are present. Lactic Acid Bacteria (LAB) has only limited biosynthetic ability, thus they evolved in environments that are rich in amino acids, vitamins, purines and pyrimidines to provide nutritional need (Gilliland 1990).

There is one important difference between subgroups of LAB, which is depended on sugar fermentation patterns. One subgroup produces only lactic acid as a major fermentation product, and is called homo fermentative. On the other hand, the other group produces ethanol and CO₂ as well as lactic acid, and it is called hetero fermentative (Facklam and Elliot 1995).

They mostly live in beneficial or harmless associations with animal, although some of them are pathogens. They may be found in different habitats such as milk and dairy products, and in decaying plant materials (Schillinger and Lucke 1987). There are some special places, which LAB play a beneficial role and can be found naturally in the intestinal tract, oral and vaginal cavity of humans (Farrow and others 1986). Moreover, LAB is one of the most common and important groups of microorganisms, which are used to ferment foods such as yogurt, cheese and beer. Besides, they have an ability to contribute to the taste and texture of fermented products. Additionally, LAB can inhibit some undesirable microorganisms like food spoilage bacteria by producing growth-inhibiting substances and large amounts of lactic acid (Essers 1982). There is an essential feature of LAB metabolism, which is productive carbohydrate fermentation coupled to substrate-level phosphorylation. Adenosine triphosphate (ATP) generated is subsequently used for biosynthesis. Lactic Acid Bacteria (LAB) has an effective capacity as a group to demote unique carbohydrates and relevant compounds such as lactic acid

that is more than 50% of sugar carbon (Facklam and others 1995). On the other hand, LAB can adapt to different conditions and produce significantly various end-product patterns because of the ability to change the metabolism.

2.4 Aerobic Plate Count (APC)

There are some kinds of methods to enumerate the number of organism in food. One of them is the Aerobic Plate Count (APC), which indicates the level of microorganisms in a product and can sometimes be used to indicate the quality and spoilage level of the product. Detailed procedures for determining the APC of foods have been developed by the Association of Official Analytical Chemists (AOAC 1990) and American Public Health Association (APHA 1984). Obtaining an estimate of the number of microorganisms in a food product will aid in evaluating sanitary practices during processing and handling, as well as determining potential sources of contamination (Jay 1986). Moreover, the APC may measure potential of bacteria flora, which can grow as visible colonies under random test conditions. Although APC is the best estimate, it is not available to measure the total bacteria population in food (AOAC 1995a). It can be effective to determine certain microorganisms such as thermophiles, mesophiles, psychrotrophiles, and proteolytic or lipolytic microorganisms (AOAC 1995). Additionally, a high APC may specify that a food product has been contaminated or has a poor quality ingredient. The suitable colony counting range was determined between 25 -250 (FDA 2012). Some conditions, which have already been altered such as temperature of incubation or composition of the agar medium, may change display of the organism, which will grow in the medium. Thanks to these kinds of altering conditions, APC can be used as the best method to determine product quality and microbial load (FDA 2001).

Table 2-1. Properties of Propylene Glycol (Source DOW 2012)

Property	Unit
Molecular Weight	76.10 g/mole
Formula	$C_3H_8O_2$
Boiling Point at 101.3 kPa	187.4 °C
Melting Point	-59.96 °C
Pour Point	< -57 °C
Density at 25 °C	1.036 gm/cm ³
Vapor Pressure at 25 °C	0.017 kPa
Viscosity at 25 °C	48.6 mPa.s(=cp)
Thermal Conductivity at 25 °C	0.2061 W/(m.K)
Heat of Formation	-422 kJ/mol

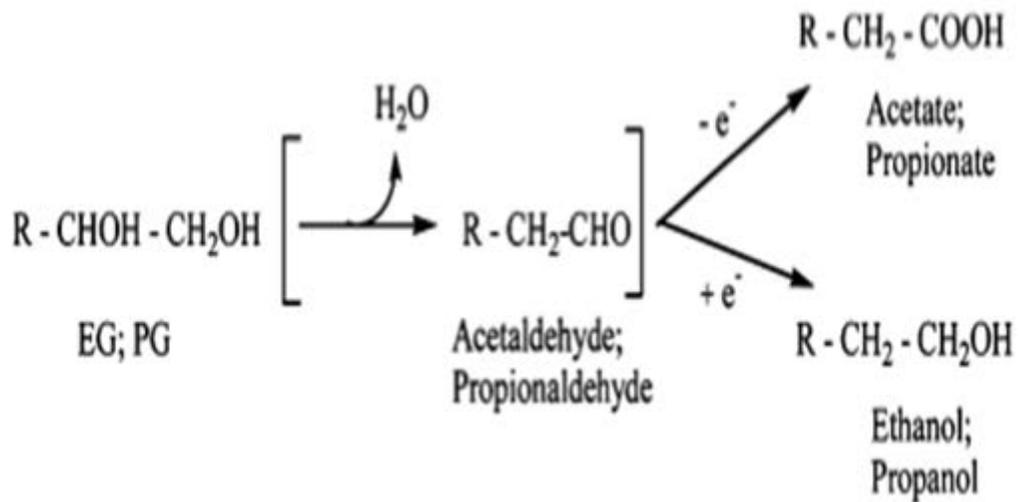


Figure 2-1. Scheme of organic products formed from anaerobic degradation of ethylene and propylene (R = H or CH₃) permitted by T. Klotzbucher and others / Geothermics 36 (2007) 348–61

CHAPTER 3 EFFECT OF PULSED LIGHT ON THE TEMPERATURE PROFILE OF PROPYLENE GLYCOL

A temperature rise has been observed with pulsed light illumination for an extended duration due to the significant proportions of infrared spectra (Li and others 2011; Shriver and others 2011). Since pulsed light by Xenon lamp consists of around 20% infrared spectra besides approximately 54% UV spectra and around 22% visible spectra.

In the pulsed generation, the electrical energy is amplified and stored in capacitor over a short period of time like few milliseconds and released as very short period pulses (several nanoseconds). The stored electrical energy is passed through a lamp filled with inert gas (xenon or krypton), which causes ionization of gas and produces a broad spectrum of light in the wavelength from UV-light to infrared region. The intensity of pulsed light has 20,000 times more powerful than that of sunlight (Dunn 1995). Generally the pulse rate is released as 1 to 20 pulses per second and the pulse width is 300 ns to 1 ms. Thus, light of pulses with high energy in several megawatts are produced though the total energy is comparable to continuous UV- light system. Use of pulsed light has been approved as a non-thermal technology as applied within short times (<10 s). In contrast, longer exposure time may cause increases in temperature when energy accumulates in foods products. Another possible explanation to changes in temperature during PL treatment is based on the fluence threshold (Wekhof 2000). As mentioned in previously publications, fluence is evaluated as the energy delivered to the sample by the system during PL exposure. However, the fluency capacity of PL may be dependent on the characteristics of sample and the extent in which energy is

absorbed. Given the limited absorption of energy in light food products, it is probable that samples did not reach a fluence threshold in any of the treatments.

In this study, effect of PL on temperature variation of naturally contaminated (FSHN Lake Water Gainesville, FL) PG was investigated. The efficiency of PL was assessed based on the increase in temperature of PG.

3.1 Material and Methods

3.1.1 Sample Preparation

Using an incubator, the inoculated PG sample (Sara Lee Foods, Peoria, IL, U.S.A) was stored at a controlled temperature of 27°C. The container containing the PG was tightly sealed in order to avoid oxidation before treatment. Each sample was loaded individually into the same weighing size dishes (Low Form Aluminum, Fluted, Fisher Scientific, U.S.A).

3.1.2 Pulsed UV Light Treatment

The PL treatments were performed at room temperature while using a pilot scale continuous PL system designed by Xenon Corp. (LH840-LMP-HSG, Xenon Corp., Wilmington, MA, U.S.A) (Figure 3-1). The PL system consists of a controller unit, treatment chamber housing with two adjustable Xenon flash lamps, and a hydraulic conveyor belt. This unit generated a broad-spectrum light (100-1100 nm) at a pulse rate of three-pulses/s and a pulse width of 360 μ s. Values of energy intensity (J/cm^2) were adopted from measurements conducted by Krishnamurthy (2006) in a PL batch system of similar characteristics.

Exposure time and distance from the PL quartz window were established as the experimental variables. The PG samples were individually centered and treated for 5,

10, 15, 20 and 25 s, at a distance of 6 and 9 cm from the light source. The thickness of the PG samples was measured as 2.5, 5 and 7.6 mm for 5, 10 and 15 mL, respectively.

3.1.3 Temperature Measurements

The temperature profile of PG samples during PL treatment was recorded by K-type thermocouple using Pico® eight-channel thermocouple data logging interface (TC-08) attached to laptop running Pico-software (Figure 3-2 vs. Figure 3-3). The K-type thermocouple was placed at the geometrical center of aluminum dish for every sample (Figure 3-4). The data recording was started and stopped 30s before and after the actual experiment. The temperature of PG samples was continuously measured using K-type thermocouples through a data acquisition system (TC-08 Thermocouple Data Logger, Pico Technology North America Inc, Tyler, TX).

3.1.4 Experimental Design

A full factorial design with three factors and 2-3 levels was utilized in this study. The factors were average temperature of the PG samples (69.9 and 93.5°C), volume of the PG sample (5, 10, and 15 mL), and treatment time (5, 10, 15, 20 and 25 s) and two different distances (6 and 9 cm) from quartz window. Three replications were performed for each condition. For the first set of experiments, the program was used to control the temperature of the PG using K-type thermocouple for 5, 10, 15, 20 and 25 s at 6 cm distance from quartz window. In the second of experiments, the distance from quartz was adjusted as 9 cm for a 30.6°C initial temperature for 5, 10, 15, 20 and 25 s PL treatment. In a third set of experiments, the treatment time was increased 50 s for volume of PG (5, 10 and 15 mL) at two distances (6 and 9 cm) from quartz window. Also, the effect of short treatment times (5, 10, 15, 20 and 25 s) was investigated by treating the microorganisms for all volumes of PG samples.

3.1.5 Statistical Analysis

Using Graph Pad Prism 5.0 Software compared the mean reduction rates. One-way ANOVA test followed by Tukey's post hoc comparison was performed to evaluate the difference between the treatment groups. The difference $p < 0.05$ was accepted as significant.

3.2 Results and Discussion

The PL treatment is considered as a non-thermal process for short period of inactivation of microorganisms. Although it is considered as a non-thermal process, there may be an increase in temperature because of high-energy content of pulses due to prolonged treatment resulting in the accumulation of energy in the sample (Krishnamurthy 2006). The sample temperature increased gradually (Figure 3-5 vs. 3-6) directly proportional with the exposure time. Also, as the thickness of the sample decreases, the temperature increased. This may be due to an increase of energy absorption because of more penetration depth allowing more energy to be absorbed by the sample. In accordance, increase in temperature is also related to the viscosity and the surface of sample (Demirci and others 2003). In contrast, an increase in sample volume resulted in a decrease in temperature rise, which may be attributed to lower penetration depth of PL into the sample (Krishnamurthy 2006). Hence, higher temperatures could be expected on samples, which are located at a closer distance (from the quartz window) and have lower thickness as seen in Figure 3-5. According to the measurements undertaken with the K-type thermocouple using Pico® eight-channel thermocouple data logging interface (PC- 08) attached to the computer with Pico-software (Figure 3-2 vs. Figure 3-3), the average initial temperature of samples was obtained as 30.6°C. At 5, 15 and 25 s, the increase in temperature of samples was

recorded as 14 ± 3.1 , 30.5 ± 1.3 and $39.9 \pm 1.2^\circ\text{C}$, respectively; using a sample volume of 5 mL was kept at 6 cm distance from quartz window. In this study, the temperature increase initiated by PL was moderately high, especially during the first seconds of exposure ($\sim 6^\circ\text{C}$). For instance, at 10 s treatment at 6 cm distance from quartz window resulted in a 20.4 ± 2.5 , 16.5 ± 1.2 and $14.8 \pm 3.2^\circ\text{C}$ increase in sample temperature for 5, 10 and 15 mL PG sample volumes, respectively (Figure 3-5). On the other hand, there was a proportional increase for all PG sample volumes during longer exposure because of the increasing photo-thermal effects of PL on the temperature at both distances (Figure 3-5 vs. 3-6). However, when the distance from the lamp source increased and treatment time is concomitantly decreased, the food samples may not be exposed to a more intense treatment (Gómez-López and others 2005a). In the second part of the study, the distance to quartz window was increased while sample volume (5, 10 and 15 mL) and treatment times were kept constant. As shown in the Figure 3-9, the temperature profiles of PG samples were determined for 50 s. As shown in the Figure 3-6, for 10 mL sample, increase in temperature was observed as 12.8 ± 0.6 , $14.8 \pm$ and $20.8 \pm 0.5^\circ\text{C}$ for 5, 15 and 25 s exposure at 9 cm distance, respectively. The critical temperature change was obtained as 7.6 , 13.2 and 16.1°C for 15 mL PG sample during 5, 10 and 20 s PL treatments at 9 cm, respectively. Figure 3-8 shows relationship between temperature and distance when distance increases from quartz window it causes a decrease in temperature. Krishnamurty (2006) reported that lower temperatures in combination with shorter treatment times and lesser volume resulted in lower reductions. As expected, in third part of this study, the ratio of temperature increase was higher when less volume of PG sample was treated, as a result of more

energy accumulation that is readily available to heat the PG sample. The temperature was raised from 55.1 to 58.9°C within fifty seconds of PL treatment for 5 mL volume at 6 and 9 cm distances, respectively (Figure 3-7 vs. 3-8). Optimizing the temperature of PG samples during PL treatment could result in less detrimental quality changes. In this study we showed that the effect of the sample thickness did not have any significant effect on increase in temperature until 10 s for 5 mL sample ($p < 0.05$) (Figure 3-5 vs. 3-6) at both distances. The temperature of the sample increased, as the treatment time increased after several seconds (Figure 3-5 vs. 3-6); however, no significant temperature change was observed during the first 5 s for all volumes. But after 15 s there was a significant increase for 5 mL sample. This may indicate that non-thermal effect of PL displays only in short treatment periods. On the other hand, a minor increase in temperature within seconds may cause a considerable thermal effect for some bacteria species such as psychrophilic organism (Struvay and Feller 2012). Another disadvantage of a high increase in temperature is that specific features of the sample may be damaged. During a 20-s treatment time, the temperature increase was about 23°C and 19°C for a 10 mL PG sample at 6 and 9 cm distance, respectively. Our statistical analysis demonstrated that the treatment duration and the interaction (treatment time*depth) had significant ($p < 0.05$) impact on temperature increase.

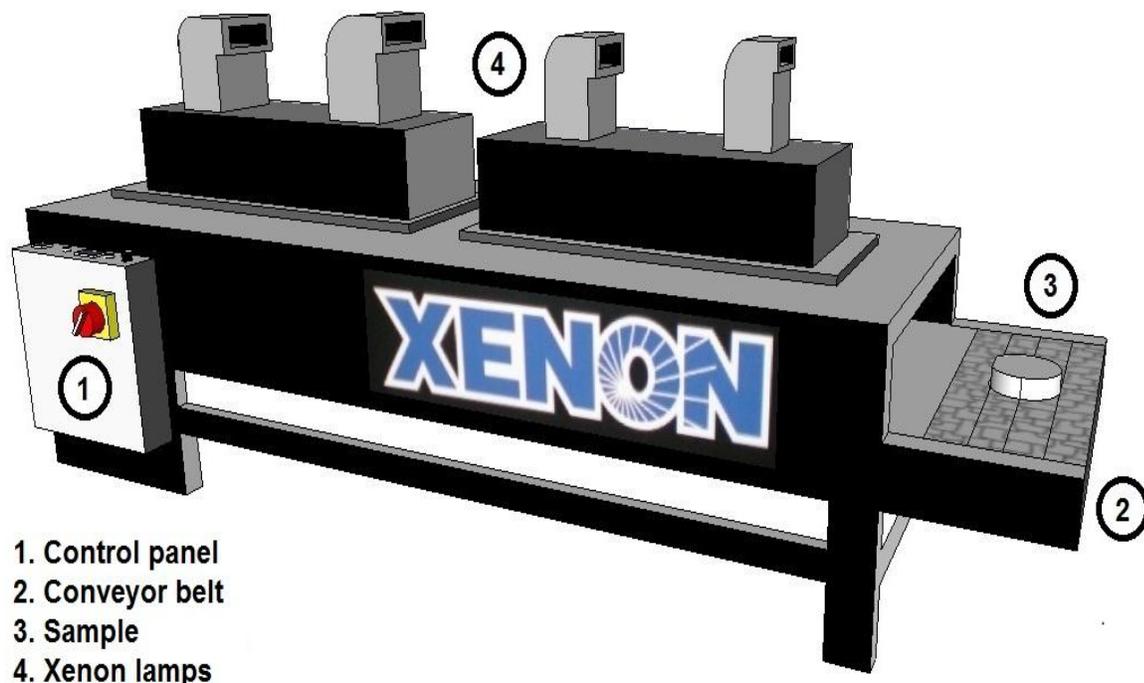


Figure 3-1. A pilot-scale PL system at the Food Science Pilot Plant, University of Florida, Gainesville, FL, U.S.A.

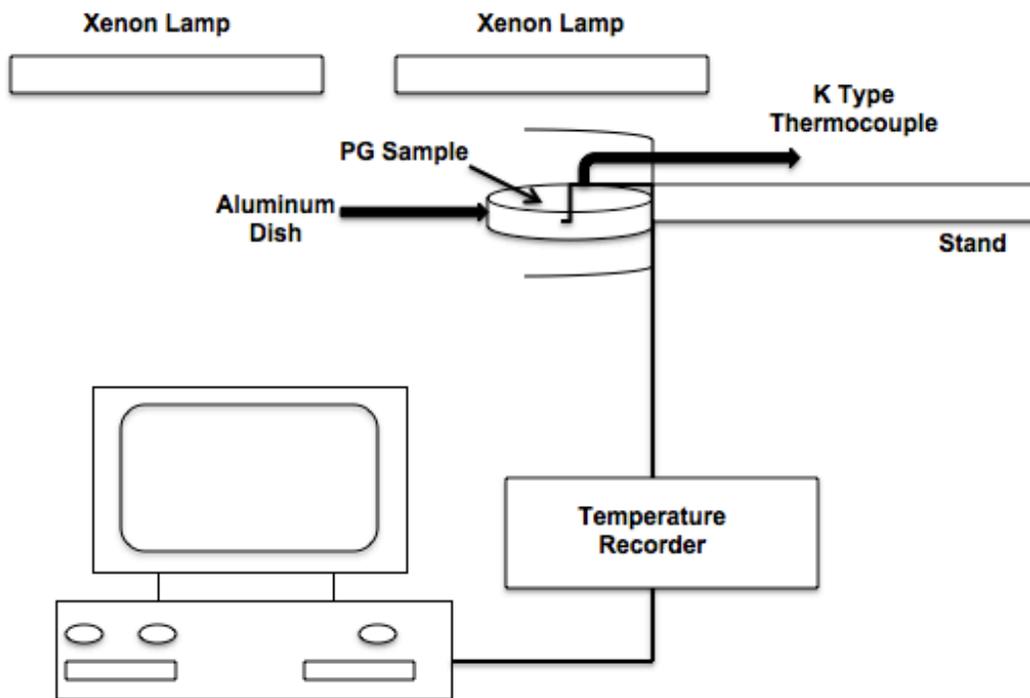


Figure 3-2. Schematics of the pulsed ultraviolet system with TC-08 thermocouple data logger

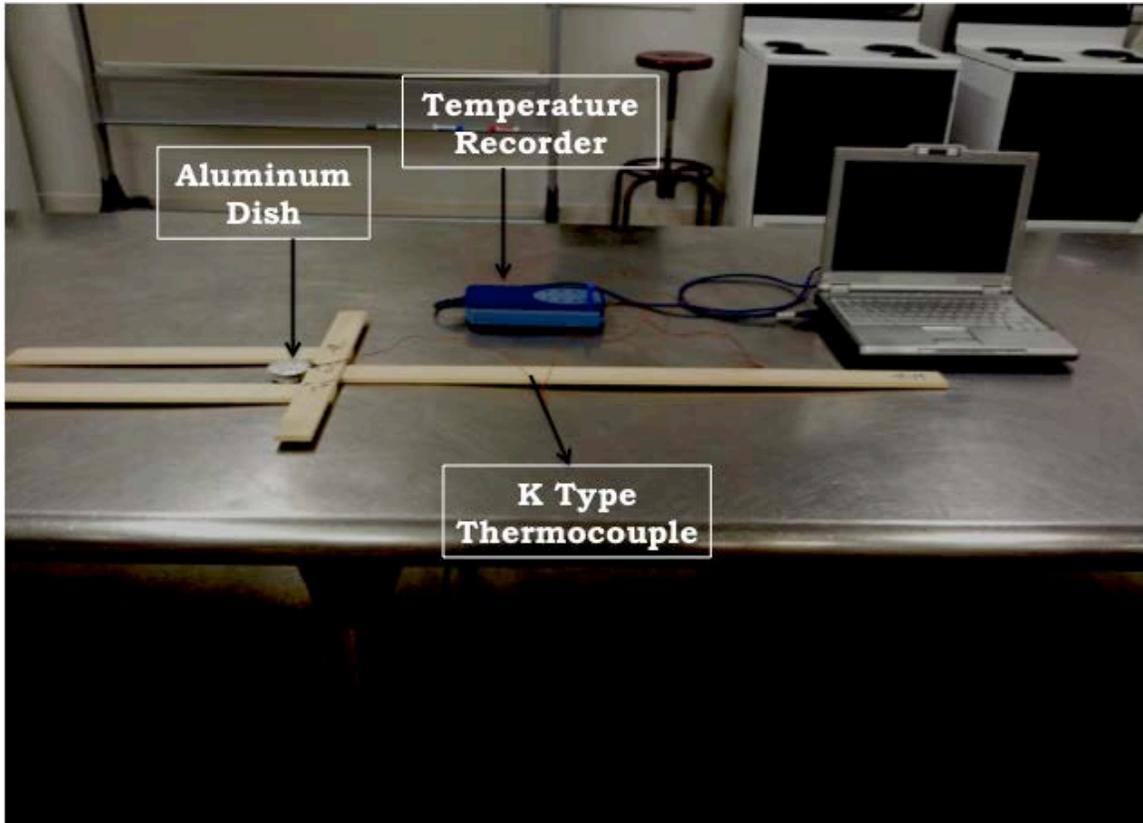


Figure 3-3. Lab scale TC-08 thermocouple data logger temperature recording system
(Photo courtesy of Samet Ozturk)

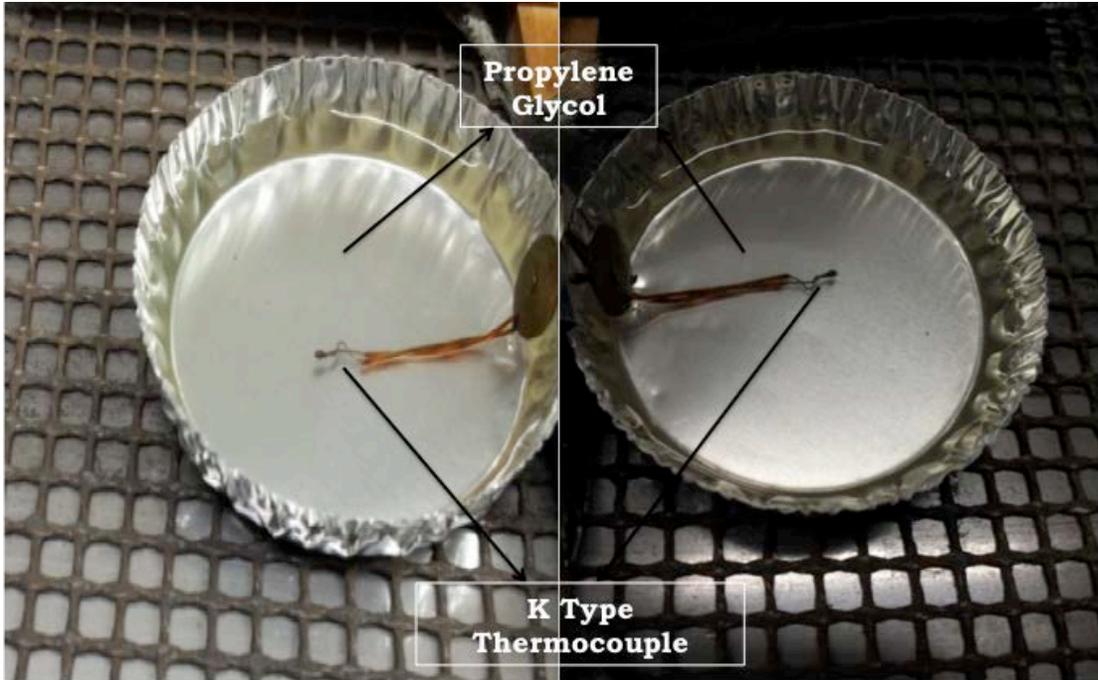


Figure 3-4. A view of K-type thermocouples immersed in propylene glycol (Photo courtesy of Samet Ozturk)

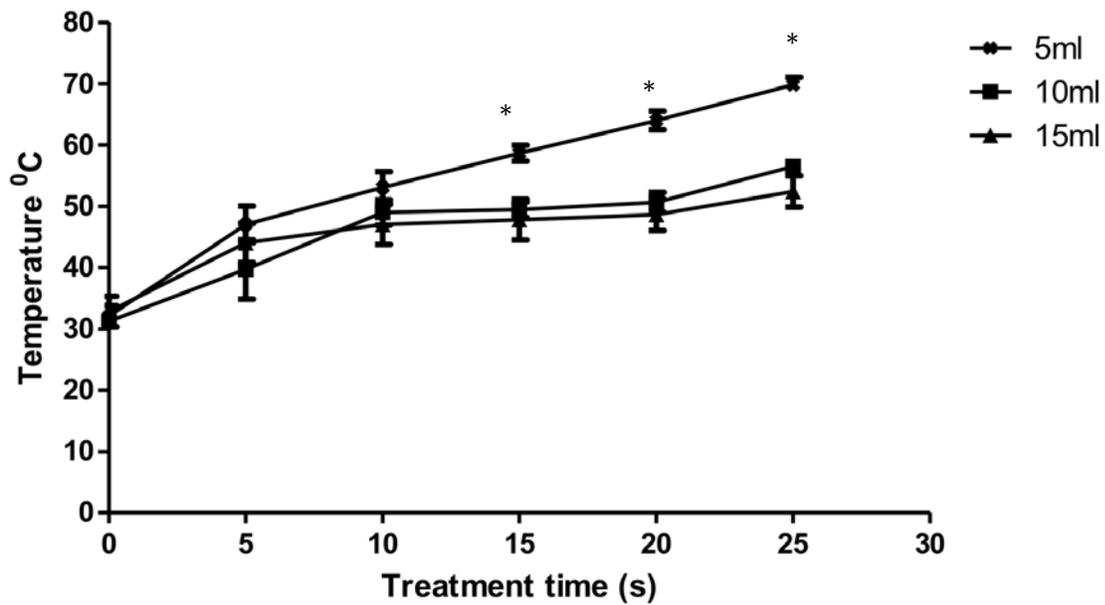


Figure 3-5. Change in initial temperature of proylene glycol samples (5,10 and 15 mL) during PL treatment at 6 cm for 25 s. Data represent the mean \pm SD (n=3). There is a significant delta temperature change in for all volumes after 10 seconds when compared with the initial temperature. * $p < 0.05$ represents significance between sample volumes.

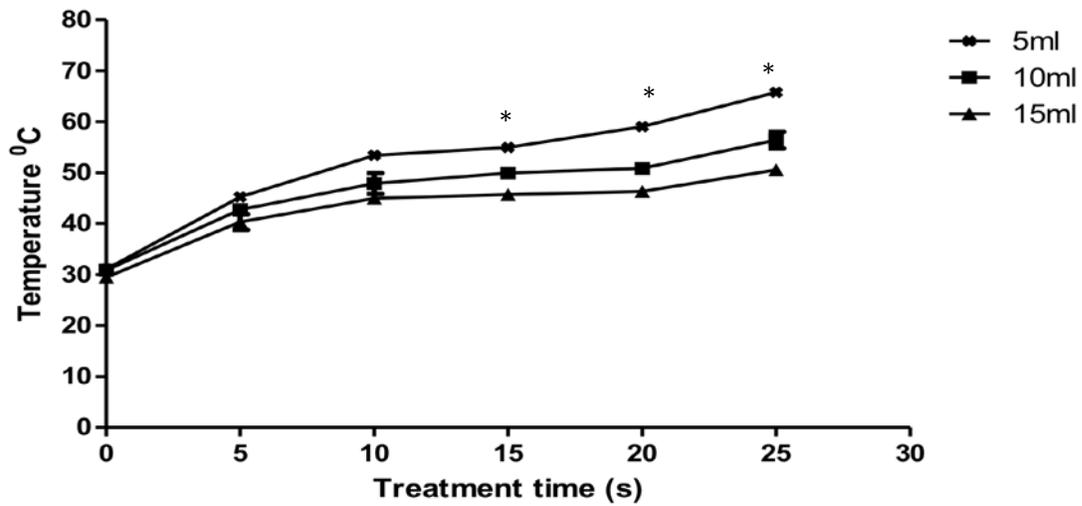


Figure 3-6. Change in initial temperature of propylene glycol samples (5,10 and 15 mL) during PL treatment at 9 cm for 25 s. Data represent the mean \pm SD (n=3). There is a significant delta temperature change in for all volumes after 10 seconds when compared with the initial temperature. * $p < 0.05$ represents significance between sample volumes.

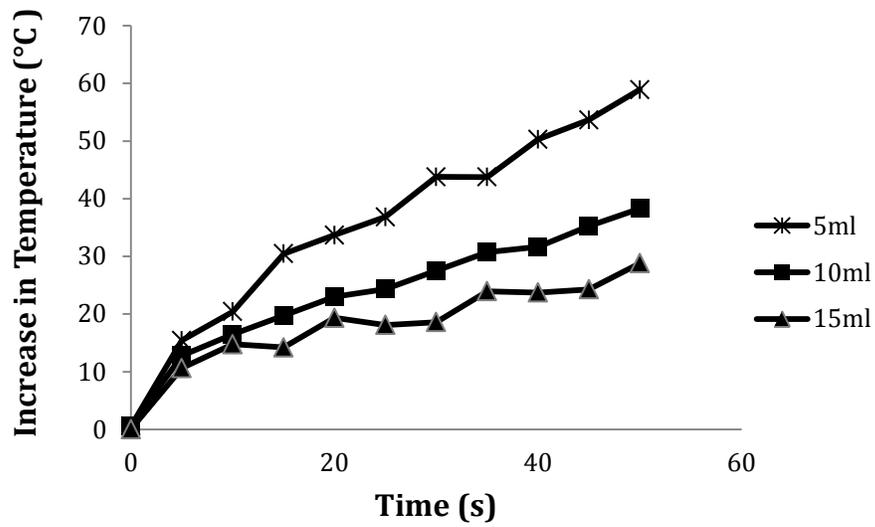


Figure 3-7. Increase in the initial temperature of propylene glycol sample during PL heating for 50 s at 6 cm. Data represents the mean (n=3).

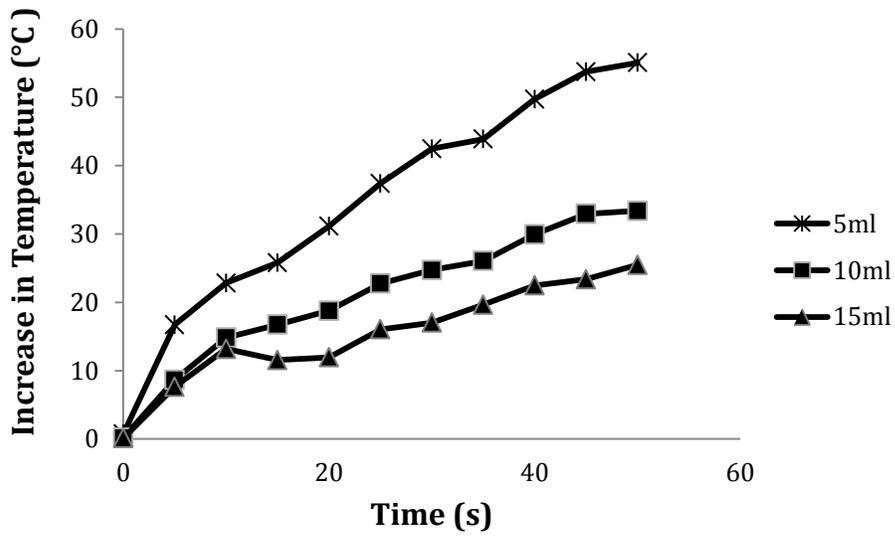


Figure 3-8. Increase in the initial temperature of propylene glycol sample during PL heating for 50 s at 9 cm. Data represent the mean (n=3).

CHAPTER 4

EFFECT OF PULSED ULTRAVIOLET LIGHT ON AEROBIC PLATE COUNT IN MICROFLORA OF PROPYLENE GLYCOL

Aerobic plate counting (APC) is a method used as an indicator of the level of microorganisms in a sample of raw material, in-process material, or finished product, which is not usually associated with food safety concerns, since it is not related to pathogenesis or toxicity of bacteria (Neusely and others 2013). However, enumeration of these microorganisms plays an integral role in determining the quality, shelf life and post-treatment processing contamination. The food industry uses this method to determine the sanitation levels between processing and the distribution steps besides determining the sufficiency of sanitation. The higher APC, there is a higher possibility to increase environmental and sanitation controls.

Closed water and chilling systems have a potential to encourage the growth of microorganisms and promote oxidation of propylene glycol in the chilling system. The level of bacterial growth is related to the rate of decomposition in PG. The FDA recalls and initiates court actions towards infections in handling systems (FDA 2001). In this study, we determined the efficiency of PL on the disinfection of APC of the microflora in PG.

4.1 Material and Methods

4.1.1 Pulsed Ultraviolet Light Treatment

Propylene glycol samples were treated with PL as described in Chapter 3.

4.1.2 Aerobic Plate Count (APC)

The AOAC 966.23 C method was used as the reference procedure in determining the APC of PG. From each PL treated PG sample, a serial dilution (1/10 mL) was made up to 10^{-4} . One mL from each diluted sample was put into sterile petri

dishes using sterile pipette. Plate Count Agar (at 45°C) (Fisher Scientific, Pittsburg, PA, U.S.A) was poured into each of petri dish containing 1 mL inoculum. The plates were allowed to solidify on a flat surface, inverted and incubated at 35°C for 48 hours. After incubation, reduction of bacteria in ten fold was calculated for different treated PG samples.

The data was analyzed using a statistical analysis system (Graph Pad Prism 5.0). One-way ANOVA followed by post hoc Tukey's multiple comparison test was performed. The level of significance was determined at $P < 0.05$.

4.1.3 Colony Forming Unit (CFU)

The colony forming of units is an estimate to use for determining the number of viable bacterial cells in a sample per mL or per gram. Hence, it tells the degree of contamination in samples of water, vegetables, soil or fruits or the magnitude of the infection in humans and animals (FDA 2012). To obtain APC, count duplicate or triplicate plating from the same dilution, which produces 25 to 300 colonies, and take the average of the plate counts. In short, 10-fold dilutions were employed in the rinse solutions (1/10, 1/100, 1/1000) initially. The PL treated and untreated samples (1 mL) were vortexed and transferred to dilutions tubes (9 mL PW) a using sterile pipette tip for each transfer. Then, 1 mL portion of dilutions was transferred to empty plates and 9-10 mL plate count agar (Fisher Scientific, Pittsburg, PA, U.S.A) was poured. After this process, plates were incubated at 48 ± 3 h at $35^\circ\text{C} \pm 1^\circ\text{C}$. To calculate the APC, the total number of colonies counted is multiplied by the reciprocal of the dilution factor on the plate.

4.1.4 Evaluation of PL Efficiency

The level of microbial inactivation (Log [N/No]) was calculated by subtracting the survivor counts (N) resultant from PL treatment from the initial counts (No) represented by the control samples. Results were expressed in log CFU/mL (PG). Additionally, survivor curves were built by creating scatter plots of $\text{log survivor counts (N)}$ versus treatment time (t).

4.1.5 Sample Preparation

The obtained PG (Sara Lee Foods, Peoria, IL, U.S.A) was contaminated with lake water to increase level of microorganism in natural flora according to chilling system uses (45 % Propylene Glycol to 55 % Lake Water). Lake water (FSHN Lake, Gainesville, FL) was obtained at 04/25/2013. Then it was incubated at 30°C up to 5 days.

4.1.6 Statistical Analysis

The data obtained was analyzed using a statistical analysis system (Graph Pad Prism 5.0). Analysis of variance (one-way ANOVA) was performed and the significant differences in the means were separated using the Tukey's studentized range test. The data was tabulated as an average of triplicates \pm standard deviation, and the level of significance was determined at $P < 0.05$.

4.2 Results and Discussion

Several treatment methods in industry are used to prevent and reduce microbial contamination in food processing equipment. These methods range from chemical treatments to heat and other non-thermal methods. However, to remove the source of microbial contamination effectively without chemicals, there is a need for novel

technologies. One example of a novel technology is the use of PL as a treatment. Use of PL has been approved to be more effective on inactivation of pathogens. It is also relatively effective on simple surfaces with 1-3 log₁₀ on complex surfaces like meat. The PL treatment was also used against *P. aeruginosa* biofilms growing on steel and helped to improve the efficiency of gentamicin against the same biofilms (Huang and others 1988). Here, the effect of PL on the microflora of PG was studied by measuring the reduction of APC. Specifically, the effects of treatment time, sample volume and distance from quartz window were studied as to their effects on APC. The initial average APC obtained was approximately 6.5 log₁₀ CFU/mL for control groups grown at 30.6°C. The complete inactivation time was found to be 20 s of PL for the 10 mL PG sample, whereas 10 s of PL treatment was adequate for inactivation for the 5 mL sample for samples treated 6 cm from the quartz window of the UV lamp (Table 4-1). For the 15 mL sample, the maximal log reduction occurred at 25 s of PL but it may have been further reduced if treatment times were extended. These results corresponded to temperature increases of the sample of 33.4, 20.1 and 16.7°C at 20 s for the treatments in the 5, 10 and 15 mL sample, respectively suggesting temperature effects 21°C (Figure 3-5). When samples were placed at 9 cm distance from quartz window, a slight drop in the log reduction occurred for the 10 mL and 15 mL samples at 20 s treatment times when compared to samples that were placed 6 cm from the quartz window under the same treatment time (Table 4-1 vs. Table 4-2). For example, the 15 mL sample treated with 20 s of PL dropped from a 5.9-log₁₀ reduction at the 6 cm treatment to a 4.7-log₁₀ reduction at the 9 cm treatment. The temperature increases were lower at the 9 cm distance compared to the 6 cm treatment in this case (22.2°C for 6 cm vs. 19.6°C for 9

cm; Figure 3-5 vs. Figure 3-6) suggesting that temperature may in part be part of the cause of the lower reduction in bacteria. However, there was still microorganisms on plate of PL treated sample (10 mL) at 9 cm distance after 20 s although there was an approximately 19.6°C increase in temperature. In addition, the poor penetration capacity of the light at the farther distance from the quartz window is also likely involved in the lower reduction of bacteria at the farther distance.

Previously, the effects of PL on the inactivation *E. coli* and *S. enteritidis* at 5 to 100 pulses with a range of 200-530 nm at different energy levels was studied in effects of pulse light on reduction in microflora of milk (Ghasemi and others 2003). For *E. coli*, a 9-log₁₀ order reduction occurred after treatment with 100 pulses of 9 J but for a lower energy pulse of 4.5J at 100 pulses there was less of a log reduction (7 log₁₀ reduction). In addition, a fewer number of pulses (5) even at higher energy (9J) resulted in a low log reduction (0.5 log₁₀ reduction). For *S. enteritidis*, 6 log CFU/mL was observed after 20 s treatment times. These results suggest that the high energy per pulse is heavily responsible for the reduction in bacteria, but require a minimum amount of pulses. In another study, when microflora of milk (3 mL) was treated for 1, 2, and 4 min of PL a 0.3, 3.4 and 8.4-log₁₀ CFU/mL reductions in bacteria were obtained, respectively (Krishnamurthy 2006).

Inactivation of microorganisms in PL is not only related to the increase in temperature because many organisms have high heat resistance (D value ↑, Heat Resistance ↑; Sungur 1994) but are inactivated under PL. For these high heat resistance organisms, the complete inactivation will require a high dose of UV treatment (Walker 1984).

Additionally, absorption of energy from the light depends on the characteristic of the bacteria and the surrounding medium; therefore, bacteria may also be over heated in different amounts (Fine and Gervais 2004). There might be some changes, which may lead to membrane destruction in the cells such as steam flow and vaporization.

In accordance with the literature, our results suggested that reduction of APC is likely based on photo-thermal and photo-physical effect of PL besides the thermal effect. Moreover, overheating of the bacteria is based on the differences in UV-light absorption by microorganism and surrounding medium; thus, microorganisms become a local vaporization center and may generate a small steam flow performing membrane destruction (Takeshita 2003). Although the thermal effect plays a vital role at extended treatments with PL (>5 s treatment time), it does not have a significant effect on shorter treatments. In this study, use of PL resulted in reduction of APC for 5 s although no significant increase in temperature for all sample volumes (<10 s) at 6 and 9 cm distance from quartz window (Figure 3-5 and 3-6) was observed. However, it may not be only related to increase in temperature. Also, it was investigated that PL was effective on killing microorganisms, which was a result of PL damage on DNA structure of microorganisms and also to bacteria cells because of absorption of energy of light by bacteria cells (Giese and others 2000; Wekhof 2000).

The present study clearly demonstrates the potential of PL for APC inactivation in PG and that complete reduction can be achieved with PL while increase in temperature (within seconds). Thus, PL treatment can be used as an alternative to thermal and chemical sterilization for microflora of PG cooling medium.

It was observed that increasing treatment time or decreasing sample volume the \log_{10} reduction rate of APC increased regardless of distance from the quartz window (Table 4-1 vs. Table 4-2). However, decreasing the distance of the sample from the quartz window increased the effect of PL on the inactivation of APC.

Table 4-1.Reduction of Aerobic Plate Count in propylene glycol after Pulsed UV Light Treatment with in increase in temperature for 6 cm distance from quartz window

Treatment Time (s)	Reduction (Log CFU/mL)		
	5 mL	10 mL	15 mL
5	3.7 ± 0.0	2.8 ± 0.0 ^{ααα}	1.8 ± 0.0 ^{ααα,βββ}
10	5.5 ± 0.9 ^{**}	4.0 ± 0.0 [*] , αα	3.0 ± 0.0 ^{***, ααα,β}
15	6.5 ± 0.00 ^{***}	5.6 ± 0.8 ^{***}	4.3 ± 0.0 ^{***}
20	6.5 ± 0.00 ^{***}	6.5 ± 0.0 ^{***}	5.9 ± 1.1 ^{***}
25	6.5 ± 0.00 ^{***}	6.5 ± 0.0 ^{***}	6.5 ± 0.0 ^{***}

One Way ANOVA followed by Tukey's test. *P<0.05, **P<0.01, *** P<0.001 vs 5s; ααα <0.001 vs 5 mL: βββ <0.001 vs 10 mL. Data are expressed as mean ± standard deviation (SD). N=3. CFU: Colony forming unit.

Table 4-2.Reduction of Aerobic Plate Count in propylene glycol after Pulsed UV Light Treatment with in increase in temperature for 9 cm distance from quartz window

Treatment Time (s)	Reduction (Log CFU/mL)		
	5 mL	10 mL	15 mL
5	3.3 ± 0.1	2.1 ± 0.0 ^{ααα}	1.4 ± 0.1 ^{ααα, βββ}
10	4.7 ± 0.0 ^{**}	3.0 ± 0.0 [*]	2.6 ± 0.0 ^{***, ααα, βββ}
15	5.7 ± 0.7 ^{***}	4.7 ± 0.0 ^{***}	3.6 ± 0.0 ^{***}
20	6.5 ± 0.0 ^{***}	5.6 ± 0.8 ^{***}	4.7 ± 0.0 ^{***}
25	6.5 ± 0.0 ^{***}	6.5 ± 0.0 ^{***}	6.1 ± 0.1 ^{***}

One Way ANOVA followed by Tukey's test.*P<0.05, ** P<0.01, *** P<0.001 vs 5s; ααα <0.001 vs 5 mL: βββ <0.001 vs 10 mL. Data are expressed as mean ± standard deviation (SD). N=3. CFU: Colony forming unit Increase in temperature presents average for all sample volumes.

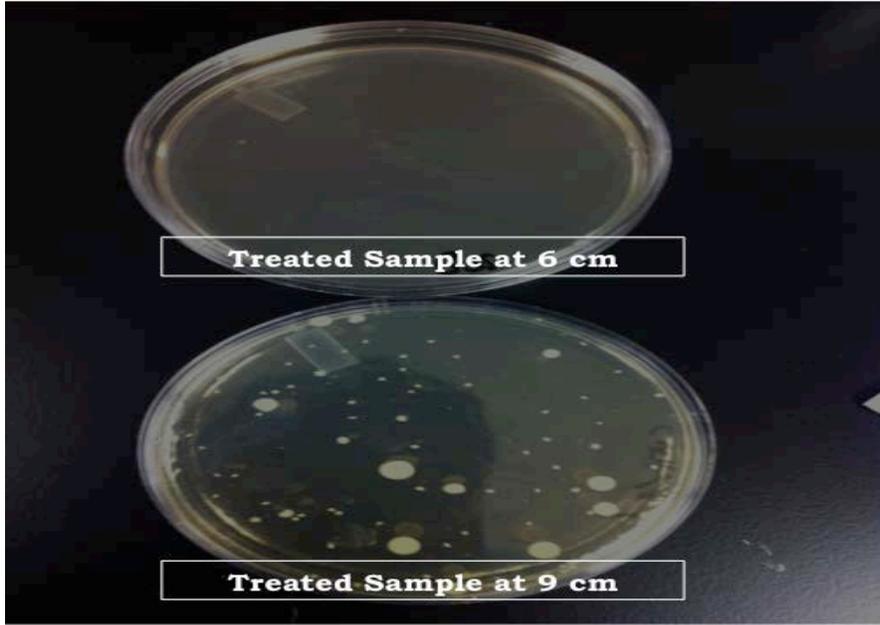


Figure 4-1. Aerobic Plate Count on the 10 mL sample volume plates after 20 s exposure at 6 and 9 cm distance (Photo courtesy of Samet Ozturk)

CHAPTER 5

EFFICACY OF PULSED UV LIGHT ON INACTIVATION OF THERMAL RESISTANCE BACTERIA IN MICROFLORA OF PROPYLENE GLYCOL

The thermal resistance of bacteria has been expressed with well-known concepts of D and z values, which are the required time (in seconds) to reduce bacteria almost 90% (D Value) and the increase in temperature (in kelvin) to provide a 90% reduction in D value (z Value), respectively (Hansen and other 1963). Also, the values of D and z can express inactivation of bacteria. Previous research has examined the resistance of pathogenic bacteria to heat such as *E. coli* O157:H7 (Ahmed and others 1995; Kotrola and others 1997), *Salmonella* strains (Roberts and others 1996), and *Listeria monocytogenes* (Farber and other 1990; Roberts and others 1996). Most of these studies have been performed using homogenized foods or liquid media. The heat resistance of lactic acid bacteria (LAB) has been widely examined due to its importance to the food industry. Franz and other (1996) investigated that heat resistance of meat spoilage for *Lactobacillus in vitro* with D-values at 57, 60 and 63°C for 52.9, 39.3 and 32.5 s treatments, respectively. In this study, we investigated the efficiency of PL on inactivation of LAB in propylene glycol.

5.1 Material and Methods

The 5, 10 and 15 mL of treated PG samples were sequentially diluted and plated on the de man, rogosa and sharpe (MRS) agar at pH 4.8 (Fisher Scientific, Pittsburg, PA, U.S.A). After the diluting and plating process, the samples were autoclaved, and then LAB strain isolation was carried out with adjusted pH to 4.8 with 10% citric acid. Plates which contained 100 mg/L cycloheximide (Fisher Scientific, Pittsburg, PA, U.S.A) to inhibit growing of microorganism such as yeasts and other fungi were then incubated anaerobically at 30°C for 3-5 days.

5.1.1 Pulsed Ultraviolet Light Treatment

Propylene Glycol (PG) samples were treated with PL as described in Chapter 3.

5.1.2 Colony Forming Unit

The colony forming of units is an estimate to use for determining the number of viable bacterial cells in a sample per mL or per gram. Hence, it tells the degree of contamination in samples of water, vegetables, soil or fruits or the magnitude of the infection in humans and animals (FDA 2012). To obtain the LAB count, duplicate or triplicate samples from the same dilution, which produces 25 to 300 colonies, were plated. In short, 10-fold dilutions were employed in the rinse solutions (1/10, 1/100, 1/1000) initially. The PL treated and untreated samples (1 mL) were vortexed and transferred to dilutions tubes (9 mL PW) a using sterile pipette tip for each transfer. Then, 1 mL portion of dilutions was transferred to empty plates and 9-10 mL plate count agar was poured. After this process, plates were incubated at 48 ± 3 h at $35^\circ\text{C} \pm 1^\circ\text{C}$. To calculate the LAB, the total numbers of colonies counted were multiplied by the reciprocal of the dilution factor on the plate.

5.1.3 Evaluation of PL Efficacy

The level of microbial inactivation (Log [N/No]) was calculated by subtracting the survivor counts (N) resultant from PL treatment from the initial counts (No) represented by the control samples. Results were expressed in log CFU/mL. Additionally, survivor curves were built by creating scatter plots of log survivor counts (N) versus treatment time (t).

5.1.4 Statistical Analysis

The data obtained was analyzed using a statistical analysis system (Graph Pad Prism 5.0). Analysis of variance (one-way ANOVA) was performed and the significant differences in the means were separated using the Tukey's studentized range test. The data was tabulated as an average of triplicates \pm standard deviation, and the level of significance was determined at $P < 0.05$.

5.2 Results and Discussion

In order to demonstrate efficiency of PL on inactivation of heat resistance bacteria in liquid medium, PL had been applied to inactivate LAB cells in PG samples up to 25 s treatment times at distance of 6 and 9 cm from the quartz window of the lamp. The initial LAB count obtained was 3.9-log_{10} CFU/mL for control groups at 30.6°C .

The efficiency of PL on inactivation of LAB in PG samples is reported based on exposure time, sample thickness and distance from the quartz window (Table 5-1 and 5-2). In accordance with the literature, when distance from the light source decreased and exposure time increased concomitantly, the samples receive more intense treatment (Gómez-López and others 2005a). Hence, as it can be seen in Figure 3-7 and 3-8, higher increase in sample temperature could be expected with extended exposure period at shorter distance from the quartz window. The temperature rise during PL treatment is also strongly dependent on properties of samples such as viscosity and color (Oms-Oliu and others 2010) but these were not factors studied here.

As shown in Table 5-1, the complete inactivation for 5 mL sample was observed after 10 s PL treatment at 6 cm distance from the quartz window and this corresponded to an increase in temperature in the sample of 17°C (Figure 3-5). After 10 s treatment of PL there was still countable LAB colonies in 15 mL PG sample likely due to the

increased sample thickness and poor penetration of pulse light into the sample (Figure 3-5 vs. 3-6). The sample depth and distance from the quartz window both had significant impact on survival ($p < 0.05$). No significant temperature increase was observed for the first 10 s between all sample volumes (Figure 3-5); however, when the treatment duration continued after several seconds there was a non-linear increase in temperature until 20 s at 6 cm distance (Figure 3-5). In contrast, a linear increase was observed for all sample volumes for the longer treatments of 50 s (Figure 3-7). In the second study we observed that while increase in distance of sample surface from lamp (9 cm) had a significant ($p < 0.05$) effect on inactivation of LAB (Table 5-2), there was no significant increase in temperature within shorter time treatment between sample volumes at 9 cm distance (Figure 3-7). As mentioned in Chapter 3 the inactivation of LAB was not likely relevant to thermal effect because of no significant increase in temperature for 5 s ($\approx 11.0^\circ\text{C}$ (Figure 3-6)). Also these increases in temperature is likely significant on reduction of some microorganism which are not resistance to heat treatment. Krishnamurthy (2006) investigated effectiveness on inactivation of *S. aureus*. The maximum log reduction of *S. aureus* ($8.6 \log_{10}$ CFU/mL) was observed in 30 mL of milk for 180 s at 8 cm sample distance from quartz window (Krishnamurthy 2006). The water content of bacteria vaporized while leading to bacterial disruption and ultimately inactivation (Takeshita and others 2003). In *Aspergillus niger* spores, the overheating due to internal explosion and resulted in “evacuation” of the cell contents during the light pulse. In this study, we did not observe any significant evacuation for PG samples because of its density and also high boiling point (187.4°C). However, while thickness of samples was kept shallow, the effectiveness of PL on inactivation of heat resistance

LAB in microflora of PG was investigated with short time PL treatment at different distances (6 and 9 cm) and also different volumes of PG samples (5,10 and 15 mL).

As mentioned Chapter 1, PL is known as novel technology, which is limited only for short time treatments. The absorption of energy for longer treatments cause increase in temperature. Pulsed UV-light treatment is considered non-thermal, but holds only for short time treatments. Temperature increases as absorbed energy accumulates during longer treatments. For 20 s treatment time caused ≈ 25.4 and 20.6°C increase while 5 s treatment time increased ≈ 12.9 and 11.0°C in all sample volumes at 6 and 9 cm distance from quartz window, respectively. Finally, our results demonstrated that the effect of treatment duration and the interaction (treatment time*depth) have significant ($p<0.05$) effect on inactivation of LAB in PG samples. A complete inactivation was obtained for samples treated with 25 s for all sample sizes. The average corresponding \log_{10} reduction was $3.92\text{-}\log_{10}$ CFU/mL (Figure 5-1).

Table 5-1. Log reduction of lactic acid bacteria in propylene glycol during Pulsed UV light treatment with in increase in temperature for 6 cm distance from quartz window

Treatment Time (s)	Reduction (Log CFU/mL)		
	5 mL	10 mL	15 mL
5	3.4 ± 0.8	2.4 ± 0.0	1.6 ± 0.0 ^α
10	3.9 ± 0.0	3.5 ± 0.8*	2.8 ± 1.0
15	3.9 ± 0.0	3.9 ± 0.0**	3.5 ± 0.8*
20	3.9 ± 0.0	3.9 ± 0.0**	3.9 ± 0.0**
25	3.9 ± 0.0	3.9 ± 0.0**	3.9 ± 0.0**

One Way ANOVA followed by Tukey's test. *P<0.05,**P<0.01 vs 5 seconds and αP<0.05 vs 5 mL.Data are expressed as mean ± standard deviation (SD). CFU: Colony forming unit

Table 5-2. Log reduction of lactic acid bacteria in propylene glycol during Pulsed UV light treatment with in increase in temperature for 9 cm distance from quartz window

Treatment Time (s)	Reduction (Log CFU/mL)		
	5 mL	10 mL	15 mL
5	2.8 ± 1.1	2.0 ± 0.1	1.0 ± 0.0 ^{α,β}
10	3.9 ± 0.0	3.0 ± 0.8*	2.1 ± 0.0 ^{***, αα}
15	3.9 ± 0.0	3.5 ± 0.8 ^{***}	3.5 ± 0.8 ^{***}
20	3.9 ± 0.0	3.9 ± 0.0 ^{***}	3.9 ± 0.0 ^{***}
25	3.9 ± 0.0	3.9 ± 0.0 ^{***}	3.9 ± 0.0 ^{***}

One Way ANOVA followed by Tukey's test. *P<0.05, **P<0.01, ***P<0.001 vs 5 seconds and P<0.05, αα P<0.01 vs 5 mL; β<0.05 vs 10 mL. Data are expressed as mean ± standard deviation (SD). CFU: Colony forming unit

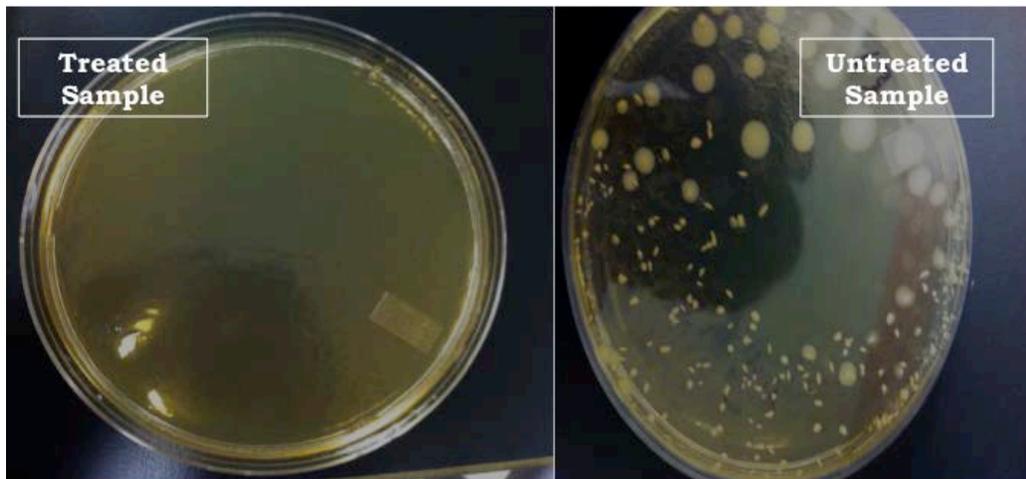


Figure 5-1. The formation of lactic acid bacteria colonies in the untreated and treated samples for 25s of PL (all samples volumes were similar) (Photo courtesy of Samet Ozturk)

CHAPTER 6 CONCLUSIONS

The main focus of this study was to investigate the potential efficacy of PL on temperature profile and microflora of PG cooling medium. All these findings demonstrated that PL treatment resulted in reduction in microflora and increase in temperature of PG. Consistent with previous studies (Krishnamurthy 2006), this effect depended on sample thickness, which affects the penetration depth of PL, distance from the quartz window and exposure time. As the sample volume increases, the inactivation ratio decreases because of poor penetration of the PL into the sample. Although there was no significant temperature rise for all volumes after 10 s treatment times, the inactivation of APC and LAB in PG microflora was achieved, indicating a non-thermal effect of PL. Thus it may be concluded that the inactivating effect of PL on microorganisms was via its photo-chemical and photo-thermal properties in shorter time course (<10 s). On the other hand, at the shorter times (<10 s) the temperature increase, although not significant, may have a considerable effect on some species like psychrophilic bacteria since they and their enzymes are sensitive to lower temperature increases. The effect of PL is also related to the microorganism type, i.e. Boeger (1999) demonstrated the *Cryptosporidium parvum*, a protozoa of major concern in water which is less resistant than *Bacillus subtilis* spores, was more susceptible to PL. There was a significant increase in the temperature in the 5 mL PG sample at 15 s indicating that extended exposure time caused this high increase in temperature. Others also have reported an increase in temperature when treatment time increased (Demirci and others 2003). There was a greater increase in temperature as the sample was located closer to light source (6 vs. 9 cm). Based on these studies, it recommended that at 6 cm

distance, 25 s treatment times showed complete reduction for APC and LAB in PG microflora. However, at 9 cm distance from the quartz window 25 s treatment times was not sufficient in complete reduction of APC bacteria. It is likely due to the poor penetration or lower thermal effect. For LAB, the treatment time of 25 s for either 6 or 9 cm distance from the light source was sufficient for complete reduction of bacterial population in PG microflora but reduction levels of both distance had differences. Therefore, further studies are required to determine exact treatment times based on type of contamination, characteristic of microorganism, location of sample from the light source, sample thicknesses and also thermal properties of sample.

CHAPTER 7 RECOMMENDATION

These results may encourage future studies, which may be conducted with initial microbial load, different microorganism targets, repair mechanisms of microorganisms and their impact on quality and safety of food and devices. The initial population of APC and LAB in PG may not be representative of real life situations since the degree of contamination on food, surface or equipment tends to be lower. The efficiency of PL treatment may be associated level of contamination on food samples, and also inappropriate environmental conditions which may contribute to light attenuation. Moreover, under inconvenient environmental conditions and handling methods microorganisms may accumulate and attach to surfaces forming biofilms making their inactivation more and more difficult. A more detailed research needs to be done to find an optimum condition for the evaluation of inactivating effects of PL for continuous flow conditions to represent commercial cases. While setting up a light source of PL around the tubes, which PG is inside, to treat used PG after circulation in the system. Based on this system, the dimer of tubes, permeability of tubes according to light and location of light source are needed to be determining before to apply in industry. Furthermore, the efficacy of the PL can be compared to equivalent energy continuous UV-light and infrared heating to conventional heating in order to demonstrate potential effects of PL on PG microflora. Also, future studies need to evaluate temperature profiling during PL illuminations using a fiber optic sensor or infrared thermal imaging technique for static or continuous systems.

In conclusion this study indicates that PL treatment may be an alternative technology to prevent contamination and also remove formed biofilms in or on closed system for future studies.

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BIOGRAPHICAL SKETCH

Samet Ozturk was originally from Sivas, Turkey. He received his bachelor's degree in food engineering from Gaziosmanpasa University in 2009. After a one-year of industry experience, he received a scholarship from the Ministry of Education of Turkey in 2010, which gave him the opportunity to study abroad, in the U.S. He attended to UC Davis Intensive English Course before he entered a Food Science Master of Science program at the University of Florida under the supervision of Dr. Wade Yang. During his matriculation in his Masters' program, Samet presented his research at the institute of Food Technologists meeting, LA, Nevada in 2012. Samet plans to continue his academic journey by pursuing his Ph.D. degree upon his completion of his master's program.