DECONTAMINATION OF MILK AND WATER BY
PULSED UV-LIGHT AND INFRARED HEATING

A Thesis in
Agricultural and Biological Engineering

by
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Abstract

Efficacy of pulsed UV-light and infrared heating for inactivation of pathogens was investigated. Pulsed UV-light was very effective in inactivating *S. aureus* on agar seeded cells and in phosphate buffer. Complete inactivation of *S. aureus* was achieved within 5-s treatments.

Raw milk inoculated with *S. aureus* was treated with pulsed UV-light by varying distance of milk sample from the quartz window, volume of milk, and treatment time. The log$_{10}$ reduction obtained varied from 0.16 to 8.55 log$_{10}$ CFU/ml. Complete inactivation of *S. aureus* was obtained at two conditions with corresponding reductions of 8.55 log$_{10}$ CFU/ml.

Continuous treatment of milk was tested in order to determine the feasibility of industrial application of pulsed UV-light treatment. Reductions of *S. aurues* in milk varied from 0.55 to 7.26 log$_{10}$ CFU/ml. Complete inactivation was achieved at two conditions. Sensory evaluation of pulsed UV-light treated pasteurized skim milk and 1% milk suggests that there was some perceivable change in the quality.

*B. subtilis* spores in water were treated with pulsed UV-light in an annular flow chamber. Flow rates up to 14 L/min resulted in complete inactivation of *B. subtilis* spores. No growth was observed during incubation under light and no-light conditions.

The efficacy of infrared heating on inactivation of *S. aureus* in milk was tested. The effect of depth of milk, infrared lamp temperature, and treatment time were investigated. Reductions of 0.10 to 8.41 log$_{10}$ CFU/ml were obtained for treatments up to 4 min.
The inactivation mechanism for pulsed UV-light and infrared heating was investigated using transmission electron microscopy (TEM) and spectroscopy. After 5-s treatment with pulsed UV-light, cell wall breakage, cytoplasm leakage, damage in the cellular membrane structure, and leakage of the cell content were observed by TEM. Infrared heat treated cells exhibited condensation of cytoplasm, cytoplasmic membrane damage, cell wall damage, and cellular content leakage occurred. The FTIR spectrometry was successfully used to classify the pulsed UV-light and infrared treated cells.
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Dedicated to

HH Romapada Swami Maharaj
1. Introduction

Food preservation techniques have been a part of the daily life of the human race for thousands of years because of food deterioration by pathogenic and spoilage microorganisms. Food preservation techniques are critical for modern mass food production and distribution. As such, various preservation technologies have been developed and adopted successfully. Although several technologies such as, heat treatment, cold temperature treatment, irradiation, microwave radiation, pulsed electric field, magnetic field, high pressure, and ohmic heating treatments are available for inactivation of these microorganisms (Juneja and Sofos, 2002), there is always a need to investigate novel technologies as an alternative to existing preservation methods to improve efficiency, minimize cost, and yield minimal quality changes.

In 2000, the United States produced 167.7 billion pounds of milk worth more than $20 billion (IDFA, 2001). Milk is used in the manufacture of dairy products as well in the manufacture of a wide variety of food products (IDFA, 2001). Improper handling and management practices could result in contamination of the udder (Bramley and McKinnon, 1990) and contamination of milk by pathogenic microorganisms. Hence, effective inactivation is central to assuring milk safety. Conventional milk pasteurization (heat treatment of milk) methods not only require a vast amount of energy, but also lack precise control and thus incur a high capital and operational cost. In addition, during the pasteurization process, the warm areas in the heat exchangers are conducive for the growth of *Staphylococcus aureus*, a pathogenic microorganism.

In general, milk is held until it reaches the specified temperature during pasteurization for a prolonged residence time, providing conducive environment for
Among these pathogens, \textit{S. aureus} is the most common cause of suppurating infections such as boils and pimples (Martin et al., 2001) and a common cause of mastitis. According to Centers for Disease Control and Prevention (CDC), there have been 17,248 cases of \textit{S. aureus} reported during 1973-87, which accounted for 14\% of all the cases because of bacterial pathogens (Bean and Griffin, 1990; Bean et al., 1990; Olsen et al., 2000). Therefore, it is necessary to inactivate \textit{S. aureus} in food to ensure safety of the public. Pulsed UV-light treatment and infrared heating may serve as potential methods for inactivation of this pathogen effectively.

UV-light has been used as a bactericidal agent from as early as 1928 (Xenon, 2003). UV-light is a portion of the electromagnetic spectrum ranging from 100 to 400 nm wavelengths and has the potential to induce damage to the DNA by forming thymine dimers (Bank et al., 1990; Miller et al., 1999). These dimers prevent the microorganism from DNA transcription and replication, which leads to cell death (Miller et al., 1999). UV-light can be applied in two modes, namely continuous UV-light mode and pulsed UV-light mode. The pulsed UV-light provides higher instantaneous energy intensities than continuous UV-light and hence is more effective in inactivating pathogens. Pulsed UV-light is produced by accumulating the energy in a capacitor and releasing it as a short duration pulse (in the order of nano seconds) to magnify the power greatly. UV-light treatment provides a cost-effective alternative to the existing heat pasteurization and preservation methods. Furthermore, taste degradation of milk subjected to UV-light treatment is minimal, because UV-light treatment can be accomplished at an ambient temperature (Hollingsworth, 2001).
Also, water is used in large quantities in the food industry for wide applications ranging from cleaning equipments to processing/formulating food. Therefore, it is necessary to disinfect water. Pulsed UV-light can be effectively used to inactivate microorganisms present in water since water has a very high transmissivity for UV-light. Continuous UV-light has been traditionally used for industrial disinfection of clean and waste water. *Bacillus subtilis* is a surrogate microorganism which can be used to study the effect of a disinfection process. As a spore former, they are resistant to many disinfectants including UV-light. Therefore, one can assure that less resistant pathogenic vegetative cells are inactivated completely by proving the inactivation of *B. subtilis* spores.

Previous studies by several researchers indicate that pulsed UV-light is up to four times more effective than continuous UV-light for inactivation of pathogenic microorganisms when the total energy provided is the same. However, there is no study done to evaluate the reasons for the enhanced effectiveness. Therefore, the effect of pulsed UV-light on *S. aureus* was investigated using spectroscopy and microscopy in order to explain the underlying inactivation mechanism. It was proposed that increased instantaneous energy and a shocking effect because of pulsing are responsible for increased inactivation.

Infrared radiation is part of the electromagnetic spectrum in the wavelength range between 0.5 and 1,000 μm (Rosenthal, 1996). Though infrared heating is mainly used for processing food materials, this approach can also be used for pathogen inactivation. However, there are no extensive studies performed on its application for pathogen inactivation in food systems. Several researchers have suggested numerous targets for
inactivation such as DNA, RNA, ribosome, cell envelope, and proteins in the cell (Sawai et al., 1995). Far-infrared radiation can be used for heating of food systems and inactivation of pathogens because of stronger absorption of microorganism and food components in the far-infrared wavelength range (3 to 1,000 μm).

Therefore, the overall objective of this study was to investigate the ability of pulsed UV-light and infrared heating as alternative technologies to inactivate pathogenic microorganisms in milk or water.
2. Literature Review

2.1. Milk and its microbiological significance

Milk is a biological fluid comprised of several components, such as lipids, fatty acids, proteins, carbohydrate, salts, and other trace elements (Banks and Dalgleish, 1990). Due to the presence of nutritious components, milk serves as a favorable medium for microbial growth. *Campylobacter jejuni, Pseudomonas* spp., *Xanthomonas maltophilia, Acinetobacter* spp., *Moraxella* spp., *Neiseria* spp., *Kingsella* spp., *Ateromonas putrefaciens, Flavobacterium* spp., *Alcaligenes* spp., *Brucella* spp., *Escherichia* spp., *Citrobacter* spp., *Salmonella* spp., *Enterobacter* spp., *Erwinia* spp., *Serratia* spp., *Micrococcus* spp., and *Staphylococcus* spp. are common microorganisms associated with milk (Gilmour and Rowe, 1990). The microbial population in milk also increases because of mastitis, where the organisms enter the udder through the duct at the teat tip and colonize within the udder. Some of the microorganisms such as *S. aureus* are pathogenic and others are spoilage microorganisms. Therefore, it is necessary to treat milk in order to inactivate these spoilage and pathogenic microorganisms. Traditionally, heat pasteurization is used to achieve this goal. However, the cost associated with pasteurization is high because of high energy demands and high capital/operational costs. Also, *S. aureus* can survive because of the prolonged holding time required during the pasteurization process. Therefore, it is necessary to find alternative processes that are cost-effective and efficient for inactivation of *S. aureus* present in milk. Pulsed UV-light and infrared heating may be used to achieve this goal.
2.2. *Staphylococcus aureus*

*Staphylococcus aureus* is a Gram-positive, spherical shaped bacterium, which appears in pairs, short chains, or grape like structures (Figure 2.1). *S. aureus* produces several toxins, including toxic shock syndrome toxin-1, staphylococcal enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SHE, SEI, SEJ), exfoliative toxins (ETA, ETB), α-, β-, δ-, and γ-hemolysins, and Panton-Valentine leukocidin (PVL) (Yarwood and Schlievert, 2001).

![Figure 2.1. *Staphylococcus aureus* (obtained with permission from Dennis Kunkel Microscopy, Inc., Kailua, HI).](image)

*S. aureus* can cause both infection (direct ingestion of vegetative cells resulting in multiplication in human body and production of toxins) and intoxication (ingestion of toxins which are already produced by bacterial growth). A toxin dose level of 1 μg can cause disease in humans. *S. aureus* exists in air, water, sewage, dust, human, and animals (CFSAN-FDA, 1992). Cows affected by mastitis are a big source of *S. aureus* contamination in milk. Nausea, vomiting, retching, abdominal cramping, prostration,
headache, muscle cramping, and transient changes in blood pressure and pulse rate are the symptoms of \( S. \text{ aureus} \) infection/intoxication. In severe cases, \( S. \text{ aureus} \) can cause death in infants, elderly, and people with weak immune system.

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### 2.3. \textit{Bacillus subtilis}

\textit{Bacillus subtilis}, a Gram positive, rod shaped, spore forming soil microorganism (Figure 2.2), though generally considered as non-pathogenic is rarely associated with food poisoning. Food poisoning because of consumption of \( B. \text{ subtilis} \) contaminated cooked turkey, muffin, custard powder, pickled onion, mayonnaise, corn flour gel, canned bean salad, synthetic fruit drink were reported in the past (Kramer and Gilbert, 1989).

![Bacillus Subtilis](https://www.microscopyconsulting.com)

Figure 2.2. \textit{Bacillus Subtilis} (Source: Microscopy consulting services Inc., www.microscopyconsulting.com).

Severe vomiting, abdominal cramps, diarrhea are common symptoms of \( B. \text{ subtilis} \) food poisoning. \( B. \text{ subtilis} \) spores are normally very resistant to several food
processing steps and able to survive pasteurization temperatures. Therefore, it is necessary to inactivate the spores in order to ensure safety. *B. subtilis* also serves as a surrogate organism to represent other dangerous microorganisms of the same genus such as *B. cereus* and *B. anthracis*. As these microorganisms are genetically similar, a process which inactivates *Bacillus subtilis* may also effectively inactivate pathogenic *B. anthracis* and *B. cereus*.

The following sections, namely, irradiation, high hydrostatic pressure, pulsed electric field, centrifugation, membrane filtration, UV-light treatment, and infrared heating will discuss conventional pasteurization techniques and technologies for the inactivation of *S. aureus*, *B. subtilis* and/or other pathogens.

### 2.4. Pasteurization of milk

Conventionally, heat treatment is used for pasteurization of milk. For pasteurization, milk is held at 62.8°C for 30 min (low temperature-long time: LTLT), 71.7°C for 15 s (high temperature-short time: HTST), or 137.8°C for 2 s (Ultra high temperature: UHT) (Varnam and Sutherland, 1994; Zall, 1990). Milk is tested for the presence of alkaline phosphatase in the pasteurized milk in order to ensure that milk has been adequately pasteurized (Cornell, 1998). Alkaline phosphatase has higher heat stability than the pathogens present in the milk and hence used as an indicator of ensuring adequate pasteurization. Normally milk is preheated in the regenerator section of the pasteurization unit in order to increase the thermal efficiency and save energy. The preheated milk flows through a heat exchanger, which consists of series of plates in which the milk and the heating medium are moving counter-currently. The milk passes
through the holding tube, where the milk is kept at the desired temperature for the minimum specified time (Varnam and Sutherland, 1994). Though pasteurization is effective in inactivating microorganisms, the method has many disadvantages. Nutritional quality of milk decreases during heat pasteurization processing and storage, losses in vitamins, proteins, lactose, and minerals especially occur (Varnam and Sutherland, 1994). Heat pasteurization also affects the structure and quality of milk because of protein denaturation, enzyme inactivation, lipid oxidation, and non-enzymatic browning. *Campylobacter* spp., *L. monocytogenes*, *Yersinia enterocolitica*, *S. aureus*, and *Bacillus cereus* may be present in pasteurized milk because of underprocessing and post-processing contamination (Varnam and Sutherland, 1994). Warm spots are created in the equipment because of prolonged residence time. The warm spots may promote microbial growth by providing a conducive environment. Therefore, there is a need for other innovative techniques to ensure the safety of milk. Among several alternative techniques available, irradiation, high hydrostatic pressure, pulsed electric field, centrifugation, membrane processing, pulsed UV-light treatment, and infrared heating are discussed in the following sections.

### 2.5. Gamma irradiation

Gamma radiation has a very high energy level and can disrupt even the most stable atom, which in turn produce ions and free radicals. These free radicals damage the DNA and RNA of bacterial cells leading to cell death (Graham, 1992). The effects of irradiation on biological materials can be direct, where the energy is directly utilized in breakage of chemical bonds in DNA or RNA; or indirect, wherein the free radicals
produced ionize water and other food components and produce further radicals, which in turn result in cell damage. Thus the free radicals damage of DNA or RNA molecules is hypothesized as a major destruction mechanism by irradiation (Desrosier and Desrosier, 1977). Irradiation dose is expressed in kiloGray (kGy) (The amount of energy equivalent to 1 kJ/kg absorbed by a material from ionizing radiation is equivalent to 1 kGy). The $D_{10}$ values of *S. aureus* and *B. cerus* spores are 0.26-0.6 and 1.6 kGy, respectively (Farkas, 2001). Generally milk and many dairy products are unsuitable for irradiation because of increased quality changes (Crawford and Ruff, 1996).

### 2.6. High hydrostatic pressure

High Hydrostatic Pressure (HHP) treatment is the application of high hydrostatic pressure in the range of several hundred Mega Pascals (MPa) on the food normally submerged in liquid. High hydrostatic pressure has the following effects on microorganisms: 1) cell envelope-related effects, 2) pressure induced cellular changes, 3) biochemical aspects, and 4) effect on genetic mechanisms (Hoover, 2001). These changes are because of increased pressure and altered cellular morphology. The cell division slows down because of HHP because of the altered cellular morphology (Hoover, 2001). HHP is very effective in pathogen reduction as demonstrated by several researchers. For example, Patterson et al. (1995) obtained $5 \log_{10}$ CFU/ml reduction of *S. aureus* at 700 MPa pressure when treated for 15 min.
2.7. **Pulsed electric fields**

Pulsed Electric Fields (PEF) involve high voltage electric pulses, which are very effective in inactivating pathogens (Molina et al., 2002). PEF induce electroporation and disruption of semi-permeable membranes leading to breakdown of organelles by swelling or shrinking (Castro, 1993). The main inactivation mechanism is the increase in cell permeability because of compression and poration (Vega-Mercado et al., 1996). Several researchers demonstrated that PEF is effective in inactivation of several pathogens. For instance, a 9 log$_{10}$ reduction of *E. coli* suspended in simulated milk ultrafiltrate was obtained by Zhang et al. (1994) when a converged electric field intensity of 70 kV/cm was applied for 160 $\mu$s. *S. aurues* population in skim milk was reduced by 3.7 log$_{10}$ CFU/ml when exposed to 3.5 kV/mm electric field strength for 450 $\mu$s when a stepwise mode fluid transmission system was used (Evrendilek et al., 2004).

2.8. **Centrifugation and membrane filtration**

Bacterial removal by centrifugation can be utilized in milk processing. Removal of bacteria and bacterial spores by high speed centrifugation is called bactofugation. It is commonly used in Europe. Centrifugation is a highly energy demanding process typically resulting in 90-95% reduction of bacterial spores (Guerra et al., 1977). Su and Ingham (2000) reported that bacterial spores can be effectively removed from milk by this process. Spores of *Clostridium tyrobutyricum*, *Clostridium butyricum*, *Clostridium sporogenes*, and *Clostridium beijerinckii* in skim milk were removed by subjecting the milk to various centrifugal forces ranging from 3,000 x g to 12,000 x g. Spore reductions of more than 97% were achieved when skim milk was centrifuged at 12,000 x g for 30
seconds for the abovementioned microorganisms. As the centrifugal force increased, spore removal also increased.

Several membrane filtration techniques such as microfiltration and ultrafiltration can be utilized for cold pasteurization of milk. This preserves the milk quality because of the absence of heat induced quality changes. Microfiltration is much more effective than centrifugation for removal of bacteria and bacterial spores (Brans et al., 2004). *Clostridium tyrobutyricum* and *Bacillus cereus* spores were reduced by more than $5 \log_{10}$ CFU/ml when the permeate flux of 400 kg/h/m² was used for 150 min in a reverse asymmetric membrane with average pore size of 0.87 µm (Guerra et al., 1997). Grant et al. (2005) achieved up to 95% reduction of *Mycobacterium avium* sbsp. *paratuberculosis* in whole milk by centrifugation of pre-heated milk at 60°C at $7000 \times g$ for 10 s or microfiltration through a filter of 1.2 µm pore size.

### 2.9. Ultraviolet-light

Ultraviolet (UV) light has been used as a bactericidal agent from as early as 1928 (Xenon, 2003). UV-light is divided into four regions namely UV-A (315-400 nm), UV-B (280-315 nm), UV-C (200-280 nm), and vacuum UV (100 to 200 nm) according to their wavelength (Perchonok, 2003). UV-light can be applied in two modes namely continuous mode and pulsed mode. In continuous mode, constant energy UV-light is released continuously in a monochromatic or polychromatic wavelength. In the pulsed mode, the electrical energy is stored in a capacitor over a short period of time (few milliseconds) and released as very short period pulses (several nanoseconds). The electrical energy is transferred 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 region of ultraviolet to near infrared. The intensity of pulsed light is 20,000 times more than that of sunlight (Dunn et al., 1995). Typically the pulse rate is 1 to 20 pulses per second and the pulse width is 300 ns to 1 ms. Therefore, light pulses with high energy in several Megawatts are produced though the total energy is comparable to continuous UV-light system. Pulsed UV-light treatment is a more effective and rapid way of inactivating the microorganisms than continuous UV-light sources because the energy is multiplied many fold (Dunn et al., 1995).

Pulsed light is a broad spectrum radiation from UV-light to infrared radiation. Pulsed light is also referred to pulsed UV-light, high intensity light, UV-light, broad-spectrum white light, pulsed white light, and near infrared light (Green et al., 2003). Typically, the wavelength of pulsed light ranges from 100 to 1100 nm. As the majority of the energy was received from UV-light portion in this study, the term pulsed UV-light was used throughout this thesis (54% Ultraviolet, 26% Visible, and 20% Infrared; Xenon, 2003). The UV portion of the pulsed UV-light has higher energy level followed by visible light and infrared region (Table 2.1).

Previous research shows that pulsed UV-light is at least two times effective as the continuous UV-light (McDonald, 2000). Pulsed UV-light may have some shocking effect on the cell wall of bacteria in addition to the effect of high intensity pulses. But more research has to be done before arriving at a conclusion. Localized heating of bacteria is induced by pulsed UV-light as the heating and cooling rate of bacteria and the surrounding matrix is different (Fine and Gervais, 2004). Pulsed UV-light is gaining attention in recent years, because it can provide sufficient antimicrobial inactivation and
commercial sterilization with no toxic by-products (FDA, 2000). It can be effectively used to inactivate pathogens on the surface of food or packaging materials. Furthermore, it can also be used for in-package sterilization if a packaging material can allow UV-light to penetrate (Butz and Tauscher, 2002).

**Interaction of light and matter**

Light consists of discrete fundamental packets of energy known as photons which have zero mass, no electric charge, and an indefinitely long lifetime. These photons contain vast amount of energy, which is determined by the wavelength of light (Equation 2.1). For instance, UV-light photon at 254 nm has energy of 470 kJ/mol (Table 2.1).

\[
E = h\nu = \frac{hc}{\lambda}
\]  
(Equation 2.1)

where, \(E\) is the energy of photon, \(h\) is the Planck’s constant \((6.626 \times 10^{-34} \text{ J.s})\), \(\nu\) is the frequency of light, \(c\) is the speed of light in vacuum, and \(\lambda\) is the wavelength of light.

Typical quantum energy of photons is given for the region of pulsed UV-light in Table 2.1. Photons in ultraviolet region have more energy (Table 2.1) than visible or infrared region and hence it accounts for predominant inactivation of pathogens.

When a pulsed light of initial intensity \((I_o)\) falls on food surface, portion of the light is transmitted through the food, while the rest is reflected back and/or scattered. As the pulsed UV-light penetrates through the food material, its intensity decays along a distance of \(x\) beneath the food surface (Palmieri et al., 1999) given by

\[
I = TI_o e^{-x}
\]  
(Equation 2.2)

where, \(T\) is the transparency coefficient of the food material, \(I\) is the intensity of light at a distance \(x\) from the surface, \(I_o\) is the initial intensity of light, and \(x\) is the distance below
the food surface. The residual amount of light is dissipated as heat and transferred to the inner layers through conduction (Palmiri et al., 1999). Therefore, the intensity of UV-light exponentially decays within the food material. UV-light is more effective for surface sterilization and sterilization of highly transparent liquids such as water.

Table 2.1. Characteristics of UV, visible, and infrared regions of electromagnetic spectrum.

<table>
<thead>
<tr>
<th>Region</th>
<th>Wavelength (nm)</th>
<th>Frequency (Hz)</th>
<th>Photon energy (eV)</th>
<th>Molar photon energy (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum UV</td>
<td>100 – 200</td>
<td>3.00x10^{16} to 3.00x10^{15}</td>
<td>124 to 12.4</td>
<td>11975 to 1197</td>
</tr>
<tr>
<td>UV-C</td>
<td>200 – 280</td>
<td>3.00x10^{15} to 1.07x10^{15}</td>
<td>12.40 to 4.43</td>
<td>1197 to 427</td>
</tr>
<tr>
<td>UV-B</td>
<td>280 – 315</td>
<td>1.07x10^{15} to 9.52x10^{14}</td>
<td>4.43 to 3.94</td>
<td>427 to 380</td>
</tr>
<tr>
<td>UV-A</td>
<td>315 – 400</td>
<td>9.52x10^{14} to 7.49x10^{14}</td>
<td>3.94 to 3.10</td>
<td>380 to 299</td>
</tr>
<tr>
<td>Visible light</td>
<td>400 – 700</td>
<td>7.49x10^{14} to 4.28x10^{14}</td>
<td>3.10 to 1.77</td>
<td>299 to 299</td>
</tr>
<tr>
<td>Near Infrared</td>
<td>700 – 1400</td>
<td>4.28x10^{14} to 2.14x10^{14}</td>
<td>1.77 to 0.89</td>
<td>171 to 171</td>
</tr>
<tr>
<td>Mid infrared</td>
<td>1400 – 3000</td>
<td>2.14x10^{14} to 9.99x10^{13}</td>
<td>0.89 to 0.41</td>
<td>85.5 to 85.5</td>
</tr>
<tr>
<td>Far infrared</td>
<td>3000 – 10000</td>
<td>9.99x10^{13} to 3.00x10^{13}</td>
<td>0.41 to 0.12</td>
<td>39.9 to 12.0</td>
</tr>
</tbody>
</table>

Less transparent foods have to be treated in a thin layer to overcome the penetration limitation. Also for liquid foods, good mixing aids in uniform exposure.

UV-light is absorbed and penetrates into the microorganism depending upon the chemical composition, size of the microorganism, wavelength of interest, and medium of introduction etc. (Table 2.2).

Table 2.2. Percent transmission to the center of selected cells and viruses*.

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>Diameter (µm)</th>
<th>Percent transmission at selected wavelengths (%)</th>
<th>200 nm</th>
<th>250 nm</th>
<th>300 nm</th>
<th>350 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus (Herpes simplex)</td>
<td>0.15</td>
<td>66</td>
<td>80</td>
<td>100</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Bacteria</td>
<td>1</td>
<td>33</td>
<td>78</td>
<td>98</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>5</td>
<td>1.6</td>
<td>69</td>
<td>97</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

*Coolhill, 1995.
Table 2.3 lists the chemical bond energy of some common chemical bonds. It is clear that UV-light has sufficient energy to break most of the chemical bonds. Hence, ultraviolet light can cause cleavage in organic compounds. Ultraviolet light has the energy in the magnitude of covalent bond energy, thus it mainly breaks the covalent bonds of the target material.

Table 2.3. Strength of common bonds in biomolecules*.

<table>
<thead>
<tr>
<th>Chemical Bond Type</th>
<th>Wavelength</th>
<th>Bond dissociation energy (kJ/mole)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N≡N</td>
<td>129</td>
<td>930</td>
</tr>
<tr>
<td>C≡C</td>
<td>147</td>
<td>816</td>
</tr>
<tr>
<td>C=O</td>
<td>168</td>
<td>712</td>
</tr>
<tr>
<td>C=N</td>
<td>195</td>
<td>615</td>
</tr>
<tr>
<td>C=C</td>
<td>196</td>
<td>611</td>
</tr>
<tr>
<td>P=O</td>
<td>238</td>
<td>502</td>
</tr>
<tr>
<td>O-H</td>
<td>259</td>
<td>461</td>
</tr>
<tr>
<td>H-H</td>
<td>275</td>
<td>435</td>
</tr>
<tr>
<td>P-O</td>
<td>286</td>
<td>419</td>
</tr>
<tr>
<td>C-H</td>
<td>289</td>
<td>414</td>
</tr>
<tr>
<td>N-H</td>
<td>308</td>
<td>389</td>
</tr>
<tr>
<td>C-O</td>
<td>340</td>
<td>352</td>
</tr>
<tr>
<td>C=C</td>
<td>344</td>
<td>348</td>
</tr>
<tr>
<td>S-H</td>
<td>353</td>
<td>339</td>
</tr>
<tr>
<td>C-N</td>
<td>408</td>
<td>293</td>
</tr>
<tr>
<td>C-S</td>
<td>460</td>
<td>260</td>
</tr>
<tr>
<td>N-O</td>
<td>539</td>
<td>222</td>
</tr>
<tr>
<td>S-S</td>
<td>559</td>
<td>214</td>
</tr>
</tbody>
</table>


As the energy of photons in UV-light range is high they can even cause ionization of molecules, whereas visible light and infrared region causes vibration and rotation of molecules, respectively. When molecules absorb the energy, it is elevated to an excited state. The excited molecule can either 1) relax back to the ground state by releasing the
energy as heat, 2) relax back to the ground state by releasing energy as photons, or 3) can induce some chemical changes.

**Inactivation mechanism of UV-light disinfection**

UV-light exhibits germicidal properties in the wavelength range of 100 to 280 nm (UV-C region). The inactivation efficiency follows a bell shaped curve where maximum inactivation occurs approximately at 254 to 264 nm range (Figure 2.3). Therefore, it is crucial to design a disinfection system, which emits higher intensities of wavelengths in the absorption region of interest. However, typical mercury UV lamps can deliver at 254 nm maximum. Therefore, it is usually mentioned that UV inactivation is at 254 nm as shown in Figure 2.3.

![Figure 2.3. Germicidal efficiency of ultraviolet light (Masschelein, 2002).](image)

As only the absorbed UV-light energy induces photophysical, photochemical, and/or photothermal effect necessary for inactivation of pathogenic microorganisms, it is
crucial to have a proper lamp design. Base pairs of DNA absorb UV-light effectively because of their aromatic ring structure. In general, pyrimidines (Thymine (DNA), cytosine (DNA and RNA) and Uracil (RNA)) are strong absorbers of photons in the Ultraviolet range and thus produce photoproducts which results in bacterial inactivation (Masschelein, 2002).

UV-light damages the DNA of bacteria by primarily forming thymine dimers and thus bacterial DNA cannot be unzipped for replication. Hence bacteria cannot reproduce (Bank et al., 1990; Jay, 2000; Demirci, 2002). Though cyclobutyl pyrimidine dimer formation is the main inactivation mechanism, there are other photoproducts formed during UV-light exposure including pyrimidine pyrimidinone [6-4]-photoproduct, Dewar pyrimidinone, Adenine-thymine heterodimer, cytosine photohydrate, thymine photohydrates, single strand break, and DNA-protein crosslink (Table 2.4). Setlow and Setlow (1962) reported that DNA chain breakage, cross-linking of strands, hydration of pyrimidines, and formation of dimers between adjacent residues in the polynucleotide chain are the major photochemical changes that occur in DNA upon UV-light exposure. Formation of photoproducts depends on wavelength, DNA sequence, and protein-DNA interactions (Mitchell, 1995). Aromatic amino acids such as phenylalanine and tryptophane also absorb ultraviolet light (Henis, 1987) and thus inactivates these amino acids present in microorganisms.
Table 2.4. Photoproduts produced in DNA because of absorption of UV-light*.

<table>
<thead>
<tr>
<th>Photoprodut</th>
<th>Percentage of total photoproduts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV-C</td>
</tr>
<tr>
<td>Cyclobutyl pyrimidine dimer</td>
<td>77</td>
</tr>
<tr>
<td>Pyrimidine pyrimidinone[6-4]-photoprodut</td>
<td>20</td>
</tr>
<tr>
<td>Dewar pyrimidinone</td>
<td>0.8</td>
</tr>
<tr>
<td>Adenine-thymine heterodimer</td>
<td>0.2</td>
</tr>
<tr>
<td>Cytosine photohydrate</td>
<td>~2.0</td>
</tr>
<tr>
<td>Thymine photohydrates</td>
<td>n/a</td>
</tr>
<tr>
<td>Single strand break</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>DNA-protein cross link</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>


Photoreactivation occurs in the wavelength range of 330 to 480 nm because of activation of DNA photolyase, which splits thymine dimers (Walker, 1984). It is also hypothesized that thermal stress induced on bacteria because of UV-light exposure leads to bacterial rupture especially at higher flux densities (0.5 J/cm²). Overheating of bacteria is caused because of differences in UV-light absorption by bacteria and surrounding medium and thus bacteria becomes a local vaporization center and may generate a small steam flow performing membrane destruction (Takeshita et al., 2003).

UV-A (315 to 400 nm) has better penetration capacity than UV-C and affects bacterial cells by causing membrane damages and/or generates active oxygen species or H₂O₂ (Kramer and Ames, 1987). However, UV-A has very little impact on microbial cells unless exogenous photosensitizers are used in the process and absorbed by the bacterial cell (Mitchell, 1978). Antimicrobial effect of pulsed UV-light is predominately caused by the UV-light portion of the broad spectrum.

The inactivation mechanism for spores is different from that of vegetative cells mainly because of the structural differences. Riesenman and Nicholson (2000) reported
that the thick protein coat induces resistance in spores of *Bacillus subtilis*. The DNA of a bacterial spore has a different conformation than the DNA of the vegetative cell. Setlow and Setlow (1987) reported that *Bacillus* species spores did not produce any detectable amount of thymine containing dimers. The predominant photoprodut was 5-thyminyl-5,6 dihydrothymine adduct; later it was termed as ‘spore photo-product’.

**UV-light penetration and absorption**

UV-light can penetrate only up to several millimeters in food material depending upon the optical properties of the food materials. UV-light can easily penetrate through water since it is transparent to the wavelengths produced by pulsed UV-light. Foods such as sugar solutions have limited penetration. The effective penetration depth for milk (2.7% fat content and 3.3% protein content) was reported by Burton (1951) (Table 2.5). As milk is opaque, UV-light cannot penetrate well hence milk has to be presented to the system as thin layer.

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Effective penetration depth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>0.036</td>
</tr>
<tr>
<td>275</td>
<td>0.038</td>
</tr>
<tr>
<td>300</td>
<td>0.041</td>
</tr>
<tr>
<td>400</td>
<td>0.050</td>
</tr>
<tr>
<td>500</td>
<td>0.058</td>
</tr>
<tr>
<td>600</td>
<td>0.065</td>
</tr>
<tr>
<td>700</td>
<td>0.073</td>
</tr>
<tr>
<td>800</td>
<td>0.080</td>
</tr>
</tbody>
</table>

* Burton, 1951.
Oppenlander (2003) suggested some of the possible arrangements of the UV-light lamp in a photo-reactor, which can be utilized to enhance the effectiveness of absorption by target material (Figure 2.4). A falling film photo-reactor design might be essential for effective penetration in milk. Other designs which reduce the thickness of milk to be treated would be crucial for the commercial success of this technology for use in opaque liquid foods. The designs proposed by Oppenlander (2003) can be extended to other liquid foods and water disinfection. Addition of some absorption enhancing agents such as edible colorants will also enhance UV-light penetration (Palmieri et al., 1999).

Another approach is to combine UV-light with other technologies such as infrared heating to achieve the desired microbial reduction. The combined treatment of UV-light and infrared heating resulted in considerable reduction in the microflora of milk (Giraffa and Bossi, 1984). For instance, a 40°C infrared heat treatment combined with 45.6 s UV-light treatment at 2000 L/h flow rate of milk resulted in reductions of standard plate count, Lactic acid bacteria, enterococci, coliforms, psychrotrophic bacteria, and spores by 92%, >99%, 70%, 95%, 99%, and 53%, respectively.
Figure 2.4. Arrangements of lamps for flow-through systems (Oppenlander, 2003).

Cross sectional and top-view of arrangements. A: continuous flow annular photoreactor with coaxial lamp position, B: External lamp position with reflector (R), C: Perpendicular lamp position, D: Contact-free photoreactor types (including falling film, flat bed). L: Lamp, RV: reactor vessel, Q: Quartz tube.

The absorption coefficient of liquid food increases as the color or turbidity of liquid increases. The penetration capacity of UV-light reduces as the absorption coefficient increases (Guerrero-Beltran and Barbosa-Canovas, 2004). Shama (1999) reported the coefficient of absorption for various liquid foods (Table 2.6). Therefore, for UV-light treatment of foods with higher coefficients of absorption, optimization of the system which will enhance the penetration depth will be beneficial. Furthermore, treatment of these food products in the form of a thin film may result in better penetration and thus increase the efficiency of microorganism inactivation.
Table 2.6. Coefficient of absorption of various liquid food products at 254 nm*.

<table>
<thead>
<tr>
<th>Liquid food product</th>
<th>Coefficient of absorption (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.007-0.01</td>
</tr>
<tr>
<td>Drinking water</td>
<td>0.02-0.1</td>
</tr>
<tr>
<td>Clear syrup</td>
<td>2-5</td>
</tr>
<tr>
<td>White wine</td>
<td>10</td>
</tr>
<tr>
<td>Red wine</td>
<td>30</td>
</tr>
<tr>
<td>Beer</td>
<td>10-20</td>
</tr>
<tr>
<td>Dark syrup</td>
<td>20-50</td>
</tr>
<tr>
<td>Milk</td>
<td>300</td>
</tr>
</tbody>
</table>

* Shama, 1999.

Inactivation studies by continuous and pulsed UV-light

McDonald et al. (2000) compared the effectiveness of a continuous UV-light source and a pulsed UV-light source for the decontamination of surfaces and reported an almost identical level of inactivation of *B. subtillis* with 4x10⁻³ J/cm² of pulsed UV-light source and 8 x10⁻³ J/cm² continuous UV-light source.

Chang et al. (1985) investigated the effect of UV-light for the inactivation of *Escherichia coli*, *S. Typhi*, *Shigella sonnei*, *Streptococcus faecalis*, and *S. aureus*. The bacteria were grown in nutrient broth at 35°C for 20 to 24 h. The culture was then filtered with a 0.45 μm filter and rinsed with sterile buffer water. Cells were resuspended in sterile buffer water and the aggregated groups of bacteria were removed using a 1.0 μm nucleopore polycarbonate membrane. After the treatment of filtrate with UV-light, samples were grown in nutrient agar and the bacterial colonies were enumerated. *E. coli*, *S. aureus*, *S. Sonnei*, and *S. Tyhi exhibited similar resistance to the UV-light and a 3log₁₀ reduction was obtained with approximately 7 x10⁻³ J/cm² energy. However, the
resistance exhibited by *S. faecalis* was higher and required a 1.4 times higher dose than the above-mentioned microorganisms to get 3 log_{10} reduction of inactivation.

The effect of pulsed UV-light emission with low or high UV content on inactivation of *L. monocytogenes*, *E. coli*, *S. Enteritidis*, *Psudeomonas aeruginosa*, *B. cerus*, and *S. aureus* were investigated by Rowan et al. (1999). Bacterial cultures were seeded separately on the surface of Tryptone soya-yeast extract agar and treated with a pulsed UV-light source with low or high UV content. Reductions of 2 and 6 log_{10} were obtained with 200 light pulses (approximate pulse duration = 100 ns) for low and high UV content, respectively. Sonenshein (2003) investigated the effect of high-intensity UV light on inactivation of *B. subtilis* spores. *B. subtilis* spores were diluted to give concentrations of 1x10^9 (Sample A), 1x10^8 (Sample B), or 1x10^7 (Sample C) using sterile deionized water. A 50 μl sample of each concentration were placed at three different positions (Position 1: on the lamp axis and at the midpoint of the lamp; Position 2: 1 cm above the lamp axis and at the midpoint of the lamp; Position 3: 1 cm above the lamp axis and 172 mm to the right of the midpoint of the lamp) and treated with pulsed UV-light. Three pulses (1 s) of UV-light resulted in more than 6.5 log_{10} CFU/ml (Sample B) and 5.5 log_{10} CFU/ml (Sample C) reductions when the samples were placed at the lamp axis and at the midpoint of the lamp.

Stermer et al. (1987) investigated the effect of UV radiation for inactivation of bacteria in lamb meat. With 4 x10^{-3} J/cm^2 energy, 99.9% of the naturally occurring floras of lamb (mostly *Pseudomonas, Micrococcus, and Staphylococcus spp.*) were inactivated. The bactericidal effectiveness of modulated UV-light was investigated by Bank et al. (1990). Bacterial monolayers of *S. epideridis, Pseudomonas aeruginosa, E. coli, S.*
*Staphylococcus aureus*, or *S. marcescens* on Trypticase soy agar (TSA) plates were exposed to modulated pulsed UV-light. A 60 s treatment time at 31 cm distance from the light source (approximately 4 x10^{-4} J/cm^2) resulted in 6 to 7 log_{10} reduction of viable bacterial population.

The effect of continuous ultraviolet energy to inhibit the pathogens on fresh produce was investigated by Yaun et al. (2004). The surfaces of red delicious apples, leaf lettuce, and tomatoes were inoculated with *Salmonella* spp. or *E. coli* O157:H7 and treated with UV-C light at a wavelength of 253.7 nm with different doses ranging from 1.5 to 24x10^{-3} W/cm^2. A 3.3 log_{10} CFU/apple reduction was obtained for *E. coli* O157:H7 at 24x10^{-3} W/cm^2, whereas, a 2.19 log_{10} CFU/tomato reduction was obtained for *E. coli* O157:H7 at 24x10^{-3} W/cm^2. Similarly, lettuce inoculated with *Salmonella* spp. and *E. coli* O157:H7 resulted in 2.65 and 2.79 log_{10} CFU/lettuce reductions, respectively.

Takeshita et al. (2003) investigated the mechanisms of damage of yeast cells induced by pulsed light and continuous UV-light. The authors reported that the DNA damage induced by continuous UV-light is slightly higher than that of pulsed light. Protein elution because of pulsed UV-light was higher than that resulting from continuous UV-light. Wekhof et al. (2000) suggested that the inactivation mechanism of pulsed UV-light includes both the germicidal action of UV-C light and rupture of microorganism because of thermal stress caused by UV components.

*Aspergillus niger* spores in corn meal were inactivated using a pulsed UV-light (Jun et al., 2003). A 4.95 log_{10} reduction of *A. niger* on inoculated corn meal with a treatment time of 100 s was achieved when the distance between the quartz window and
sample was kept at 8 cm and the input voltage was 3800 V (Jun, 2003). Sharma and Demirci (2003) investigated the effect of pulsed UV-light on inactivation of *E. coli* O157:H7 on alfalfa seeds. Reductions of 0.09 to 4.89 log$_{10}$ CFU/g were obtained for various thickness and treatment time combinations. Four thicknesses (1.02, 1.92, 3.61, and 6.25 mm) and 7 treatment times (5, 10, 30, 45, 60, 75, and 90 s) were used in the experiment. Complete inactivation of *E. coli* O157:H7 was obtained within 30 s treatment time when the seed thickness was maintained at 1.02 mm, which corresponds to 4.80 log$_{10}$ CFU/g. An increase in treatment time resulted in a higher log$_{10}$ reduction for all the thicknesses (p<0.05). This can be concluded since more than 4.0 log$_{10}$ CFU/g reduction was obtained at the highest treatment time for all the thicknesses. Hillegas and Demirci (2003) studied the effect of pulsed UV-light on inactivation of *Clostridium sporogenes* in honey. An 87.6% reduction of *Clostridium sporogenes* was obtained when a 2 mm depth of honey sample was kept at 8 cm distance from the quartz window and treated with UV-light for 45 s (initial inoculum level was 6.24 log$_{10}$ CFU/g); whereas, 180 s treatment time was required to achieve 89.4% reduction at 20 cm distance from the quartz window.

Smith et al. (2002) used a pulsed laser excimer source to created pulsed UV-light at 248 nm to treat bulk tank bovine milk. After exposed to 25 J/cm$^2$ energy (114 s exposure), there was no growth observed for cultures of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella Dublin*, *Yersinia enterocolitica*, *Staphylococcus aureus*, *Aeromonus hydrophillia*, and *Serratia marcescens*. The corresponding microbial reduction is more than 2 log$_{10}$ CFU/ml. UV-light can also degrade toxins. Yousef and Marth (1985) reported that upon UV-light exposure, reductions of 3.6 to 100% of
alfatoxin M₁ was achieved in milk with treatments of 2 to 60 min. Pulsed UV-light inactivation depends on several factors such as, transmissivity of the food product, geometry of the waveguide, lamp power, and exposed wavelength range.

**Effect of UV on food components and quality**

In the presence of air, depolymerization of starch occurs under UV-light (Tomasik, 2004). Presence of sensibilizers (metal oxides, particularly ZnO) enhances this process. Usually, photooxidation followed by photodecomposition occurs. UV-light exposure initiates free radical oxidation and catalyzes other stages of the process. UV-light forms lipid radicals, superoxide radicals (SOR), and H₂O₂ (Kolakowska, 2003). SOR can further induce carbohydrate crosslinking, protein crosslinking, protein fragmentation, peroxidation of unsaturated fatty acid, and loss of membrane fluidity function. UV radiation may also denature proteins, enzymes, and amino acids (especially amino acids with aromatic compounds) in milk, leading to textural changes. Water also absorbs UV photons and produces OH- and H+ radicals, which in turn aids changes in other food components. Therefore, UV-light treatment not only changes the chemistry of food components, but also leads to product quality deterioration when it is applied at high doses. However, most of the changes in the components are detrimental to microbial growth. Therefore, proper optimization of the disinfection process is necessary in order to maintain the quality of food products while ensuring its safety. Normally, microbial inactivation can be achieved within seconds to minutes depending upon the opacity of the food products and microorganism type.
UV-light may cause off flavors and color changes in food (Ohlsson and Bengtsson, 2002). During UV-light treatment, oxygen radicals are formed which leads to formation of ozone, especially between 185 to 195 nm, which causes off-flavors in milk. In the wavelength region of 280 to 310 nm, cholesterol (provitamin) changes into natural vitamin D₃ and hence enriches milk with vitamin D. UV-light exposure was used for vitamin D enrichment in milk in early days until vitamin D production became cheaper. UV-light enrichment of other products can also be achieved by ultraviolet light exposure. Jasinghe and Perera (2006) successfully fortified edible mushrooms with Vitamin D₂. The Shitake, oyster, button and abalone mushrooms were treated with UV-A (315-400 nm), UV-B (290-315 nm), and UV-C (190-290 nm) for a period of up to 1 h on each side of the mushrooms. The vitamin D₂ content ranged from 22.9±2.7 to 184.0±5.7 for various mushroom and UV-light combinations. The authors noted that even treatment of 5 g of shitake mushrooms for 15 min with UV-A or UV-B can provide more than the recommended allowance of vitamin D for adults (10 µg/day).

Light induced flavor 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. Peroxides produced during UV-light exposure may also attack fat soluble vitamins and colored compounds and may lead to change in nutritional quality and/or discoloration. Furthermore, long treatments with UV-light may increase the temperature of food product which will lead to temperature related quality changes such as cooked flavor, change in color because of non-enzymatic browning. UV-light can also degrade vitamins by photodegradation. Especially vitamins A, C, and B₂ are affected by UV-light.
Prolonged treatment with UV-light can result in discoloration of food materials. Cuvelier and Berset (2005) reported that paprika gels started to fade upon UV-light exposure after 3 h. Fat soluble vitamins and colored compounds can be affected by the peroxides produced during extended UV-light treatment. Undesirable changes in food may occur when food is treated with UV-light for an extended period of time. Foods may need to be treated for less time to achieve the desired decontamination level, and hence there will not be any adverse change in food quality.

The in-package UV-light treatment of white bread slices with the PureBright® system resulted in bread slices with a fresh appearance for more than two weeks, however, the control slices were dried out and got moldy (Rice, 1994). The author also suggested that the quality of tomatoes treated with pulsed light was acceptable up to 30 days in storage at refrigerated temperature.

Choi and Nielsen (2005) investigated the efficacy of UV-light for pasteurization of apple cider since FDA has approved the use of UV-light for reduction of pathogens in fruit and vegetable juices (Federal register, 2000). The consumer panel sensory evaluation with 40 panelists suggested that there was no significant difference (p<0.05) for the odor, color, cloudiness, sweetness, acidity, overall flavor, and overall product ratings for untreated, pasteurized, and UV-light treated apple ciders stored for 4 days. Furthermore, the pasteurized apple cider received less acceptable rating for color, cloudiness, and overall product preference than control and UV-light treated samples. This clearly indicates that the quality changes are minimal in UV-light treated apple cider at optimum conditions when compared to thermal pasteurized cider. Consumer panelists significantly preferred UV-light treated apple cider over thermally pasteurized apple cider.
for color, cloudiness, sweetness, acidity, overall flavor, and overall likeness than pasteurized cider (Choi and Nielsen, 2005).

The efficacy of pulsed UV-light for decontamination of minimally processed vegetables was investigated by Gomez-Lopez et al. (2005). They conducted a sensory evaluation procedure with a semi-trained panel of 4 to 6 people. The panelists evaluated the quality of minimally processed white cabbage and iceberg lettuce. Off-odors were present for the pulsed light treated white cabbage just above the acceptable limit. This limited the shelf life of white cabbage to a maximum of seven days. The panelists described the off-odor as “plastic”, which was distinctive immediately after the pulsed light treatment, but the off-odor faded away after a couple of hours in storage. Therefore, the off-odor can be assumed to disappear before consumption by the consumers. It is interesting to note that pulsed UV-light treated iceberg lettuce received better scores than the control samples for off-odor, taste, and leaf edge browning. This clearly indicates pulsed UV-light treatment helps in preserving the lettuce quality.

In general, UV-light treatment of food may not cause any adverse effect, if applied in moderate amounts, which is required for microbial inactivation as strongly suggested by previous studies. However, modification and optimization of the UV-light treatment might be necessary for successful implementation of the process in some foods.

**Water disinfection by UV-light**

Water is abundantly used in almost all the processing industries including food industries. Water may contain several pathogenic microorganisms including protozoa, bacteria and viruses. UV-light, ozonation, and chlorination are some of the commonly
used disinfection methods for inactivation of these microorganisms. Ultraviolet light does not produce any toxic by-products. UV-light has been used for disinfection of drinking water since the early 1900s. In addition to the disinfection of drinking water, UV-light can also be used for disinfection of water used in semiconductor, pharmaceutical, food or other industries and for sanitation of waste water. Currently low pressure (typically a monochromatic source at 254 nm) and medium pressure lamps (typically a polychromatic source with 40 to 50% of energy directly used for disinfection) commonly used for disinfection of water (Masschelein, 2002), which are continuous UV-light sources. Pulsed UV-light sources are now gaining attention as the effectiveness of disinfection can be enhanced several folds. Furthermore, pulsed UV-light provides a mercury free disinfection system. The UV-light dose required for inactivation of microorganisms is listed in Table 2.7.

The UV-light treated microorganisms, upon exposure to light in the range of 330 to 480 nm results in photoreactivation (Walker, 1984). The photoreactivated microorganisms are much more resistant to UV-light and thus require higher dose for inactivation (Guerrero-Beltran and Barbosa-Canovas, 2004; Hoyer, 1998; Sastry et al., 2000). As it can be seen from Table 2.7, cells require more energy for inactivation after being treated once and reactivated. Therefore, higher UV doses are needed for complete inactivation of pathogenic microorganisms.
Table 2.7. Ultraviolet light exposure (at 254 nm) required for 4-log$_{10}$ reduction of pathogens in drinking water*.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Exposure required without reactivation (J/cm$^2$)</th>
<th>Exposure required with reactivation (J/cm$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citrobacter freundii</td>
<td>80</td>
<td>250</td>
</tr>
<tr>
<td>Enterobacter cloacae</td>
<td>100</td>
<td>330</td>
</tr>
<tr>
<td>Enterococccus faecium</td>
<td>170</td>
<td>200</td>
</tr>
<tr>
<td>Escherichia coli ATCC 11229</td>
<td>100</td>
<td>280</td>
</tr>
<tr>
<td>Escherichia coli ATCC 23958</td>
<td>50</td>
<td>200</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>110</td>
<td>310</td>
</tr>
<tr>
<td>Mycobacterium smegmatis</td>
<td>200</td>
<td>270</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>110</td>
<td>190</td>
</tr>
<tr>
<td>Salmonella Typhi</td>
<td>140</td>
<td>190</td>
</tr>
<tr>
<td>Salmonella Typhimurium</td>
<td>130</td>
<td>250</td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>130</td>
<td>300</td>
</tr>
<tr>
<td>Vibrio cholerae wild isolate</td>
<td>50</td>
<td>210</td>
</tr>
<tr>
<td>Yersinia enterocolitica</td>
<td>100</td>
<td>320</td>
</tr>
</tbody>
</table>


**Economics of UV-light disinfection system**

Compared to other available disinfection technologies, the cost of UV-light disinfection systems is competitive or sometimes cheaper. Dunn (1997) estimated that the treatment cost at 4 J/cm$^2$ with the PureBright® pulsed UV-light treatment system is 0.1¢/ft$^2$ of treated area. The cost includes conservative estimate of electricity, maintenance, and equipment amortization. Lander (1996) also estimated 0.1¢/ft$^2$ as the cost of treatment with the PureBright® system, where the estimated cost includes the electricity, maintenance, and investment in a hooded high intensity lamp and power unit.

Choi and Nielsen (2005) suggested that it will be cost-effective for the apple cider industry to utilize UV-light pasteurization, because UV-light pasteurizers cost about $15,000 (Higgins, 2001). Anonymous (1989) reported that the annual power consumption and lamp replacement costs based on the minimum dosage of 30,000
MW.s/cm² for multilamp and single lamp continuous UV-light disinfection sources as $2,465 and $3,060 for an 8,000 h run time.

The processing cost 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 (Taghipour, 2004). This clearly indicates that the UV-light treatment is cost-effective for inactivation of pathogenic microorganisms.

### 2.10. Infrared heating

Infrared radiation can be divided into three categories namely near infrared (NIR; 0.75 – 1.4 µm; temperature less than 400°C), mid infrared (MIR; 1.4 – 3 µm; temperature in the range of 400°C to 1000°C), and far infrared (FIR; 3 – 1000 µm; temperature more than 1000°C) (Skjoldebrand, 2001; Sakai and Hanzawa, 1994). Shorter wavelength infrared radiation gives greater penetration depth while giving lesser temperature when compared to longer wavelength radiation (Skjoldebrand, 2002). Infrared heating has several advantages such as, 1) higher heat transfer capacity, 2) instant heating because of direct heat penetration, 3) high energy efficiency, and 4) faster heat treatment, 5) fast regulation response, 6) better process control, 7) no heating of surrounding air, 8) equipment compactness, 9) uniform heating, 10) preservation of vitamins, and 11) less chance of flavor losses from burning of foods (Dagerskog and Osterstrom, 1979; Afzal et al., 1997; Skjoldebrand, 2002). Far infrared radiation has been used in the industry since 1950s in United States (Skjoldebrand, 2001).
Infrared radiation basics

Temperature of the heating element determines the wavelength at which maximum radiation occurs as described by Planck’s law, Wien’s displacement law, and Stefan-Boltzmann’s law (Sakai and Hanzawa, 1994; Dagerskog and Osterstrom, 1979). Stefan-Boltzmann’s law (Equation 2.3) states that the amount of heat emitted from a black body (a theoretical object that absorbs 100% of the radiation that fall on it) can be determined using

\[ Q = \alpha A T^4 \]  
(Equation 2.3)

where, \( Q \) is the rate of heat emission in Js\(^{-1}\), \( \alpha \) is the Stefan-Boltzman’s constant \((5.7 \times 10^{-8} \text{ Js}^{-1} \text{m}^{-2} \text{K}^{-4})\), \( A \) is the surface area in m\(^2\), and \( T \) is the absolute temperature in \(^o\)K. This law clearly indicates that even a small increase in temperature can lead to higher rate of heat emission.

Infrared heating lamp emits radiation in range of wavelengths with varying intensities. The wavelength at which maximum radiation occurs depends on the lamp temperature according to Planck’s law (Equation 2.4). As the temperature of the lamp increases, the wavelength at which maximum radiation occur decreases and the total energy emitted increases (Figure 2.5).

\[ E_{b\lambda}(T, \lambda) = \frac{2\pi h c_o^2}{n^2 \lambda^5 \left[ e^{\left(\frac{h c_o}{n k T}\right)} - 1 \right]} \]  
(Equation 2.4)

where, \( E \) is the emissive power of a black body, \( h \) is the Planck’s constant \( (6.626 \times 10^{-3}) \)
Js), \( C_0 \) is the speed of light in \( \text{km}s^{-1} \), \( n \) is the refractive index of the medium, \( \lambda \) is the wavelength in \( \mu\text{m} \), \( k \) is the Boltzmann’s constant \( (1.3806 \times 10^{-23} \text{ JK}^{-1}) \) and \( T \) is the absolute temperature in °K. Figure 2.5 shows the Planck’s curve for black body radiation at different temperatures.

Wien’s law states that the wavelength corresponding to maximum emission is inversely proportional to the black body temperature (Equation 2.5).

\[
\lambda_{\text{max}} = \frac{2900}{T}
\]

(Equation 2.5)

where, \( \lambda_{\text{max}} \) is the wavelength corresponding to maximum emission in \( \mu\text{m} \) and \( T \) is the absolute temperature of the black body in °K.
Interaction of infrared radiation with food materials

As food is exposed to infrared radiation, it is absorbed, reflected, or scattered (blackbody does not reflect or scatter). Absorption intensities at different wavelengths by food components differ. As the infrared radiation is absorbed by food components, the vibration and rotation of the molecules change because of a decrease or increase in the distance between atoms, movement of atoms, or vibration of molecules (Skjoldebrand, 2002). A significant portion of the infrared radiation is reflected back depending upon the wavelength. For instance, at NIR wavelength region (\( \lambda \leq 1.25 \, \mu m \)), approximately 50% of the radiation is reflected back, while less than 10% radiation is reflected back at FIR wavelength region (Skjoldebrand, 2002). Most organic materials reflect 4% of the total reflection producing shine of polished surfaces. The rest of the reflection is because of the body reflection where radiation enters the food material and scatters producing different color and patterns (Dagerskog, 1978).

The rate of heat transfer to food material depends on several factors such as the composition, temperature of the infrared lamp, moisture content of the food material, shape and surface characteristics of the food material. A crucial factor in infrared heating of food is the penetration depth which is defined as the 37% of the unabsorbed radiation energy. As indicated earlier, penetration depth depends on the wavelength region. Penetration depth of NIR is approximately ten times higher than the FIR wavelength region (Skjoldebrand, 2002).
Inactivation mechanism of infrared heat treatment

Infrared radiation consists of wavelengths between 0.5 and 1000 μm (Rosenthal, 1992). Far Infrared Radiation (FIR) (3 to 1000 μm) is used in the pasteurization of food products, because the absorption of infrared energy by water and organic materials is high in this wavelength region (Sawai et al., 1995). Infrared heating is effective in the inactivation of bacteria. Though, the effect of FIR irradiation is not clear, several researchers suggested several possible targets for inactivation such as DNA, RNA, ribosome, cell envelope, and proteins in the cell (Sawai et al., 1995). The pasteurization effect of far infrared irradiation is much greater than thermal conduction because of the absorption of infrared energy within a thin domain near the surface and bulk temperature of the suspension. This was verified by the fact that inactivation of bacteria was observed even when the bulk temperature was negligible because of cooling (Sawai et al., 1995). The sensitivity of *E. coli* to rifampicin and chlorompenicol increased following heat treatment with FIR irradiation and thermal conduction, suggesting that the damage to RNA polymerase and ribosome occurred which leads to cell death (Sawai et al., 1995).

Infrared heat treatment

As the wavelengths of radiation emitted in the NIR and MIR ranges can be utilized for instantaneous heating within seconds, they have been an interest to the food industry. NIR has a penetration depth of up to 5 mm for some food products (Skjoldebrand, 2001). Infrared heating can be utilized for several applications in the food industry such as 1) drying vegetables, fish, pasta, and rice, 2) heating flour, 3) frying meat, 4) roasting cereals, coffee, and cocoa, 5) baking pizza, biscuits, and breads, 6)
thawing, and 7) surface pasteurization of bread and packaging materials (Skjoldebrand, 2001). Skjoldebrand et al. (1994) reported that baking time was 25-50% shorter for infrared radiation when compared to a conventional oven for comparable energy consumption. The weight loss associated with infrared radiation was 10-15% lower than with an oven, and the quality of the final products from both methods were comparable.

*E. coli* O157:H7 suspended in 0.05 M physiological phosphate buffered saline was treated with infrared radiation with a radiative power of $3.22 \times 10^{-1}$ J/s-cm$^2$ on the surface of bacterial suspension (Sawai et al., 1995). The temperature of the bacterial suspension increased exponentially from approximately 280$^\circ$K to 336$^\circ$K during a 10 min infrared irradiation treatment. After pasteurization by FIR, the cell suspension was diluted with physiological saline solution and plated on the agar surface. Two types of agar plates were used: selective and non-selective. Sensitivity disk agar-N containing penicillin G (PCG), chloramphenicol (CP), nalidixic acid (NA), and riampicin (RFP) were used as a selective agar, and sensitivity disk agar-N was used as a non-selective agar. Selective agar with the reagents was used in order to characterize the type of injury of *E. coli* O157:H7. PCG, CP, NA, and RFP inhibited the synthesis of cell wall, protein, DNA, and RNA, respectively. After infrared irradiation, *E. coli* O157:H7 becomes sensitive to RFP and CP, which may be because of the damage of RNA polymerase and ribosome in *E. coli* by infrared radiation. Approximately, $3 \log_{10}$ reduction of *E. coli* was obtained after an 8 min treatment, when chloramphenicol selective agent was used. The validity of this result was verified by the comparison of the effect with UV-light treatment. Approximately, a $1 \log_{10}$ reduction of *E. coli* was obtained after 20 s when a chloramphenicol selective agent was used. The authors concluded that the FIR
pasteurization effect was because of a heating effect after comparison with conventional convective heating.

The efficacy of infrared heating on microbial reduction of milk was investigated by Giraffa and Bossi (1984). They reported that infrared heat treatment of milk at 65°C and 1000 L/h flow rate resulted in reductions of standard plate count, Psychotrophic bacteria, Coliforms, Enterococci, Lactic acid bacteria (grown in MRS agar), Lactic acid bacteria (grown in M17 agar), and spores by 63%, 96% 99.9%, 80%, 71%, 60%, and 21%, respectively.

Rosenthal et al. (1996) investigated the surface pasteurization effect of infrared radiation in cottage cheese. Surface heating of the cheese was done by Philips infrared spotlights (250 J/s) at a distance of 2.5 to 3 cm from the cheese surface. After the treatment, cheeses were examined for yeast, mold, and coliform counts during 8 weeks of storage at 4°C. The initial yeast and mold counts before infrared pasteurization were <10 cells/g. Even after 8 weeks storage at 4°C, less than 100 yeast and mold cells/g were found, suggesting that infrared pasteurization was effective for surface sterilization. The corresponding log10 reductions of yeasts and molds were approximately 3. However, the number of yeast and mold cells was reduced to only approximately 1 log10 CFU/g at a 1 cm depth from the cheese surface.

Hashimoto et al. (1992a) investigated the effect of far-infrared irradiation on pasteurization of bacteria suspended in liquid medium below lethal temperature. E. coli 745 and S. aureus 9779 cultures were suspended in 0.05 M phosphate buffer (pH=7.0) and dispensed in a stainless steel Petri dish with a thermally insulated side walls. The Petri dish was covered with aluminum foil to reflect infrared radiation. The Petri dish
was placed on a temperature controlled plate, where the temperature was maintained from 263 to 283°K by a coolant, and the plate was on a reciprocating shaker. The infrared irradiation apparatus was kept at a 15 cm distance from the bacterial suspension, and the source temperature was from 773 to 943°K. The bacterial suspension was infrared treated under agitation (180 rpm) and cooled rapidly using a plate kept at 278 °K immediately after the treatment. A reduction of more than 4.5 log\textsubscript{10} CFU/ml of \textit{S. aureus} was achieved with an irradiation power of approximately 7.5x10^{-7} J/s.cm\textsuperscript{2} when using selective agar (standard method agar enriched with 8% sodium chloride); whereas, a reduction of approximately 3.5 log\textsubscript{10} CFU/ml was achieved using non-selective agar (standard method agar). Similarly, a log\textsubscript{10} reduction of approximately 2 log\textsubscript{10} CFU/ml and approximately 0.5 log\textsubscript{10} CFU/ml was obtained for \textit{E. coli} 745 and \textit{S. aureus} 9779, respectively, when selective agar (Nutrient agar enriched with 0.06% sodium deoxycolate) and non-selective agar (Nutrient agar) were used.

The FIR effect on the pasteurization of bacteria on or within wet-solid medium was evaluated by Hasimoto et al. (1992b). \textit{E. coli} 745 and \textit{S. aureus} 9779 culture were surface plated on nutrient agar and standard method agar, respectively. After surface plating, the bacteria were covered with agar medium to a thickness between 1 to 5 mm. An FIR heater with a reflector which irradiated 2.46x10\textsuperscript{3} to 7.01x10\textsuperscript{-1} J/s.cm\textsuperscript{2} power on the agar plate was used in the experiment. A complete inactivation of \textit{E. coli} was obtained with irradiation at 4.36x10\textsuperscript{-1} J/s-cm\textsuperscript{2} for 6 min., which corresponds to approximately 2 log\textsubscript{10} CFU/plate when there is no medium added. However, when the medium thickness was 1 mm and 2 mm, approximately, 1 log\textsubscript{10} CFU/plate was obtained under the same experimental condition.
Hashimoto et al. (1993) also investigated the irradiation power effect on IR pasteurization below reaching the lethal temperature of bacteria. *E. coli* 745 and *S. aureus* 9779 culture were suspended in 0.05 M phosphate buffer (pH=7.0). FIR and Near Infrared Radiation (NIR) were used in the experiment. The irradiation power for the NIR heater was calculated using surface temperature and emissivity of the heater. The irradiation power of FIR was calculated using a Fourier transform infrared spectrophotometer. Approximately 4 log\textsubscript{10} CFU/ml and 1 log\textsubscript{10} CFU/ml reductions of *S. aureus* were obtained by FIR and NIR, respectively, when the irradiation power was 7.57x 10\textsuperscript{-1} J/s-cm\textsuperscript{2}, which indicates that the FIR heating is more effective in inactivating the microbial population than NIR heating.

Jun and Irudayaraj (2003) studied inactivation of *Aspergillus niger* inoculated on corn meal by infrared radiation with or without a bandpass filter (5.45 to 12.23 μm). A reduction of approximately 2 log\textsubscript{10} CFU/g was obtained with and without the filter after a 5 min treatment time; however, the log\textsubscript{10} reduction obtained with a filter was higher than the log\textsubscript{10} reduction obtained without a filter. Similarly, *Fusarium proliferatum* was inoculated on corn meal and treated with infrared radiation with or without a bandpass filter and resulted in approximately 1.5 and 2 log\textsubscript{10} CFU/g reductions, respectively, with a 5 min treatment time.
2.11. **Determination of mode of microbial inactivation**

It is necessary to understand the basic inactivation mechanism of an emerging technology in order to optimize the system for better inactivation of pathogenic microorganisms. Selected examples are discussed below.

**Transmission electron microscopy**

Transmission electron microscopy (TEM) can be used effectively for investigation of the mode of bacterial inactivation, as these microscopes can help in viewing cellular level details of microorganisms. A TEM is a modern, sophisticated microscope, which uses an electron beam to observe internal structures of microorganisms. The resolution of TEM is 1,000 times better than the light microscope enabling it to distinguish points closer than 0.5 nm (Prescott et al., 1999). As the electrons are absorbed and scattered by microorganisms very easily, very thin slices in the order of 20 to 100 nm need to be used. The bacterial cell is treated with various chemicals to stabilize the cell structure, cut into a thin slice using a diamond or glass knife, stained to increase the contrast, and exposed to an electron beam. As the composition of the cell components differ, the intensity of electron scattering also varies and thus produces an image of the internal structure of the bacteria. Several researchers have used the electron microscopy techniques successfully for the investigation of inactivation mechanisms of several novel technologies such as pulsed electric field, antimicrobial agents, etc. (Brouillette et al., 2004; Calderon-Miranda et al., 1999; Liu et al., 2004). The effect of pulsed electric field and nisin on *Listeria innocua* in skim milk was investigated using TEM by Calderon-Miranda et al. (1999). They noticed several
futures of pulsed electric field damaged cell such as lack of cytoplasm, cell wall damage, cytoplasmic clumping, increase in cell membrane thickness, poration of cell wall and leaching of cellular content. The ruptures of cell wall and cell membrane were observed at selected electric field intensities. Liu et al. (2004) explained that the inactivation of bacteria by chitosan is because of cell wall damage by using TEM. The outer membrane of chitosan treated *E. coli* was altered after the chitosan treatment. The cell membrane of chitosan-treated *Staphylococcus aureus* was disrupted and the cellular contents were leaked.

**Fourier transform infrared spectroscopy**

Infrared spectroscopy is a chemical analytical method which can be used to determine the chemical and structural information of the target material based on vibration transitions. Various food/microbial components absorb infrared light at specific wavelengths. Thus, infrared spectroscopy produces a fingerprint of spectral absorption characteristics of the biological components by providing absorption/transmission characteristics over time. The spectrum obtained is transformed from the time domain into frequency domain by Fourier transformation, so that absorption with respect to a particular wavelength could be assessed.

Fourier Transform Infrared Spectroscopy (FTIR) can be used to discriminate pathogenic microorganisms by spatially resolving the structural and compositional information of the microorganisms at molecular level (Yu and Irudayaraj, 2005). Liu et al. (2004) utilized FTIR for determination of the interaction between chitosan and a synthetic phospholipids membrane in an effort to understand the basic inactivation
mechanism. Several researchers successfully utilized FTIR for the discrimination of microorganisms and to investigate the changes in chemical composition because of several processes. Therefore, the use of TEM and FTIR will be beneficial for the investigation of inactivation mechanisms.

2.12. Summary of literature review

Contamination of pathogens in food and water is a serious threat to the industry from a health and economic perspective. *Staphylococcus aurues* food poisoning alone causes 185,060 illnesses, 1,753 hospitalizations, and 2 deaths in United States annually (Mead et al., 1999) estimated to cost about $1.2 billion (Buzby et al., 1996). Several novel disinfection methods are utilized by the industry to inactivate the pathogens effectively; however, the challenge posed in the preservation of food quality limits their application. Therefore, there is always a need to optimize the existing processing method and/or identify a new method for inactivation of pathogenic microorganisms while preserving the quality of the food.

UV-light has been used as a bactericidal agent for over a century, because it is effective for inactivation of pathogens on a surface and in clear liquid. However, it has a poor penetration capacity and hence was not utilized for treatment of opaque solutions. Pulsed UV-light is the application of broadband UV-light in a pulsed mode, wherein the instantaneous intensity of the UV-light is increased significantly. Increased UV-light intensity and the shocking effect of pulses may aid in enhancing the effectiveness of UV-light on microbial inactivation. Optimization of pulsed UV-light and a proper equipment design may result in a disinfection process for opaque food products such as milk. Pulsed
UV-light may provide a cheaper alternative to existing pasteurization methods as the capital and operational costs are comparatively less than existing technologies. Furthermore, pulsed UV-light treatment also fortifies the Vitamin D content of the food being treated.

Infrared heating is a cost effective method of heat treatment which has several advantages such as high heat transfer rate and energy savings. Infrared heating can also be utilized to heat selectively a particular food component or target of interest such as bacterial cells without heating other components. Therefore, selective heating may result in better product quality as only the bacterial cells are heated. Though not widely utilized for bacterial inactivation, previous studies indicate that far infrared heating is very effective on bacterial inactivation. Though, the energy consumption is almost 50% less than the regular heating process, far infrared heating also results in better product quality. Therefore, successful application of infrared heat treatment will result in cost reduction and better quality food products.

FTIR and TEM can be successfully used for the investigation of inactivation mechanisms of microorganisms. Several researchers have used these techniques to identify the cause of microbial inactivation for several emerging inactivation technologies. Identification of inactivation of mechanism of pulsed UV-light and infrared heating will result in better understanding of the underlying process, which in turn results in better process optimization.

As the literature review substantiates, there was not much research work done in optimization of pulsed UV-light and infrared heating inactivation of *S. aureus* in milk. In this study, *S. aureus* culture inoculated in milk or milk foam was treated with pulsed UV-
light and infrared heating and the degree of *S. aureus* inactivation were determined.

Furthermore, the inactivation mechanism of *S. aureus* using pulsed UV-light was investigated using spectroscopic and microscopic studies. The efficacy of the pulsed UV-light for inactivation of resistant *B. subtilis* spores was also investigated to determine the applicability of UV-light for inactivation of spores.
3. Inactivation of *Staphylococcus aureus* by Pulsed UV-Light Sterilization*

3.1. Abstract

Pulsed UV-light is a novel technology to inactivate pathogenic and spoilage microorganisms in a short time. Efficacy of pulsed UV-light for the inactivation of *Staphylococcus aureus* as suspended or agar seeded cells was investigated. A 12, 24, or 48 ml cell suspension in buffer was treated under pulsed UV-light for up to 30 s and a 0.1 ml of sample was surface plated on Baird-Parker agar and incubated at 37°C for 24 h to determine log$_{10}$ reductions. Also, a 0.1 ml of cell suspension in peptone water was surface plated on Baird-Parker agar plates and the plates were treated under pulsed UV-light for up to 30 s. The treated and untreated plates were incubated as before. A 7 to 8 log$_{10}$ CFU/ml reduction was observed for suspended and agar seed cells treated for 5 s or higher treatment times. In the case of suspended cells, the sample depth, time, treatment, and interaction were significant (p<0.05). In case of agar seeded cells, the treatment time was significant (p<0.05). This study clearly indicates that pulsed UV technology has potential for inactivation of pathogenic microorganisms.

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3.2. Introduction

Foodborne diseases are estimated to cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths annually in the United States (Mead et al., 1999). Therefore, foods contaminated with pathogenic microorganisms, such as, *Salmonella* spp., *Clostridium perfringens*, *Staphylococcus aureus*, *Campylobacter jejuni*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* remain a major public health concern. Among these pathogens, *S. aureus* is one of the common causes of disease (Martin et al., 2001). According to Centers for Disease Control and Prevention (CDC), there have been 17,248 and 1,413 cases of *S. aureus* cases reported during 1973-87 and 1993-97, respectively, accounting for 14% and 1.6% of all cases because of bacterial pathogens (Bean and Griffin, 1990; Olsen et al., 2000).

Several technologies have been evaluated for the inactivation of these pathogens, such as, heat treatment, cold temperature treatment, irradiation, microwave radiation, pulsed electric field, magnetic field, high pressure, and ohmic heating treatments (Juneja and Sofos, 2002). In this study, effect of pulsed UV-light treatment on inactivation of a pathogen, *S. aureus*, was investigated. Though, UV-light treatment is effective in inactivation of pathogens, it may have several disadvantages such as oxidation of unsaturated fats, poor penetration capability of UV-light, and destruction of some vitamins. However, these may be reduced or eliminated by shorter treatment time and thin layer treatment.

UV-light has been used as a bactericidal agent since as early as 1928 (Xenon, 2003). UV-light is a portion of the electromagnetic spectrum ranging from 100 to 400 nm wavelengths. UV-light in the wavelength range of 100 to 280 nm has germicidal
implications (EPA, 1999). UV-light sterilization provides a cost effective alternative to the existing heat pasteurization techniques and preservation methods. Also, taste degradation of the food material subjected to UV-light treatment is minimal, since UV-light treatment can be accomplished at an ambient temperature (Hollingsworth, 2001). UV-light damages the DNA by forming pyrimidine dimers (McDonald et al., 2000). This dimer prevents the microorganism from DNA transcription and replication, which leads to cell death. Several researchers demonstrated that UV-light can be used for the inactivation of pathogens without adversely affecting the quality of food (Allende and Artes, 2003; Rowan et al., 1999; Smith et al., 2002; Yaun et al., 2003).

The UV-light treatment can be accomplished in two modes namely continuous UV-light or Pulsed UV-light. Continuous UV-light has several disadvantages such as poor penetration depth and low dissipation power, whereas pulsed UV-light sterilization has higher penetration depth and dissipation power. Pulsed UV-light treatment is more effective and rapid for microorganism inactivation compared to continuous UV-light sources, since the energy is multiplied many fold (CFSAN-FDA, 2000; Dunn et al., 1995). Power dissipation from a continuous UV-light system ranges from 100 to 1000 W (Demirci, 2002); however, a pulsed UV-light system can produce peak power distribution as high as 35 MW (McDonald et al., 2002; McGregor et al., 1997). In pulsed UV-light treatment, the energy is stored in a high power capacitor and released constantly in a short period of time (nano to microseconds). This produces several high energy flashes per second and hence the microorganisms are inactivated effectively because of high UV-content during the flash and constant disturbance caused by pulses. Moreover, the pulsed UV-light provides cooling periods between pulses and hence reduces the temperature
build-up as compared to continuous UV-light because of short pulse duration and cooling period between pulses. McDonald et al. (2000) compared the effectiveness of a continuous UV light source and a pulsed UV-light source for the decontamination of surfaces. The authors reported that the almost identical level of inactivation of *Bacillus subtilis* was obtained with an energy level of $4 \times 10^{-3}$ J/cm$^2$ of pulsed UV-light source instead of $8 \times 10^{-3}$ J/cm$^2$ continuous UV-light source.

Chang et al. (1985) investigated the effect of continuous UV light on inactivation of *E. coli*, *S. Typhi*, *Shigella sonnei*, *Streptococcus faecalis*, and *S. aureus*. *E. coli*, *S. aureus*, *S. sonnei*, and *S. Tyhi* exhibited similar resistances to the UV-light and required approximately $7 \times 10^{-3}$ J/cm$^2$ energy to obtain 3 log$_{10}$ reduction. However, the resistance exhibited by *S. faecalis* was higher and required 1.4 times higher dose than the above mentioned microorganism to get 3 log$_{10}$ reduction.

On the other hand, a 6 log$_{10}$ reduction of *L. monocytogenes*, *E. coli*, *S. Enteritidis*, *Psudeomonas aeruginosa*, *B. cerus*, and *S. aureus* seeded on agar surface was obtained after a 20 μs treatment with pulsed UV-light (Rowan et al., 1999). Sonenshein (2003) investigated the effect of high-intensity pulsed UV light with an energy level of 15.8 J/cm$^2$.s on inactivation of *B. subtilis* spores. A complete inactivation (7 to 8 log$_{10}$ reduction) was obtained with 1 s exposure of UV-light when the samples were placed at the lamp axis and at the midpoint of the lamp. The effectiveness of pulsed UV-light for the inactivation of *E. coli* O157:H7 on inoculated alfalfa seeds was demonstrated by Sharma and Demirci (2003). A complete inactivation of *E. coli* O157:H7 (4.80 log$_{10}$ CFU/g) was obtained after 30 s treatment when the thickness of alfalfa seeds was kept at 1.02 mm.
Stermer et al. (1987) investigated the effect of pulsed UV radiation on inactivation of bacteria in lamb meat. With 4 x 10^{-3} \text{ J/cm}^2 energy, 99.9\% of the naturally occurring flora of lamb (mostly \textit{Pseudomonas}, \textit{Micrococcus}, and \textit{Staphylococcus} species) was inactivated. The bactericidal effectiveness of pulsed UV-light was investigated by Bank et al. (1990). Bacterial monolayers of \textit{S. epideridis}, \textit{Pseudomonas aeruginosa}, \textit{E. coli}, \textit{S. aureus}, or \textit{Serratia marcescens} on Trypticase soy agar plates were exposed to pulsed UV-light. A 60 s treatment time at 31 cm distance from the light source resulted in 6 to 7 \log_{10} reduction of viable bacterial population (~4 x 10^{-4} \text{ J/cm}^2).

Though, Bank et al. (1990) and Rowan et al. (1999) investigated the effect of pulsed UV-light on inactivation of \textit{S. aureus} on solid surface, there is a need to investigate the effect of pulsed UV-light on liquid medium, which represents liquid food systems such as milk. In addition to liquid medium, the effect of pulsed UV-light on solid agar surface was also investigated as a comparative method. The overall purpose of this study was to investigate the possibility of using pulsed UV-light as an alternative method for the inactivation of \textit{S. aureus} and to investigate the effect of various parameters such as depth of sample, time of exposure, and medium of introduction (solid [agar seeded cells] or liquid [suspended cells]).
3.3. Materials and Methods

Microorganism

*Staphylococcus aureus* (ATCC 13311) was obtained from the Penn State Food Microbiology Culture Collection. Cells were grown in 150 ml of Tryptic Soy Broth (TSB) (Difco, Sparks, MD) at 37°C for 24 h, and harvested by centrifugation (Sorvall STH750, Kendro Lab Products, Newtown, CT) at 3,300 x g for 25 minutes at 4°C.

Sample preparation

The cells to be treated by pulsed UV-light were prepared as suspension cells and agar seeded cells, which represent liquid and solid food systems.

i) Suspended Cell: After centrifugation, the pellet was resuspended in 100 ml of sterile 0.1 M % phosphate buffer (pH=7.0) to obtain viable cell populations of approximately 7 to 8 log_{10} CFU/ml of *S. aureus*. A 12, 24, or 48 ml cell suspension in buffer was transferred to sterile aluminum containers with a diameter of 7 cm (VWR International, Buffalo Grove, IL).

ii) Agar seeded cell: The pellet was resuspended in 100 ml of sterile 0.1% peptone water. The resulting inoculum solution had approximately 7 to 8 log_{10} CFU/ml of *S. aureus* and the cell suspension was serially diluted up to 10^{-5} dilution. A 0.1 ml of cell suspension from each dilution and inoculum was surface plated on Baird-Parker agar plates. To facilitate direct counting of treated and untreated plates, regular plastic Petri dishes were used rather than aluminum containers.
Pulsed UV-light treatment

Pulsed UV-light treatment was carried out with a lab scale, batch pulsed light sterilization system (SteriPulse®-XL 3000, Xenon Corporation, Woburn, MA). The system generated 1.27 J/cm² per pulse at 1.8 cm below the quartz window for an input voltage of 3,800 V and with 3 pulses per second as per the manufacturer. The distance between the quartz window and the centre axis of the UV-light strobe was 5.8 cm. The output from the pulsed UV-light system followed a sinusoidal wave pattern with 1.27 J/cm² per pulse being the peak value of the pulse. Power values of UV-light treatments used in this manuscript are based on peak value of the pulse (1.27 J/cm² per pulse). The pulse width (duration of pulse) was 360 µs. The suspended cell samples with varying volumes were treated under pulsed UV-light for 1, 2, 3, 4, 5, 10, 15, or 30 s at a distance of 8 cm from the quartz window. Similarly, agar seeded plates are treated under pulsed UV-light for 1, 2, 3, 4, 5, 10, 15, or 30 s at a distance of 8 cm from the quartz window. During the pulsed UV-light treatment, the temperature of agar plates and the suspended cell liquid were monitored using a type K thermocouple (Omegaette HH306, Omega Engineering Inc., Stamford, CT).

Microbiological analysis

i) Suspended cell: Untreated samples and samples immediately after pulsed UV-light treatment were analyzed for surviving populations of *S. aureus*. A 1 ml sample from the sample cup was serially diluted with 0.1 M phosphate buffer. This is followed by surface-plating of a 0.1 ml sample on Baird-Parker agar. After incubating at 37°C for 24
h, the colonies were enumerated. The log$_{10}$ reduction was calculated by subtracting the treatment and control plate counts. Each experiment was replicated three times.

ii) **Agar seeded cell:** Pulsed UV-light treated agar plates were incubated for 24 h at 37°C. The higher dilution plates with no colonies were discarded and the colonies in the low dilution plates were counted and reported. Three replications for each experiment were performed.

**Statistical analysis**

Statistical significant differences between treated and untreated cells were tested using the General Linear Model of ANOVA with 2-way interaction with MINITAB software (version 13.3, Minitab Inc., State College, PA). A 95% confidence interval was used.

### 3.4. Results and Discussion

**Suspended cell treatment**

In order to demonstrate effectiveness of pulsed UV-light on liquid medium, *S. aureus* cells were suspended in 0.1 M phosphate buffer and treated by pulsed UV-light up to 30 s at a distance of 8 cm from UV strobe. A complete inactivation was obtained for samples treated with 5 s for all sample sizes. The average corresponding log$_{10}$ reduction was 7.50 log$_{10}$ CFU/ml (Figure 3.1).
Figure 3.1. Log$_{10}$ reductions of *Staphylococcus aureus* after pulsed UV-light sterilization in suspended cell.

The pulsed UV-light was very effective in inactivating *S. aureus* in suspension. Complete inactivation was also observed for 1 and 2 s treatments of 12 and 24-ml samples, respectively. However, after 1-s treatment, a 4.6 log$_{10}$ and 1.5 log$_{10}$ reductions were obtained in 24 and 48 ml samples, respectively. For the 48 ml sample, the log$_{10}$ reduction increased exponentially in 5 s. As expected, the log$_{10}$ reduction increased with treatment time because of increase in number of pulses. The effect of the sample depth was significant (p<0.05). The temperature of the sample increases, as the treatment time increases after several seconds (Figure 3.2), however, no significant temperature increase was observed during the first 5 s. Pulsed UV-light treatment is considered non-thermal, but holds only for short time treatments. Temperature increases as absorbed energy accumulates during longer treatments. During a 20-s treatment time, the temperature
increase was about 20°C for a 12 ml phosphate buffer sample. Finally, statistical analysis suggested that the effect of treatment time and the interaction (treatment time*depth) were significant (p<0.05).

Figure 3.2. Temperature profile of phosphate buffer and Baird-Parker agar base during pulsed UV-light treatment.

**Agar seeded cells**

In order to demonstrate the effectiveness of pulsed UV-light on solid surfaces, agar seeded *S. aureus* cells were treated by pulsed UV-light for up to 30 s at a distance of 8 cm from the UV strobe. The corresponding power available at 8 cm distance away from the quartz window were 0.99, 1.98, 4.95, and 29.7 J/cm²/s for 1, 2, 5, and 30 s treatment time, respectively. A 5-s treatment inactivated all *S. aureus* to yield about 8.5 log₁₀ reduction (Figure 3.3) contributing to an effective inactivation of *S. aureus* (P<0.05)
on the agar surface, when the distance between agar seeded cells and UV strobe was 8 cm. Bank et al. (1990) obtained 6 to 7 log\textsubscript{10} reduction of agar seeded cells after 60 s treatment, when the distance from UV strobe was 31 cm. The corresponding power obtained by the sample is 6.67x10\textsuperscript{-6} J/cm\textsuperscript{2}/s. Since, the distance of quartz window from the sample for our system was different from Bank et al. (1990), comparing the log\textsubscript{10} reduction from both the cases will be futile. A 5 log\textsubscript{10} reduction was obtained after a 1-s of treatment. As expected, the log\textsubscript{10} reduction of \textit{S. aureus} increased exponentially with the treatment time similar to suspended cell solution and complete inactivation was achieved within 5 s.

![Graph](image)

**Figure 3.3.** Log\textsubscript{10} reductions of \textit{Staphylococcus aureus} after pulsed UV-light sterilization in seeded agar plates.

The temperature of the agar increased for longer treatment times (Figure 3.2). However, there is no significant increase in the temperature during the first 5 s and the inactivation was hypothesized to occur primarily because of the pulsed UV-light and not
because of the synergistic effect from the temperature increase. Experiments indicated that a few colonies survived along the edge of the plate possibly because of the obstruction of light by the elevated edges of the plate. The intensity of UV-light reduced with the radial distance from the central axis of the lamp.

Pulsed UV-light treatment is more effective on surface sterilization than sterilization of liquid medium. Log_{10} reductions of 5.0 and 1.35 CFU/ml were obtained for agar seeded cells and suspended cells (when sample volume was 48 ml), respectively after a 1 s treatment. However, as expected when the depth of the suspended cell was kept to minimum by having smaller suspended cell volume, suspended cell inactivation yielded similar results as agar seeded cells. As the sample depth of suspended cell increases, inactivation level of \textit{S. aureus} decreases, because of poor penetration capacity of pulsed UV-light. Therefore, one can increase the effectiveness of pulsed UV-light on inactivation of suspended cells by minimizing the sample depth and/or increasing the treatment time.

### 3.5. Conclusions

The present study clearly shows the potential of pulsed UV-light for \textit{S. aureus} inactivation in liquid and solid systems. Complete inactivation of \textit{S. aureus} can be achieved within seconds with pulsed UV-light. Therefore, pulsed UV-light can be used as an alternative to thermal sterilization processes. However, there is a need for optimization of the experimental parameters to achieve the target inactivation level for specific applications. Further research is in progress to evaluate the applicability of pulsed UV-light for the sterilization of various food products.
3.6. References


4. Inactivation of *Staphylococcus aureus* in Milk and Milk Foam by Pulsed UV-light Treatment and Surface Response Modeling

4.1. Abstract

Pulsed UV-light is a novel technology that can be used to inactivate pathogens in a short time. In this study, efficacy of pulsed UV-light was investigated for inactivation of *S. aureus* in milk and milk foam, as it is a pathogen of concern in milk and milk products. A 12, 30, or 48-mL of cell suspension in milk was treated under pulsed UV-light for 30, 105, or 180 s. The reduction obtained varied from 0.16 to 8.55 log$_{10}$ CFU/ml demonstrating the ability of pulsed UV-light to inactivate *S. aureus*. Complete inactivation was obtained at (1) 8-cm sample distance from quartz window, 30-ml sample volume, and 180-s time combination and (2) 10.5-cm sample distance from quartz window, 12-ml sample volume, and 180-s treatment time combination. In case of milk foam, reductions up to 6.61±0.11 log$_{10}$ CFU/g were obtained at several conditions. It was noted that there was a significant increase in the milk temperature during pulsed UV-light treatment, which might have also contributed to microbial inactivation.
4.2. Introduction

In 2000, the United States produced 76 million tons of milk worth more than $20 billion (IDFA, 2001). Milk is used in the manufacture of dairy products as well in the manufacture of a wide variety of food products (IDFA, 2001). Milk may contain spoilage and pathogenic microorganisms because of improper handling and contamination from both inside and outside of udder (Bramley and McKinnon, 1990). Conventionally, thermal pasteurization is used to inactivate microorganisms in the milk. However, thermal pasteurization has several disadvantages such as high energy demand and high capital and operational cost. In addition, during thermal pasteurization, warm areas in the heat exchangers are conducive for the growth of \textit{Staphylococcus aureus}, a pathogenic microorganism, which produces heat-resistant toxins.

Foodborne diseases are estimated to cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,200 deaths annually in the United States (Crutchfield and Roberts, 2000). Therefore, foods contaminated with pathogenic microorganisms, such as \textit{Salmonella} spp., \textit{Clostridium perfringens}, \textit{S. aureus}, \textit{Campylobacter jejuni}, \textit{Escherichia coli} O157:H7, and \textit{Listeria monocytogenes} remain a major public health concern. Among these pathogens, \textit{S. aureus} is the most common cause of suppurating infections (Martin et al., 2001). Based on the data from the Centers for Disease Control and Prevention (CDC), 185,060 illnesses, 1,753 hospitalizations, and 2 deaths occur annually because of staphylococcal food poisoning, respectively (Mead et al., 1999). \textit{S. aureus} infections and intoxications from food sources are estimated to cost about 1.2 million dollar annually (Buzby et al., 1996). Milk and milk products often associate with \textit{S.}}
*aureus* contamination. Therefore, it is necessary to inactivate the pathogens including *S. aureus* in milk more effectively in order to ensure its safety.

Although there are several technologies available for inactivation of *S. aureus* and other pathogens, they may change the color, flavor, and/or texture of food products. Therefore, there is a need for novel processes that can eliminate pathogens while maintaining the quality of milk. Pulsed UV-light treatment is a potential microbial inactivation method. The bactericidal effect of UV-light was known as early as 1892 (Kime, 1980). UV-light is a portion of electromagnetic spectrum ranging from 100 to 400 nm wavelengths and provides a cost-effective alternative to existing heat pasteurization techniques and preservation methods. Also, taste degradation of the milk subjected to UV-light treatment can be minimal, since UV-light treatment can be accomplished at ambient temperature (Hollingsworth, 2001). UV-light damages the DNA by forming thymine dimers (Bank et al., 1990; Miller et al., 1999). These dimers prevent the microorganism from accomplishing DNA transcription and replication and thereby lead to cell death (Miller et al., 1999).

The UV-light sterilization can be accomplished in two modes: continuous UV-light or pulsed UV-light. Pulsed UV-light is a more effective and rapid way of inactivating microorganisms than continuous UV-light, since the instantaneous power is multiplied many fold in case of pulsed UV-light (CFSAN-FDA, 2000; Dunn et al., 1995). McDonald et al. (2000) compared the effectiveness of continuous UV-light and the pulsed UV-light sources for surface decontamination. The authors reported that similar levels of inactivation of *Bacillus subtilis* were obtained with 4 mJ/cm² of pulsed UV-light and 8 mJ/cm² continuous UV-light.
Chang et al. (1985) investigated the effect of continuous UV light for the inactivation of *E. coli*, *Salmonella Typhi*, *Shigella sonnei*, *Streptococcus faecalis*, and *S. aureus*. Among these microorganisms, *E. coli*, *S. aureus*, *S. sonnei*, and *S. Typhi* exhibited similar resistance to the UV-light and required approximately 7 mJ/cm² energy to get a 3 log₁₀ reduction. However, the resistance exhibited by *S. faecalis* was higher and required 1.4 times higher dose than the above mentioned microorganisms to get a 3 log₁₀ reduction.

The effect of pulsed UV-light emission with low or high UV content on inactivation of *L. monocytogenes*, *E. coli*, *Salmonella Enteritidis*, *Psudeomonas aeruginosa*, *Bacillus cereus*, and *S. aureus* were investigated by Rowan et al. (1999). A reduction of 2 or 6 log₁₀ CFU/plate was obtained with 200 light pulses (duration of pulse ~100 ns) for low or high UV content, respectively. Treatment with three 360 µs duration pulses resulted in more than 6 log₁₀ CFU/ml reduction of *B. subtilis* for an energy input of 1.2 J/cm²/s (Xenon, 2005).

Stermer and others (1987) investigated the effect of UV radiation on inactivation of bacteria on lamb meat surfaces. With 4 mJ/cm² energy, 99.9% of the naturally occurring flora of lamb (mostly *Pseudomonas*, *Micrococcus*, and *Staphylococcus* species) were inactivated. The bactericidal effectiveness of pulsed UV-light was investigated by Bank and others (1990) on bacterial monolayers of *S. epidermidis*, *Pseudomonas aeruginosa*, *E. coli*, *S. aureus*, or *S. marcescens*. A 60-s treatment time at 31 cm distance from the light source (~4 J/m²) resulted in 6 to 7 log₁₀ reduction of viable bacterial population.
Aspergillus niger spores in corn meal were inactivated using a pulsed UV-system (Jun et al., 2003). A 4.95 \log_{10} reduction of A. niger on inoculated corn meal with a treatment time of 100-s was achieved when the distance between the quartz window and sample was kept at 8 cm with an input voltage of 3800 V. Sharma et al. (2003) investigated the effect of pulsed UV-light on inactivation of E. coli O157:H7 on alfalfa seeds. Reductions of 0.09 to 4.89 \log_{10} CFU/g were obtained for various thickness and treatment time combinations. Complete inactivation of E. coli O157:H7 was obtained within 30-s treatment time, when the seed thickness was maintained at 1.02 mm, which corresponds to 4.80 \log_{10} CFU/g. Hillegas and Demirci (2003) studied the effect of pulsed UV-light on inactivation of Clostridium sporogenes in honey. An 87.6% reduction of Clostridium sporogenes was obtained when 2 mm depth of honey sample was kept at 8 cm distance from the quartz window and treated with UV-light for 45-s, whereas an 180-s treatment was required to achieve 89.4% reduction at 20 cm distance from the quartz window. Krishnamurthy et al. (2005; chapter 3) reported that complete inactivation of S. aureus (about 8 \log_{10} CFU/ml reduction) can be obtained within 2 or 5 s of pulsed UV-light treatment, when treated as agar seeded cells and cell suspension, respectively. It was suggested that the pulsed UV-light treatment is non-thermal for a short time applications (<5 s treatment). These studies demonstrate that pulsed UV-light treatment can be utilized for the inactivation of S. aureus in both solid and liquid food systems.

As the opacity of the liquid increases, penetration depth of UV-light in food decreases. Therefore, only the top portion of the liquid and associated pathogen are well exposed to UV-light because of poor penetration. So, pathogens may still survive in the
bottom portion and hence reduce the overall inactivation level of the pathogen in food. Thus, better overall inactivation of a pathogen can be achieved in a less opaque food matrix. For instance, Krishnamurthy et al. (2004; chapter 3) reported that a 5-s treatment time was sufficient to achieve a 7 to 8 $\log_{10}$ reduction of $S.\ aureus$ in 0.1 M phosphate buffer (pH = 7.0). However, Hillegas and Demirci (2003) obtained less than one $\log_{10}$ reduction of $C.\ sporogenes$ in honey treated for 45 s at 8-cm distance when a 2-mm depth of sample was used. Jun et al. (2003) reported that voltage applied to the pulsed UV-light system is an important parameter in pulsed UV-light treatment. Reductions of 1.35 and 4.95 $\log_{10}$ CFU/ml of $A.\ niger$ spores were obtained in corn meal when treated for 100 s and sample distance was kept as 8 cm for 2000 and 3800 V applied voltage, respectively. Results of this study and other findings indicate that sample volume, treatment time, sample distance from quartz window, opacity of the sample, and applied voltage are some parameters which influence the inactivation of pathogens. By optimizing these parameters one can effectively utilize pulsed UV-light treatment for a better inactivation of pathogens.

As the literature review substantiates, there is not much research work done on the inactivation of $S.\ aureus$ in milk using pulsed UV-light treatment. Therefore, the overall goal of this study was to investigate the possibility of using pulsed UV-light as an alternative method for inactivation of $S.\ aureus$ and determining the effect of various parameters such as volume of sample, time of exposure, and distance from the UV-light source on inactivation of $S.\ aureus$. 

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4.3. Materials and Methods

Preparation of inoculum

*Staphylococcus aureus* (ATCC 13311) was obtained from the Penn State Food Microbiology Culture Collection. Stock cultures were maintained on tryptic soy agar (TSA) (Difco) slants at 4°C. Also, stock cultures in 20% glycerol as a cryoprotectant were maintained at -80°C in an ultra-low freezer (Sanyo Scientific Inc., Chatsworth, CA). Cells were grown in 150 ml Tryptic Soy Broth (TSB; Difco, Sparks, MD) at 37°C for 24 h, and harvested by centrifugation (Sorvall STH750, Kendro Lab Products, Newtown, CT) at 3,300 x g for 25 min at 4°C. The pellet was resuspended in 100 ml of raw milk after decanting the supernatant to yield approximately 8 to 9 log_{10} CFU/mL of *S. aureus*.

Experimental design

A Box-Behnken surface response method design was utilized for experimental design. This method has several advantages such as, reduced number of samples and reduced number of replicates. This method recommends the treatments at the mid-points of the edges (12 edges for 3 factors with 3 levels; hence, 12 data points) and the center of the factor space (centre point is replicated three times; hence 12 + 3 = 15 data points total) (Figure 4.1). A Box-Behnken design for three factor levels is 15 data points whereas a full factorial design would have 27 data points (3 factors x 3 levels x 3 replicates = 27 treatments).
In general, the Box-Behnken design will have a combination of minimum, maximum, and middle values specified for each factor. By using this method, one can model the system effectively with fewer data points. MINITAB® (version 13.3; Minitab Inc., State College, PA) statistical software was used to design the experiments.

**Production of milk foam**

Milk foam was produced by heating the milk to 65°C and adding 2% of whey protein isolate (Alacen™ 895, NZMP, Lemoyne, PA). After mixing slowly the mixture for 10 min, the milk was kept at 45°C for 10 min. Foam was created by bubbling air at 1 psi through the milk. The average temperature of the foam was 45 ± 2°C. The foam was then weighed and transported aseptically to pulsed UV-light treatment chamber in an aluminum dish.
Pulsed UV-light treatment

Pulsed UV-light treatment was carried out with SteriPulse®-XL 3000 Pulsed Light Sterilization System (Xenon Corporation, Wilmington, MA; Figure 4.2). The sterilization system generated 1.27 J/cm² per pulse for an input voltage of 3,800 V and with 3 pulses/s setting at 1.8 cm from the quartz window as per the manufacturer’s specifications. It should be noted that the distance between the centre axis of the UV-strobe and the quartz windows is 5.8 cm. The lamp produced a polychromatic radiation in the wavelength range of 100 to 1100 nm, with 54% of the energy being in the UV-light region (Panico, 2002). Volumes of 12, 30, and 48-mL of cell suspension in milk was treated under pulsed UV-light for 30, 105, and 180 s according to the surface response design (Table 4.1).

Figure 4.2. Schematic of Steripulse® XL-RS-3000 pulsed UV-light system.

The samples were placed inside the sterilization chamber in sterile aluminum containers with vertical walls (7-cm diameter x 1.6-cm depth) at three sample distances from quartz window levels: 8, 10.5, and 13-cm. The calculated depth levels
corresponding to 12, 30, and 48-mL of milk were 3.12, 7.79, and 12.47-mm, respectively. The effect of pulsed UV-light on milk foam was also tested by evaluating the effect of the following variables and levels, distance of milk foam from the quartz window (5, 8, and 11 cm), treatment time (30, 105, 180 s), and weight of milk foam (3, 5, and 7 g). Three replications were performed for each condition.

**Microbiological analysis**

Immediately after pulsed UV-light treatment, surviving populations of *S. aureus* were enumerated. A 10 ml of 0.1% peptone was added gradually to milk foam in order to recover the microorganism and serially diluted in 0.1% peptone water. In case of milk, 1-ml of sample was serially diluted using 0.1% peptone water. Both the samples were spiral-plated on Baird-Parker agar by using Autoplate® 4000 (Spiral Biotech, Norwood, MA). After incubating at 37°C for 24 h, the colonies were enumerated by Q-Count™ (Spiral Biotech). The log₁₀ reduction was calculated by determining the difference between average log₁₀ value of untreated samples and average log₁₀ value of pulsed UV-light treated samples. Also, enrichment was performed for zero or low counts of *S. aureus* by transferring 1-ml of sample into 9-ml of TSB and incubating at 37°C for 24 h.

**Temperature measurements**

Milk temperature during pulsed UV-light treatment was monitored using K-type thermocouples and the data were stored using a data-logger (Omegatette HH306, Omega Engineering Inc., Stamford, CT).
**Water bath studies**

As the temperature of the milk increased during pulsed UV-light treatment, the effect of heat treatment alone was investigated using a water bath study. A 50-ml milk sample kept in a sterile 250-ml beaker (Kimax Model No. 14000, VWR international, West Chester, PA) was inoculated with 1-ml of *S. aureus* culture and immediately placed in the water bath (Aquabath Model No. 18800; Labline Instruments Inc., Melrose park, IL) maintained at the room temperature. The inner diameter of the beaker was approximately 6.5 cm, and the depth of milk was approximately 1.7 cm. The water in the water bath was kept at least 1 cm above the milk surface to make sure that the milk was always surrounded by hot water. The water bath was immediately turned on with the temperature setting of 87°C, and the temperature was allowed to rise freely to reach the target milk temperature of 85°C. Milk was constantly stirred in order to maintain homogenous temperature. Three replications were used, and the temperature of the milk was monitored regularly using a thermometer. A 1-ml milk sample was taken at regular intervals (1, 2, 4, 8, 16, 32, and 64 min) and analyzed for surviving population of *S. aureus*.

**Energy measurements**

The available broad band energy (100 to 1100 nm) at different tray levels of the pulsed UV-light system was measured by using a radiometer (Ophir PE50, Ophir Optronics Inc., Wilmington, MA). The pyroelectric detection head and the centre axis were aligned and the detection head was placed at the centre. Measurements were taken at this location, as all the experiments done throughout this study. The radiometer was
calibrated at 254 nm. The UV-light intensity at 254 nm was determined by comparing the UV-light intensity obtained using another radiometer (SED240/ACT5/W detector head, International lights, Newburyport, MA) as per the data given by Xenon Corporation (Wilmington, MA).

**Statistical analysis**

Statistical significance was tested using the Surface Response Method using MINITAB® software. A 95% confidence level was used to determine the significance. Two independent data sets were used in model development and one independent data set was used for validation for static milk treatment. For milk foam treatment, a surface response model was developed with an independent data set.

### 4.4. Results and Discussion

The effects of sample volume, treatment time, and distance from the light source were investigated for milk foam. The effects of foam weight, treatment time, and distance were investigated for milk foam. A surface response methodology design was used to design the experiments.

**Static milk treatment**

The log$_{10}$ reductions obtained varied from 0.16 to 8.55 log$_{10}$ CFU/ml (Table 4.1). This demonstrated the ability of pulsed UV-light to inactivate *S. aureus*. The effects of treatment time and time*volume interaction were significant (p<0.05). The log$_{10}$ reduction increased as the treatment time increased, sample volume decreased, or the
sample distance from the quartz window decreased. Maximum log reductions of 8.55 log_{10} CFU/ml were obtained for 1) 8-cm sample distance from quartz window, 30-ml sample volume, and 180-s treatment time combination and 2) 10.5-cm sample distance from quartz window, 12-ml sample volume, and 180-s treatment time combination.

Table 4.1. Surface response method analysis of pulsed UV-light treatment of milk.

<table>
<thead>
<tr>
<th>Distance (cm)</th>
<th>Time (s)</th>
<th>Volume (ml)</th>
<th>Log_{10} reduction(^1)</th>
<th>Validation log_{10} reduction (experimental)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>30</td>
<td>30</td>
<td>1.13 ± 0.45</td>
<td>0.89</td>
</tr>
<tr>
<td>8</td>
<td>105</td>
<td>12</td>
<td>2.13 ± 0.28</td>
<td>1.87</td>
</tr>
<tr>
<td>8</td>
<td>105</td>
<td>48</td>
<td>0.16 ± 0.07</td>
<td>0.12</td>
</tr>
<tr>
<td>8</td>
<td>180</td>
<td>30</td>
<td>8.55 ± 0.27(^*)</td>
<td>8.63</td>
</tr>
<tr>
<td>10.5</td>
<td>30</td>
<td>12</td>
<td>0.19 ± 0.01</td>
<td>0.23</td>
</tr>
<tr>
<td>10.5</td>
<td>105</td>
<td>48</td>
<td>0.93 ± 0.71</td>
<td>1.01</td>
</tr>
<tr>
<td>10.5</td>
<td>105</td>
<td>30</td>
<td>1.68 ± 0.37</td>
<td>1.50</td>
</tr>
<tr>
<td>10.5</td>
<td>105</td>
<td>30</td>
<td>0.62 ± 0.31</td>
<td>0.78</td>
</tr>
<tr>
<td>10.5</td>
<td>105</td>
<td>30</td>
<td>1.04 ± 0.34</td>
<td>1.35</td>
</tr>
<tr>
<td>10.5</td>
<td>180</td>
<td>12</td>
<td>8.55 ± 0.27(^*)</td>
<td>8.63</td>
</tr>
<tr>
<td>10.5</td>
<td>180</td>
<td>48</td>
<td>0.94 ± 0.42</td>
<td>0.79</td>
</tr>
<tr>
<td>13</td>
<td>30</td>
<td>30</td>
<td>0.14 ± 0.06</td>
<td>0.21</td>
</tr>
<tr>
<td>13</td>
<td>105</td>
<td>12</td>
<td>3.01 ± 1.00</td>
<td>2.89</td>
</tr>
<tr>
<td>13</td>
<td>105</td>
<td>48</td>
<td>0.19 ± 0.14</td>
<td>0.31</td>
</tr>
<tr>
<td>13</td>
<td>180</td>
<td>30</td>
<td>0.61 ± 0.58</td>
<td>0.46</td>
</tr>
</tbody>
</table>

\(^*\) Complete inactivation of *Staphylococcus aureus* was obtained at these conditions (Enrichments were negative)
\(^1\)Mean and standard deviation of two replications are given. Mean was used to develop the model.

**Effect of sample volume**

As the volume of the sample decreased the log_{10} reduction increased at longer treatment times (Figure 4.3A). For example, pulsed UV-light treatment of *S. aureus* at 10.5- cm distance and 180-s treatment time resulted in 8.55 and 0.94 log_{10} CFU/ml reductions for 12 and 48-ml sample volumes, respectively. The treatment time*volume of
sample interaction was significant (p<0.05). In other words, the effect of treatment volume also depends upon the treatment time. For example, log$_{10}$ reductions of 1.13 and 8.55 log$_{10}$ CFU/ml were obtained for 30 and 180-s treatments, respectively, when sample distance was kept at 8 cm and the sample volume was 30 ml. Also at lower volumes, there is a rapid increase in log$_{10}$ reduction for increase in treatment time, when compared to higher volumes because of poor penetration capacity of UV-light.

**Effect of treatment time**

As the treatment time increased, the log$_{10}$ reduction increased (p<0.05) because of increased cumulative energy absorption by the bacteria (Figure 4.3B). Also, the distance*time interaction was significant (p<0.05). Reductions of 0.19 and 8.55 log$_{10}$ CFU/ml were obtained when treated for 30 s and 180 s, respectively, at 10.5-cm sample distance from quartz window and 12-ml sample volume. Data clearly show that there is an exponential relationship between log$_{10}$ reduction and treatment time. This can be verified by the rapid increase in log$_{10}$ reduction with the time of exposure.

**Effect of distance**

The amount of energy received by the sample decreased as the sample distance from the quartz window increased (Figure 4.3B). When compared to other distances from the UV-strobe, the 8-cm distance exhibited higher log$_{10}$ reduction, since the sample was closer to the UV lamp than samples at 10.5 and 13-cm; the samples hence received more energy.
Figure 4.3. Inactivation of *S. aureus* in milk: A) Interaction of treatment time and sample volume (hold value: distance: 10.5 cm), B) Interaction of distance from quartz window and treatment time (hold value: volume: 30 ml), and C) Interaction of distance from quartz window and sample volume (hold value: time: 105 s).
For instance, 180-s treatment of 30-ml sample resulted in 8.55 and 0.61 \( \log_{10} \) CFU/ml reduction when treated at 8 and 13-cm distance from the quartz window, respectively.

The effect of distance was found to have a significant impact on \( \log_{10} \) reduction during longer treatment time compared to shorter treatment times (Figure 4.3B). The \( \log_{10} \) reduction increased as the volume of sample and the sample distance from quartz window decreased (Figure 4.3C).

These results are compared with studies of Smith et al. (2003) and Bank et al. (2000). Smith et al. (2003) obtained ~2.0 \( \log_{10} \) reduction of \textit{S. marcescens} when inoculated in raw bulk tank milk after 28-s treatment time (6.6 J/cm\(^2\) dose level) with a pulsed UV laser light. Bank et al. (2000) reported that a 60-s treatment time at 31-cm distance from the light source (approximately 4 x10\(^{-4}\) J/cm\(^2\)) resulted in 6 to 7 \( \log_{10} \) reduction of \textit{S. aureus} on seeded TSA plates.

**Temperature profile during pulsed UV-light treatment**

The pulsed UV-light treatment for inactivation of microorganisms is considered a non-thermal process for short times (less than 5 or 10-s) (chapter 3). However, increase in cumulative energy results in temperature increase. Therefore, as treatment time increased, the temperature increased gradually (Figure 4.4). The temperature of the sample was 28\(^\circ\)C, 58.1\(^\circ\)C, and 91.2\(^\circ\)C when treated for 10, 60, and 180 s, respectively, when a sample volume of 12-mL was kept at 8 cm distance. Also, as the distance from the quartz window decreases, the temperature increases as the energy absorption increases because of more energy available to the sample. Sample temperatures were 72.3\(^\circ\)C, 69.6\(^\circ\)C, and 38.6\(^\circ\)C after 100 s treatment when the sample volume was 30-mL for
sample distances of 8, 10.5, and 13-cm, respectively. Similarly, an increase in sample volume resulted in a decrease in temperature rise. For example, a 180-s treatment at 8-cm sample distance from the quartz window resulted in 91.2°C, 73.2°C, and 57°C sample temperatures, when the sample volumes were 12, 30, and 48 ml, respectively.

![Temperature profile during pulsed UV-light treatment](image)

Figure 4.4. Temperature profile during pulsed UV-light treatment

**Heat treatment of *S. aureus* in water bath**

There was a considerable increase in the temperature of a milk sample during pulsed UV-light treatment. Therefore, the inactivation also might have been partly contributed to the temperature increase. In order to investigate this possibility, milk inoculated with *S. aureus* was heated in a water bath to the same temperature achieved during pulsed UV-light treatment, which was increased to about 85°C after a 3 min of pulsed UV-light treatment.

The temperature increase during the pulsed UV-light treatment was gradual. Therefore, the amount of time required for the gradual increase in temperature for
inactivation of *S. aureus* was investigated. The water bath temperature was set at 88°C, so that the temperature of the milk sample can reach up to 85°C gradually. It took 64 min for complete inactivation of *S. aureus* (Table 4.2). The temperature reached 85°C after 40 min.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Log10 reduction</th>
<th>Growth after enrichment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.75</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>0.70</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>0.62</td>
<td>Yes</td>
</tr>
<tr>
<td>16</td>
<td>1.20</td>
<td>Yes</td>
</tr>
<tr>
<td>32</td>
<td>3.21</td>
<td>Yes</td>
</tr>
<tr>
<td>64</td>
<td>8.12</td>
<td>No</td>
</tr>
</tbody>
</table>

These results indicate that the temperature rise in milk also contributed to inactivation of *S. aureus*. However, it took about 3 min for pulsed UV-light treatment, whereas it took more than 40 min for the heat treatment in a water bath.

**Surface response model and validation for milk**

A full quadratic surface response model with constant, linear, interactions, and squared terms was developed. Variables used in the model were sample distance from the quartz window (Distance “D”, cm), treatment time (Time “T”, s), and volume of sample (Volume “V”, ml). Response surface modeling was done using MINITAB with uncoded units and the following regression equation was obtained.

\[
\text{Log}_{10} \text{ reduction} = \beta_0 + \beta_1 D + \beta_2 T + \beta_3 V + \beta_2 T^2 + \beta_12 DT + \beta_13 DV + \beta_23 TV + \varepsilon.
\]

Where, \(\varepsilon = \text{Error and } \beta_0, \beta_1, \beta_2, \beta_3, \beta_{22}, \beta_{12}, \beta_{13}, \text{ and } \beta_{23} \) are coefficients (Table 4.3)
D² and V² terms are not included in the model, because they were not significant. Although T² term was not significant, it was used in the model because the Time (T) term was significant.

Table 4.3. Regression coefficients of response surface model for inactivation of *S. aureus* in milk.

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>β₀</td>
<td>-8.83684</td>
<td>0.247</td>
</tr>
<tr>
<td>β₁</td>
<td>0.71367</td>
<td>0.296</td>
</tr>
<tr>
<td>β₂</td>
<td>0.11970</td>
<td>0.047*</td>
</tr>
<tr>
<td>β₃</td>
<td>0.13097</td>
<td>0.471</td>
</tr>
<tr>
<td>β₁₁₂</td>
<td>0.00024</td>
<td>0.097</td>
</tr>
<tr>
<td>β₁₁₃</td>
<td>-0.00927</td>
<td>0.040</td>
</tr>
<tr>
<td>β₂₂₃</td>
<td>-0.00472</td>
<td>0.767</td>
</tr>
<tr>
<td>β₃₃</td>
<td>-0.00155</td>
<td>0.019</td>
</tr>
</tbody>
</table>

*Bold values indicate terms which are statistically significant (p<0.05).*

**Defining optimum or maximum log reduction:** The model predicted a mathematically optimum condition of 8 cm distance, 12 ml volume, and 180 s treatment time, yielding an estimated reduction of 10.74 log₁₀ CFU/ml. Not surprisingly, this is the closest distance, smallest volume, and longest time the model was allowed to consider and was a more severe treatment than any condition in the experiment. Complete inactivation of *S. aureus* was obtained when the UV-light treatment was conducted at the above mentioned optimum condition, which corresponds to 8.70 log₁₀ CFU/ml reduction. The observed log₁₀ reduction (8.70 log₁₀ CFU/ml) was less than the predicted log₁₀ reduction (10.74 log₁₀ CFU/ml) because of the limitation of maximum bacterial density. As mentioned earlier, as the volume of the sample decreases the log₁₀ reduction increases. These indicate that one should expect to get complete inactivation of *S. aureus* at the model generated optimal condition.
An $R^2$ value (coefficient of determination) of 0.88 was obtained with this model, which implies that 88% of the sample variation was taken into account by the model. Only treatment time, time*distance interaction, and time*volume interaction were significant ($p<0.05$) (Table 4.3). An independent set of data was collected in order to validate the model developed. The predicted data reasonably agrees with the experimental data with an $R^2$ of 0.87 (Figure 4.5) and a slope of 0.90, when the trend line passes through the origin.

Figure 4.5. Validation of the response surface model

A perfect fit would have an $R^2$ of 1.0 and a slope of 1.0 for the same condition. When the data points were forced to fit the perfect fit (experimental = predicted) line, the model yielded an $R^2$ of 0.85, which indicates that predictive ability of the model is reasonably good.
4.5. **Inactivation of S. aureus in milk foam**

UV-light has a poor penetration capacity. Therefore, it is necessary to facilitate vigorous mixing of a milk sample in order to expose most of the sample to UV-light during continuous flow treatment of milk. Foam is produced during this vigorous mixing and it may behave differently as the composition is different from that of the regular milk sample. Therefore, Inactivation of *S. aureus* in milk foam was studied in order to investigate the effect of UV-light on foam. Reductions of 1.05 to 6.61 log$_{10}$ CFU/g were obtained (Table 4.4 and Figure 4.6).

Table 4.4. Inactivation of *S. aureus* in milk foam.

<table>
<thead>
<tr>
<th>Distance (cm)</th>
<th>Time (s)</th>
<th>Weight (g)</th>
<th>Reduction (Log$_{10}$ CFU/g)</th>
<th>Validation reduction (Log$_{10}$ CFU/g - experimental)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>30</td>
<td>5</td>
<td>1.19±0.01</td>
<td>1.17</td>
</tr>
<tr>
<td>5</td>
<td>105</td>
<td>3</td>
<td>6.61±0.11*</td>
<td>7.10*</td>
</tr>
<tr>
<td>5</td>
<td>105</td>
<td>7</td>
<td>5.00±0.26</td>
<td>4.94</td>
</tr>
<tr>
<td>5</td>
<td>180</td>
<td>5</td>
<td>6.39±0.11*</td>
<td>6.88*</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>3</td>
<td>1.67±0.05</td>
<td>1.68</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>7</td>
<td>1.05±0.23</td>
<td>1.31</td>
</tr>
<tr>
<td>8</td>
<td>105</td>
<td>5</td>
<td>6.39±0.11*</td>
<td>6.88*</td>
</tr>
<tr>
<td>8</td>
<td>105</td>
<td>5</td>
<td>6.39±0.11*</td>
<td>6.88*</td>
</tr>
<tr>
<td>8</td>
<td>180</td>
<td>3</td>
<td>6.61±0.11*</td>
<td>7.10*</td>
</tr>
<tr>
<td>8</td>
<td>180</td>
<td>7</td>
<td>6.24±0.11*</td>
<td>6.73*</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>5</td>
<td>1.70±0.33</td>
<td>1.72</td>
</tr>
<tr>
<td>11</td>
<td>105</td>
<td>3</td>
<td>6.61±0.11*</td>
<td>7.10*</td>
</tr>
<tr>
<td>11</td>
<td>105</td>
<td>7</td>
<td>2.13±0.44</td>
<td>2.53</td>
</tr>
<tr>
<td>11</td>
<td>180</td>
<td>5</td>
<td>6.39±0.11*</td>
<td>6.88*</td>
</tr>
</tbody>
</table>

*No growth observed on agar plates after incubation. Further enrichment was positive for most of the samples indicating sublethal injury and cells were able to repair themselves.
Figure 4.6. Inactivation of *S. aureus* in milk foam: A) Interaction of distance and treatment time (hold value: weight of foam: 5 g), B) Interaction of distance from quartz window and weight of foam (hold value: treatment time: 105 s), and C) Interaction of foam weight and treatment time (hold value: distance: 8 cm).
As expected, increase in treatment time resulted in higher inactivation of *S. aureus* (Figure 4.6A). The effect of treatment time on inactivation of *S. aureus* was statistically significant (p<0.05). For instance, reductions of 1.19 and 6.39 log$_{10}$ CFU/g were obtained for 30 and 180 s treatment, respectively, when the distance from the quartz window was 5 cm for 5 g of milk foam. The cumulative energy absorbed by *S. aureus* is increased as the treatment time increases, resulting in a higher probability of photon absorption for effective inactivation.

Weight of the milk foam did not have a significant effect on inactivation of *S. aureus* (p > 0.05). For example, reductions of 6.61 and 6.24 log$_{10}$ CFU/g were obtained after treatment of 3 and 7 g of milk foam, respectively, when the samples were treated for 180 s at 8 cm distance from the quartz window (Figure 4.6C). As the UV-light can easily penetrate through the foam because of its structure, increasing the volume of foam did not change the inactivation significantly.

In general, increasing the distance of the sample from the quartz window did not significantly affect the inactivation as the effect of treatment time was predominant (Figure 4.6A) (p>0.05).

**Surface response model and validation for milk foam**

A full quadratic equation was developed for the response surface model using MINITAB with uncoded units. The following variables are used in developing the model, 1) sample distance from the quartz window (Distance “D”, cm), 2) treatment time (Time “T”, s), and 3) weight of sample (Weight “W”, g). The response surface model obtained was as follows:
\[ \text{Log}_{10} \text{ reduction} = \beta_0 + \beta_1 D + \beta_2 T + \beta_3 W + \beta_{22} T^2 + \beta_{12} DT + \beta_{13} DW + \beta_{23} TV + \epsilon. \]

where, \( \epsilon = \text{Error} \) and \( \beta_0, \beta_1, \beta_2, \beta_3, \beta_{22}, \beta_{12}, \beta_{13}, \text{and} \beta_{23} \) are coefficients (Table 4.5).

Table 4.5. Regression coefficients of response surface model for inactivation of \( S. auures \) in milk foam.

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant ( \beta_0 )</td>
<td>-11.4171</td>
<td>0.132</td>
</tr>
<tr>
<td>Distance ( \beta_1 )</td>
<td>1.6927</td>
<td>0.156</td>
</tr>
<tr>
<td>Treatment time ( \beta_2 )</td>
<td>0.1043</td>
<td>0.019*</td>
</tr>
<tr>
<td>Weight ( \beta_3 )</td>
<td>2.1202</td>
<td>0.207</td>
</tr>
<tr>
<td>Distance*Distance ( \beta_{11} )</td>
<td>-0.0709</td>
<td>0.261</td>
</tr>
<tr>
<td>Treatment time*Treatment time ( \beta_{22} )</td>
<td>-0.0003</td>
<td>0.015</td>
</tr>
<tr>
<td>Weight*weight ( \beta_{33} )</td>
<td>-0.1651</td>
<td>0.247</td>
</tr>
<tr>
<td>Distance* treatment time ( \beta_{12} )</td>
<td>-0.0006</td>
<td>0.803</td>
</tr>
<tr>
<td>Distance*weight ( \beta_{13} )</td>
<td>-0.1194</td>
<td>0.199</td>
</tr>
<tr>
<td>Treatment time*weight ( \beta_{23} )</td>
<td>0.0004</td>
<td>0.902</td>
</tr>
</tbody>
</table>

*Bold values indicate terms which are statistically significant (p<0.05)

The surface response model had an \( R^2 \) of 94% indicating that the model was able to explain 94% of the variation in the model. The developed model predicted the inactivation rate within a reasonable tolerance as indicated by an \( R^2 \) of ~0.93 and slope of ~0.99 (Figure 4.7).

**Energy measurement**

It is very important to know the actual energy available at the surface of food material since a considerable portion of the energy is wasted during pulsed UV-light treatment. As different pulsed UV-light systems have different configurations, the energy available is not the same for the same amount of exposure at the same distance. Therefore, by specifying the energy available for microbial inactivation, one can
standardize the pulsed UV-light inactivation. The broadband energy measurements for various distances from the quartz window are given in Table 4.6. As the distance from the quartz window increases, the energy available is decreased gradually. For instance, energy levels of 0.40, 0.33, and 0.19 J/cm² were available at 3, 8, and 13 cm distances from the quartz window, respectively.

![Graph](image)

Figure 4.7. Experimental vs. predicted log₁₀ reduction of *S. aureus* in milk foam.

Absorption at 254 nm may be an indicator of DNA absorption. Therefore, it is important to know what portion of the energy is available at 254 nm, which contributes to the photochemical changes in the DNA. Only 2% of the total broadband energy is available in the 254 nm wavelength region in the pulsed UV-light system. For instance, the broadband energy (100 to 1100 nm range) at 3 cm distance from the quartz window was 0.40 J/cm², whereas only 0.008 J/cm² energy was present 254 nm.
Table 4.6. Broadband energy measurement during pulsed UV-light treatment.

<table>
<thead>
<tr>
<th>Approximate distance from Quartz window (cm)</th>
<th>Energy (J/pulse)</th>
<th>Energy (J/cm² per pulse)</th>
<th>Energy at 254 nm (J/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>7.19</td>
<td>0.40</td>
<td>0.008</td>
</tr>
<tr>
<td>5.0</td>
<td>6.48</td>
<td>0.36</td>
<td>0.007</td>
</tr>
<tr>
<td>7.0</td>
<td>5.93</td>
<td>0.33</td>
<td>0.007</td>
</tr>
<tr>
<td>8.0</td>
<td>5.93</td>
<td>0.33</td>
<td>0.007</td>
</tr>
<tr>
<td>10.5</td>
<td>4.85</td>
<td>0.27</td>
<td>0.005</td>
</tr>
<tr>
<td>13.0</td>
<td>3.47</td>
<td>0.19</td>
<td>0.004</td>
</tr>
</tbody>
</table>

1Radiometer was calibrated at 254 nm and measured the broadband energy in the wavelength range of 100 to 1100 nm.
2The distance between the quartz window and the centre axis of the UV-strobe is 5.8 cm.
3Energy was averaged over 30 pulses; three independent measurements were taken and average is reported.
4Surface area of the radiometer detector head was 18.096 cm².

4.6. Conclusions

The potential of pulsed UV-light as an alternative process for the inactivation of *S. aureus* in milk was demonstrated. A surface response model was developed and validated successfully. Pulsed UV-light is highly effective in reducing the pathogens in milk, which can be demonstrated by the complete inactivation of *S. aureus* obtained after 180-s of treatment time in two cases (Table 4.1) and as predicted by model. Though the holding time for HTST pasteurization is at 71°C for 15 s, the total time required for the pasteurization process will be several minutes as the raw milk at 4°C has to be preheated in the regenerator section and heated by steam or hot water to achieve the required temperature. However, pulsed UV-light treatment takes about 3 minute for inactivation of pathogens even though milk was under static conditions. Pulsed UV-light was also effective on inactivation of *S. aureus* on milk foam. The temperature increase during pulsed UV-light treatment also played a significant role on inactivation of *S. aureus*. 

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Therefore, both temperature increase and photochemical changes account for microbial inactivation during longer treatment. However, for a short period of pulsed UV-light exposure, the effect of temperature increase is negligible and hence the inactivation occurs mainly because of photochemical changes in DNA. If the system is designed for continuous milk pasteurization then this can be validated. Further research needs to be done to find an optimum condition for the inactivation of *S. aureus* for continuous flow conditions to represent commercial cases. Effect of the pulsed UV-light process on the quality and nutritional attributes of milk must be examined to assess any adverse effects of UV-light.

**4.7. References**


5. Inactivation of *Staphylococcus aureus* in Milk Using Flow-through Pulsed UV-Light Treatment System

5.1. Abstract

This study investigated the efficacy of pulsed UV-light for continuous-flow milk treatment for the inactivation of *Staphylococcus aureus*, pathogenic microorganism frequently associated with milk. Pulsed UV-light is a novel technology which can be used for inactivation of this pathogen in milk in a short time. Pulsed UV-light damages the DNA of the bacteria by forming thymine dimers which leads to bacterial death. The effect of sample distance from the UV-light source, number of passes, and flow rate were investigated. A response surface method was used for design and analysis of the experiments. Milk was treated at 5, 8, or 11 cm distance from UV-light strobe at 20, 30, or 40 ml/min flow rate and treated up to three times by recirculation of milk to find the effect of number of passes on inactivation efficiency. Log_{10} reductions varied from 0.55 to 7.26 log_{10} CFU/ml. Complete inactivation was obtained in two cases and growth was not observed mostly following the enrichment protocol. A response surface method was used for the design of the experiments. The predicted data were in agreement with the experimental data. Overall, this work demonstrated that pulsed UV-light has a potential for inactivation of milk pathogens.
5.2. Introduction

Foodborne diseases are estimated to cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,200 deaths annually in the United States (Mead et al., 1999). There have been several outbreaks associated with milk or milk products. Milk may contain spoilage microorganisms and pathogenic microorganisms because of improper handling and contamination from both inside and outside the udder (Bramley and McKinnon, 1990). Therefore, it is necessary to process milk to inactivate these microorganisms. Conventionally, milk pasteurization is done using heat exchangers. However, pasteurization is a high energy demanding process with high capital and operational costs involved. Therefore, there is a need for an alternative processing method that is simple, cost-effective, and has high inactivation efficiency.

The Center for Disease Control and Prevention (CDC) estimates that there have been 17,248 and 1,413 cases of Staphylococcus aureus reported during 1973-87 and 1983-87, respectively, which accounted for 14% and 1.6% of all the cases because of bacterial pathogens (Bean and Griffin, 1990; Bean et al., 1990; Olsen et al., 2000). Pulsed UV-light treatment could serve as a potential method for the inactivation of S. aureus in milk. UV-light has been used as a bactericide as early as 1928 (Xenon, 2003). UV-light is the portion of the electromagnetic spectrum ranging from 100 to 400 nm wavelengths and possesses germicidal properties in the wavelength region of 220 to 280 nm. UV-light damages the DNA by forming thymine dimers, which leads to cell death (Bank et al., 1990; Miller et al., 1999).

There are two modes of application of UV-light: continuous or pulsed UV-light mode. Continuous UV-light is the conventional one, which delivers the UV-light in a
continuous mode. In the pulsed UV-light mode, the UV-light is stored in a capacitor and released as intermittent pulses, thus increasing the instantaneous energy. Therefore, pulsed UV-light treatment is a more effective and rapid way of inactivating microorganisms than continuous UV-light sources since the energy is multiplied many fold (Dunn, 1995; CFSAN-FDA, 2000). McDonald et al. (2000) reported that the almost identical level of inactivation of Bacillus subtilis was obtained with 40 J/m² of pulsed UV-light source and 80 J/m² continuous UV-light sources.

Bank et al. (1990) reported that a 60-s pulsed UV-light treatment at 31 cm distance from the light source (∼4 J/m²) resulted in 6 to 7 log₁₀ reduction of viable bacterial populations of Staphylococcus epideridis, Pseudomonas aeruginosa, Escherichia coli, S. aureus, or Serratia marcescens on Trypticase soy agar plates.

Jun et al. (2003) investigated the effect of pulsed UV-light on inactivation of Aspergillus niger spores in corn meal and reported a 4.95 log₁₀ reduction for a treatment time of 100 s when the distance between the quartz window and sample was kept at 8 cm and the input voltage was 3800 V. Sharma et al. (2003) investigated the effect of pulsed UV-light on inactivation of E. coli O157:H7 on alfalfa seeds. Reductions of 0.09 to 4.89 log₁₀ CFU/g were obtained for various thickness and treatment time combinations. Four thicknesses (1.02, 1.92, 3.61, and 6.25 mm) and 7 treatment times (5, 10, 30, 45, 60, 75, and 90 s) were used in the experiment. Complete inactivation of E. coli O157:H7 was obtained within 30 s of the treatment time, when the seed thickness was maintained at 1.02 mm, which corresponds to 4.80 log₁₀ CFU/g. Increase in treatment time resulted in higher log₁₀ reduction for all the thicknesses (p<0.05). Hillegas and Demirci (2003) obtained 87.6% reduction of Clostridium sporogenes in honey when 2 mm depth of
honey sample was kept at 8 cm distance from the quartz window and treated with pulsed UV-light for 45 s.

Krishnamurthy et al. (2004; chapter 3) reported that pulsed UV-light is a non-thermal process during a short period treatment (<10 s) and obtained more than 8 log\textsubscript{10} reduction of \textit{S. aureus} in a phosphate buffer or agar seed cells within 5 s treatment time. They also reported that complete inactivation of \textit{S. aureus}, which corresponds to 8.55 log\textsubscript{10} CFU/ml, was obtained in two cases, when static milk samples were treated with pulsed UV-light (Chapter 4). The conditions at which complete inactivation was obtained were as follows: 1) 8 cm sample distance from the quartz window, 30 ml sample volume, and 180 s treatment time combination, and 2) 10.5 cm sample distance from the quartz window, 12 ml sample volume, and 180 s treatment time combination.

The significance of the safety of food products reveals ample potential for the proposed study on continuous milk treatment using pulsed UV-light, which could be implemented in a food plant for continuous processing. Understanding the efficacy of pulsed UV-light in pathogen inactivation and developing test protocols in a beta-site will help in the development of a commercial UV system for use in a food plant. In this study, the inactivation of artificially inoculated \textit{S. aureus} in milk with respect to key design parameters was demonstrated.
5.3. Materials and Methods

Preparation of inoculum

*Staphylococcus aureus* (ATCC 25923) was obtained from the Penn State Food Microbiology Culture Collection and maintained at 4°C on tryptic soy agar (TSA; Difco, Sparks, MD) slants. Cells were grown in 150 ml Tryptic Soy Broth (TSB; Difco) at 37°C for 24 h, and harvested by centrifugation (Sorvall STH750, Kendro Lab Products, Newtown, CT) at 3,300 \(x\) g for 25 min at 4°C and the supernatant was decanted.

Raw milk was obtained from the University Creamery or dairy farm at Pennsylvania State University and stored in the refrigerator at 4°C for a maximum of three days. The centrifuged *S. aureus* cell pellet from the previous step was suspended in 100 ml raw milk to yield approximately 8 to 9 log\(_{10}\) CFU/ml and was vortexed vigorously to ensure homogeneity.

Experimental design

In order to reduce the number of treatments, a response surface method (Box-Behnken) design was used (MINITAB\textsuperscript{®} version 13.3; Minitab Inc., State College, PA). Distance of the sample from the UV-light strobe (5-11 cm), number of passes (1-3 passes), and flow rate (20-40 ml/min) are the three variables used in the design.

Pulsed UV-light treatment

Pulsed UV-light treatment was carried out with the SteriPulse\textsuperscript{®}-XL 3000 pulsed light sterilization system (Figure 5.1; Xenon corporation, Woburn, MA), which produced polychromatic radiation in the wavelength range of 100 to 1100 nm, with 54% of the
energy being in the UV-light region. The sterilization system generated three pulses per second and 1.27 J/cm² per pulse at 1.8 cm from the quartz window surface for an input voltage of 3,800 V as per the manufacturer. A flow-through system was installed in the pulsed UV-light chamber (Figure 5.2).

Figure 5.1. SteriPulse®-XL 3000 Pulsed UV-light sterilization system (Xenon Corporation®)

Figure 5.2. Schematic diagram of continuous milk treatment system.
Milk inoculated with *S. aureus* was pumped through a quartz tube (1.14-cm i.d., 1.475-cm o.d.) exposed to pulsed UV-light using a peristaltic pump (Model No. 7524-50; Masterflex® L/S®, Barnant Co., Barrington, IL). Only 28 cm of the quartz tube length was exposed to UV-light, and the rest of the tubing including flexible connection tubing was covered with aluminum foil to block UV-light exposure. Also, a V-groove reflector with polished surface was used to hold the quartz tube in order to reflect the UV-light back to the quartz tube and hence increase the energy absorbed by the sample. The V-groove reflector had angles of approximately 56 and 117° (Figure 5.4). The reflector consisted of a sharp channel to hold the quartz tube in place and the top portion of the reflector was more flat to avoid any shadow effect. The reflector was designed by the manufacturer of the pulsed UV-light system (Xenon corporation, Wilmington, MA) based on trial and error to maximize the UV-light energy absorption.

Both the angles play an important role in determining how much energy can be reflected back to the sample. Typically an ellipse shaped reflector would serve the purpose better as all the energy can be focused back to the sample. Further investigation on the design of the V-groove reflector is warranted for optimization of the pulsed UV-light processing.
The central axis of the quartz tube was aligned with that of the UV-light lamp in order to maximize the energy absorption. The receiving container was held at an altitude of 47 cm in order to reduce the bubble formation during pumping. Depending upon the surface response design, milk was treated up to three times by recirculation.

**Experimental design**

A surface response design was used in this study (Table 5.1) with the following variables: 1) distance of sample from the quartz window, 2) number of passes, and 3) flow rate. A Box-Behnken design was used with Minitab® (version 13.3; Minitab Inc., State College, PA) statistical software.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance from Quartz window*</td>
<td>5 cm</td>
<td>11 cm</td>
</tr>
<tr>
<td>Number of passes</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Flow rate</td>
<td>20 ml/min</td>
<td>40 ml/min</td>
</tr>
</tbody>
</table>

* Distance between quartz window and the center axis of the UV-lamp is 5.8 cm.

**Temperature measurement**

The temperature profile of the milk was monitored using a K-type thermocouple connected with the data logger (Omegaette HH306, Omega Engineering Inc., Stamford, CT). The thermocouples were inserted in the tubing immediately outside the pulsed UV-light system at the same distance to measure the inlet and outlet temperatures. The bulk temperature of the milk was also measured using a thermometer after pulsed UV-light treatment by adequately mixing to ensure homogenous temperature.
**Microbiological analysis**

Populations of *S. aureus* were enumerated before and immediately after pulsed UV-light treatment. A 1-ml sample was serially diluted using 0.1% peptone water and spiral-plated on Baird-Parker agar using Autoplate® 4000 (Spiral Biotech, Norwood, MA). The colonies were enumerated using Q-Count™ (Spiral Biotech) following incubation at 37°C for 24 h. The \( \log_{10} \) reduction was calculated by determining the difference between average \( \log_{10} \) value of untreated samples and average \( \log_{10} \) value of pulsed UV-light treated samples. Enrichment protocol was followed for zero or low counts of *S. aureus* by transferring 1-ml of pulsed UV-light treated milk sample into 9-ml of TSB and incubating at 37°C for 24-48 h.

**Statistical analysis**

MINITAB® software was used to test the statistical significance of the parameters. A 95% confidence level was used to determine the significance. Three replications were done and two independent data sets were used in the model development and one independent data set was used for the validation of the developed model.

**5.4. Results and Discussion**

For continuous treatment of milk by pulsed UV, the variables tested were: 1) distance of sample from the quartz window, 2) number of passes, and 3) flow rate. The \( \log_{10} \) reduction obtained from the pulsed UV-light treatment ranged from 0.55 to 7.26 \( \log_{10} \) CFU/ml (Table 5.2). Complete inactivation of *S. aureus* was obtained at 1) 8 cm...
sample distance from quartz window, single pass, and 20 ml/min flow rate, and 2) 11 cm sample distance from quartz window, 2 passes, and 20 ml/min flow rate combinations.

Table 5.2. Log$_{10}$ reductions of *S. aureus* in milk by pulsed UV-light treatment.

<table>
<thead>
<tr>
<th>Run Order</th>
<th>Distance</th>
<th>Passes</th>
<th>Flow rate</th>
<th>Log$_{10}$ reduction$^{++}$</th>
<th>Validation data set (experimental)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>3</td>
<td>30</td>
<td>0.63 ± 0.40</td>
<td>0.48</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>3</td>
<td>20</td>
<td>2.07 ± 0.33</td>
<td>1.56</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>1</td>
<td>40</td>
<td>0.55 ± 0.27</td>
<td>0.65</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>1</td>
<td>20</td>
<td>7.23 ± 0.36*</td>
<td>7.09*</td>
</tr>
<tr>
<td>5</td>
<td>11</td>
<td>2</td>
<td>40</td>
<td>0.73 ± 0.26</td>
<td>0.43</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>2</td>
<td>40</td>
<td>0.77 ± 0.06</td>
<td>0.76</td>
</tr>
<tr>
<td>7</td>
<td>5</td>
<td>2</td>
<td>20</td>
<td>2.48 ± 0.66</td>
<td>2.11</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>2</td>
<td>30</td>
<td>4.72 ± 0.69</td>
<td>4.48</td>
</tr>
<tr>
<td>9</td>
<td>8</td>
<td>2</td>
<td>30</td>
<td>5.87 ± 0.08</td>
<td>5.60</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>3</td>
<td>30</td>
<td>2.40 ± 0.01</td>
<td>2.63</td>
</tr>
<tr>
<td>11</td>
<td>11</td>
<td>1</td>
<td>30</td>
<td>0.72 ± 0.18</td>
<td>0.99</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>1</td>
<td>30</td>
<td>1.25 ± 0.07</td>
<td>0.98</td>
</tr>
<tr>
<td>13</td>
<td>11</td>
<td>2</td>
<td>20</td>
<td>7.26 ± 0.32*</td>
<td>7.09*</td>
</tr>
<tr>
<td>14</td>
<td>8</td>
<td>3</td>
<td>40</td>
<td>1.36 ± 0.10</td>
<td>1.17</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>2</td>
<td>30</td>
<td>4.76 ± 0.35</td>
<td>4.62</td>
</tr>
</tbody>
</table>

*Indicates complete inactivation. In most of the cases, there was no growth even after enrichment.

$^{++}$Three replications were performed. Two used for model development and one for model validation. Mean and standard deviation are given. Mean was used for the model development.

Statistical analysis revealed that the sample distance from the UV-light source is the only variable which has statistical significance at the 95% confidence level (p<0.05). However, the distance*distance interaction and passes*passes interaction had a slightly higher p values than the threshold (p=0.05), where the corresponding p values are 0.053 and 0.052, respectively.

The log$_{10}$ reduction varied as a function of the distance from the quartz window, wherein the microbial inactivation curve resembles a bell shaped curve (Figure 5.4).
Though, the log$_{10}$ reduction increased as the distance increased, after reaching certain distance, log$_{10}$ reduction decreased, indicating that maximum reduction can be obtained within the limits of the variable. For instance the log$_{10}$ reductions of 1.25 and 0.72 log$_{10}$ CFU/ml were obtained at 5 and 11 cm distance from UV-light source, respectively, when the flow rate was 30 ml/min in a single pass UV-light treatment (Table 5.2).

The log$_{10}$ reduction at 8 cm distance from the quartz window, 30 ml/min flow rate and single pass was 3.66 log$_{10}$ CFU/ml. This clearly shows that the maximum log$_{10}$ reduction was obtained at the middle point of the surface plot where the number of passes was 2, distance from UV-light source was 8 cm and the flow rate was 30 ml/min.

As expected, the lower flow rate resulted in better inactivation in most of the cases as the absorbed energy is high because of longer residence time (Figures 5.5 and 5.6). The residence time corresponding to the flow rates of 20, 30, and 40 ml/min are 5.52, 3.68, and 2.76 minutes, respectively resulting in a processing volume of ~110 ml.
The volume of milk processed for a given treatment time increased in continuous milk processing when compared to static milk processing significantly. In case of static milk processing (Chapter 4), the maximum volume treated was 48 ml for a 3 min pulsed UV-light treatment. Despite of increased processing volume, microbial reduction obtained with continuous milk treatment was comparable to that of static milk treatment.

The effect of flow rate was not statistically significant (p>0.050). Also there was an interaction between number of passes and flow rate (p=0.098). In other words, the log_{10} reduction at a particular pass also depends on the flow rate. For instance, log_{10} reductions of 2.07 and 1.36 log_{10} CFU/ml were achieved at 20 and 40 ml/min, respectively, when the number of passes were 3 and the distance from the UV-light source was 8 cm. However, log_{10} reductions of 7.23 and 0.55 log_{10} CFU/ml were obtained at the same conditions when the milk was treated in a single pass.

As indicated earlier, the effect of the number of passes was also investigated to mimic industrial scale situations where milk may be treated until the desired log_{10} reduction is achieved to meet the regulatory standards. However, there was no significant difference (p>0.05) in the number of passes (Figure 5.4 and Figure 5.6).
The interaction between number of passes and flow rate was also assessed using Minitab®. At a single pass treatment, decreased flow rate of the milk increased the log\(_{10}\) reduction. However, when the number of passes were three, there is no significant change in the log\(_{10}\) reduction when the flow rate is changed.
**Surface response model**

The following surface response model with constant, linear, interactions, and squared terms was developed using two independent data sets. Variables used in the model are 1) distance of sample from the UV-light source strobe (D, cm), 2) number of passes (P), and 3) flow rate (F, ml/min).

\[
\text{Log}_{10} \text{ reduction} = \beta_0 + \beta_1D + \beta_2P + \beta_3F + \beta_{11}D^2 + \beta_{22}P^2 + \beta_{33}F^2 + \beta_{12}DP + \beta_{13}DF + \beta_{23}PF + \epsilon.
\]

where, \(\epsilon = \text{Error}\) and \(\beta_0, \beta_1, \beta_2, \beta_3, \beta_{11}, \beta_{12}, \beta_{13},\) and \(\beta_{23}\) are coefficients (Table 5.3).

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\beta_0)</td>
<td>-16.21481</td>
<td>0.255</td>
</tr>
<tr>
<td>(\beta_1)</td>
<td>4.94370</td>
<td><strong>0.029</strong></td>
</tr>
<tr>
<td>(\beta_2)</td>
<td>3.68625</td>
<td>0.429</td>
</tr>
<tr>
<td>(\beta_3)</td>
<td>0.05371</td>
<td>0.922</td>
</tr>
<tr>
<td>(\beta_{11})</td>
<td>-0.21440</td>
<td>0.053</td>
</tr>
<tr>
<td>(\beta_{22})</td>
<td>-1.93708</td>
<td><strong>0.052</strong></td>
</tr>
<tr>
<td>(\beta_{33})</td>
<td>-0.00377</td>
<td>0.643</td>
</tr>
<tr>
<td>(\beta_{12})</td>
<td>-0.10333</td>
<td>0.691</td>
</tr>
<tr>
<td>(\beta_{13})</td>
<td>-0.04017</td>
<td>0.162</td>
</tr>
<tr>
<td>(\beta_{23})</td>
<td>0.14925</td>
<td>0.098</td>
</tr>
</tbody>
</table>

*Bold values indicate terms which are statistically significant (p<0.05).*

The developed model was validated using an independent data set. The model predicted data reasonably agree with the experimental data. An \(R^2\) of 0.836 (Figure 5.7) indicates that the model explained 83.6% variation in the data. In an ideal situation, a perfect fit line will have an \(R^2\) of 1 and slope of 1. In this case, the slope was 0.984 which suggest a clear correlation.
Temperature measurement

The temperature of the milk increased gradually during pulsed UV-light treatment. For instance, the temperature of the milk treated at 8 cm distance from the quartz window at 30 ml/min flow rate was 1.5, 5.6, and 27.8°C for 1, 60, and 300 sec treatments, respectively (Figure 5.8). The temperature increases during infrared heat treatment for single pass milk treatment were given in Figure 5.8. Increase in temperature because of pulsed UV-light was up to 38°C. Temperature increase might have a synergistic effect on inactivation of *S. aureus* in some cases. The initial temperature of the milk increased as the number of passes increased because of absorption of thermal energy (Table 5.4). For instance, the final bulk temperature of the milk was 22, 32, and 36°C, for 1, 2, and 3 passes, respectively, when the milk was treated at 20 ml/min at 5 cm distance from the quartz window. This temperature build-up might
have caused some changes in the quality of the milk. Therefore, it is beneficial to eliminate the temperature build-up in order to preserve the quality of the food.

Table 5.4. Average bulk temperature of milk after pulsed UV-light treatment.

<table>
<thead>
<tr>
<th>Distance from quartz window (cm)</th>
<th>Flow rate (ml/min)</th>
<th>1 pass</th>
<th>2 passes</th>
<th>3 passes</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>20</td>
<td>22</td>
<td>32</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>27</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>24</td>
<td>33</td>
<td>36</td>
</tr>
<tr>
<td>8</td>
<td>20</td>
<td>26</td>
<td>35</td>
<td>39</td>
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<tr>
<td></td>
<td>30</td>
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<td>35</td>
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</tr>
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<td>47</td>
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<tr>
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<td>45</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>29</td>
<td>39</td>
<td>46</td>
</tr>
</tbody>
</table>

Figure 5.8. Typical temperature profile during pulsed UV-light treatment of milk.
5.5. **Conclusions**

This study explored the possibility of using pulsed UV-light treatment as an alternative method for inactivation of *S. aureus* in milk. In general, pulsed UV-light effectively inactivated *S. aureus* in milk, which can be verified by the complete inactivation obtained in two cases. No growth was observed after enrichment in most cases. Pulsed UV-light treatment could be an attractive alternative to conventional pasteurization in terms of energy and cost. It is simple and can deliver lethal doses of pulsed UV-light for effective inactivation of *S. aureus* and hence an important technique in milk processing. The results of this study also suggest that pulsed UV-light treatment can be potentially applied in commercial scale for continuous milk pasteurization. An appropriate design of the equipment which will aid better penetration and shorter treatment time is needed for commercial success. Furthermore, the pulsed UV-light system can also operate in conjunction with the existing pasteurization system to ensure the safety of the product. Pulsed UV-light may inactivate pathogens which are resistant to heat treatment. Further studies have to be done to verify the applicability of pulsed UV-light treatment in an industrial scale.

5.6. **References**


6. Disinfection of Water by Flow-through Pulsed Ultraviolet Light Sterilization System*

6.1. Abstract

Disinfection of water is an important task for semiconductor, pharmaceutical, food or other industries for various purposes. Pulsed ultraviolet light is a novel technology which offers a rapid and effective solution to achieve sterilization of water and to provide reductions in the organic load of the water. In this study, efficacy of pulsed UV-light was studied for inactivation of *Bacillus subtilis* spores by using a flow-through, pulsed UV light chamber. Various flow rates up to 14 L/min were evaluated. The pulsed UV treatment results demonstrated the complete inactivation of *B. subtilis* for all the flow rates evaluated, which yielded 5.5 log<sub>10</sub> CFU/ml reduction or more. Furthermore, there was no growth observed after enrichment in either light or no-light conditions, which indicated that there were no injured cells and no recovery of the spores because of the photorepair mechanism. Absorption of pulsed UV-light treated water at 254 nm reduced significantly for most of the cases suggesting decrease in the turbidity. Therefore, this study clearly demonstrated that pulsed UV-light has a potential to be utilized for sterilization of water.

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6.2. **Introduction**

Water is used abundantly in many industries. Water may contain several pathogenic microorganisms including *Vibrio cholerae, Salmonella, Shigella, Campylobacter*, and *Escherichia coli O157:H7*. Therefore, it is necessary to disinfect the water before using it for industrial applications to ensure the safety and purity of water. Ultraviolet (UV) light disinfection is gaining interest among public water systems and commercial applications to disinfect the drinking water. UV-light does not form harmful by-products while inactivating pathogenic microorganisms (EPA, 1999). Therefore, UV-light is viewed as an alternative to chemicals such as chlorine. Continuous UV-light has been used for disinfection of drinking water since 1906. In addition to the disinfection of drinking water, UV-light can also be used for disinfection of water used in semiconductor, pharmaceutical, food, or other industries and for sanitation of wastewater.

UV-light can be applied in two different modes; namely continuous and pulsed modes. Recently the use of pulsed UV-light has been getting attention since it can inactivate pathogenic microorganisms in a short period of time with its better penetration than continuous UV-light. Pulsed light is a broad spectrum of radiation covering UV, visible, and infrared regions with typical wavelength range from 100 nm to 1100 nm. Energy is stored in a capacitor and released as very short intermittent pulses (typical pulse duration ranges from several hundred nanoseconds to microseconds), so the instantaneous peak energy will be in the order of several Megawatts (McDonald et al., 2000). However, the total energy is comparable to that of continuous UV-light (in the order of several watts). Therefore, by using the same amount of total energy, one can achieve better inactivation using pulsed UV-light because of higher peak energy and
constant disturbance caused by pulses. Krishnamurthy et al. (2004; chapter 3) reported that pulsed UV-light can inactivate *Staphylococcus aureus*, a foodborne pathogenic microorganism within several seconds. Within 5 s treatment, up to 8.5 log_{10} CFU/ml reduction was achieved. This clearly indicated the effectiveness of pulsed UV-light.

The objective of this study was to investigate the efficacy of the pulsed UV-light for sterilization of water during continuous water treatment. As spores are more resistant to UV-light than vegetative cells, *Bacillus subtilis* spores were used in the study to evaluate the efficacy of the pulsed UV-light system in a worst case scenario.

### 6.3. Materials and Methods

**Microorganism**

*Bacillus subtilis* (ATCC 6633) was obtained from American Type Culture Collection (Manassas, VA) and kept as frozen culture at -80°C. The culture was transferred to 150 ml of Tryptic soy broth (TSB, Difco, Sparks, MD) and grown for 24 h at 37°C and then transferred to tryptic soy agar (TSA) slants. After incubating at 37°C for 24 h, the slants were stored in the refrigerator until further use. Sub-culturing was performed on TSA slants every other week in order to ensure the culture viability.

**Spore preparation**

Four different methods of spore preparation were tested by changing the growth media, wash buffer, and/or number of days of incubation in order to maximize the spore count. Using the culture stored on TSA slants at 4°C, streak plating was done on TSA followed by incubation at 37°C for 24 h. A single colony from the plate was transferred
to 10 ml of TSB and incubated. All the incubation was done at 37°C for 24 h unless noted.

**Method 1:** The prepared culture was spread on TSA plates and incubated for 3 days. Then, the plates were rinsed with 5 ml of KCl/0.5 M NaCl solution and disturbed gently with a sterile spreader to remove the spores from the plates. Rinsing was repeated with another 5 ml of KCl/0.5 M NaCl solution and the rinse solution was transferred to sterile centrifuge bottles. The solution was vortexed in order to maintain the homogeneity followed by centrifugation at 3,800 \( \times \) g, at 4°C for 10 min (Sorvall Super T 21, ST-H750, Kendro Lab Products, Newton, CN). After centrifugation, the cells were washed with 250 ml of 950 mM Tris-HCl/EDTA buffer and re-centrifuged. Washing with 250 ml of 950 mM Tris-HCl/EDTA buffer was repeated two more times. The washed cells were resuspened in phosphate buffer (supplemented with Tween 20, pH 7.4, Sigma-Aldrich, St. Louis, MO) and the cells were heat shocked to produce spores at 80°C for 10 min. The spore suspension was stored at 4°C until further use. It was believed that the high amount of Tris-HCl and EDTA in the buffer resulted in injury to the cell leading to very low final spore concentration. Therefore, a lower concentration of Tris-HCl and EDTA were used in method 3.

**Method 2:** The prepared culture was grown in TSB for 7 days at 37°C. The sample was centrifuged at 3,800 \( \times \) g at 4°C. After centrifugation, the cells were washed with 250 ml of phosphate saline buffer and re-centrifuged. Washing with 250 ml of phosphate saline buffer was repeated two more times. The washed cells were resuspended in phosphate saline buffer and the cells were heat shocked at 80°C for 10 min to produce spores. The spore suspension was stored at 4°C until further use.
**Method 3:** The prepared culture was spread on TSA plates and incubated for 7 days at 37°C. The plates were rinsed with 5 ml of KCl/0.5 M NaCl solution and disturbed gently with a sterile spreader. In order to remove the spores from the plates, rinsing was repeated with another 5 ml of KCl/0.5 M NaCl solution and the rinse solution was transferred to sterile centrifuge bottles. The solution was vortexed to maintain homogeneity and followed by centrifugation at 3,800 \( \times \) g at 4°C for 10 min. After centrifugation, the cells were washed with 250 ml of 10 mM Tris-HCl/EDTA buffer and re-centrifuged. Washing with 250 ml of 10 mM Tris-HCl/EDTA buffer was repeated two more times. The washed cells were resuspended in phosphate saline buffer and the cells were heat shocked to produce spores at 80°C for 10 min. The spore suspension was stored at 4°C until further use.

**Method 4:** The prepared culture was spread on TSA plates (50 plates/batch) and incubated for 7 days at 37°C. The plates were rinsed with 5 ml of KCl/0.5 M NaCl solution and disturbed gently with a sterile spreader to remove the spores from the plates. Rinsing was repeated with another 5 ml of KCl/0.5 M NaCl solution and the rinse solution was transferred to sterile centrifuge bottles. The solution was vortexed to maintain the homogeneity and followed by centrifugation at 3,800 \( \times \) g at 4°C for 10 min. After centrifugation, the cells were washed with 250 ml of phosphate saline buffer and re-centrifuged. Washing with 250 ml of phosphate saline buffer was repeated two more times. The washed cells were resuspended in phosphate saline buffer and the cells were heat shocked to produce spores at 80°C for 10 min. The spore suspension was stored at 4°C until further use.
Pulsed UV-light treatment system

Pulsed UV-light treatment was done with a SteriPulse®-RS 4000 pulsed light sterilization system (Xenon Corporation, Wilmington, MA) (Figure 6.1). The system generated 1.27 J/cm²/pulse of radiant energy at 1.8 cm below the quartz window surface of the UV-lamp and produced polychromatic radiation in the wavelength range of 100 to 1100 nm, with 54% of the energy being in the UV-light region. The system produced 3 pulses of 360 µs duration per second.

![Pulsed UV-light sterilization system](image)

Figure 6.1. Pulsed UV-light sterilization system.

The system chamber had an annular cylinder arrangement with an UV lamp being placed at the center (Figure 6.1). The water disinfection system was made up of stainless steel and had 10.2 cm outer diameter and 40.6 cm length. A site glass was provided to facilitate the observation of water flow and to measure the UV-light intensity.
The annular space between the UV-lamp and the outer wall of the vessel were separated by a quartz sleeve to enhance the transmission of UV-light to water. The maximum volume of water in the disinfection chamber at any given time was 2.9 L (i.d. of the outer vessel was 9.8 cm, o.d. of the inner quartz tube was 2.5 cm, length of the chamber was 40.6 cm, and added volume because of the site glass was 0.05 L). The water was moved with a centrifugal pump (TE5-5C-MD, Emerson motor company, St. Louis, MD), and the flow rate was adjusted by a control valve. The water from a 10 or 20 L carboy container (Cole-Parmer, Vernon Hills, IL) was pumped through the water vessel. The flow of the water was measured and adjusted using a flow meter (F-41017L, Blue white industries, Huntington Beach, CA).
Cleaning of the flow-through pulsed UV-light system

In order to avoid any cross contamination in the pulsed UV system, pulsed UV-light was coupled with chlorine solution followed by sterile D.I. water rinse to remove any chlorine residues after several different combinations were investigated to get a sterile system. The final cleaning procedure was determined as follows: 1) circulate 10 L sterile deionized (D.I.) water with pulsed UV-light on for 10 L/min for 10 min for 10 L of water and 30 min for 20 L of water, 2) circulate 10 L of 200 ppm chlorine solution for 10 min, 3) circulate 10 L of sterile D.I. water for 10 min, 4) pump sterile D.I. water to adjust the target flow rate.

Pulsed UV-light treatment

D.I. water was autoclaved at 121°C for 60 min and cooled overnight at room temperature. Ten ml of the prepared spore suspension for 10 L of D.I. water or 20 ml of the prepared spore suspension for 20 L of D.I. water was added and mixed well to ensure homogeneity. The initial spore population in water determined by plating on TSA was 5.5-6.5 log₁₀ CFU/ml. The UV-light system was activated, and the inoculated water was pumped through the system at the set flow rates of 2, 4, 6, 8, 10, and 14 L/min, which yielded 88, 44, 29, 22, 18, and 13 s residence times in the chamber, respectively. After 50% of the water was passed through, about one liter of sample was collected. The pulsed UV-treated water was analyzed for microbial reduction by plating on TSA followed by incubation at 37°C. Two replications were performed for each treatment. Enrichment was performed for all treatments by transferring 1 ml of pulsed-UV treated water into 9 ml of TSB and incubating at 37°C for 24 h. Enrichment was performed to ensure that there
were no injured cells. In order to find out that the cells would be recovered by repairing
the UV damage under light exposure because of the photorepair mechanism, enrichment
was also performed under light.

**Turbidity measurement**

The absorption of the untreated and the treated samples were measured at 254 nm
using a UV-Vis spectrophotometer (DU series 500, Beckman, Fullerton, CA) to monitor
the turbidity of the water.

**Radiant energy measurement**

UV-light energy absorbed by the water was measured by using a radiometer
(Ophir PE50, Ophir Optronics Inc., Wilmington, MA) by measuring the broadband UV-
light intensity for each pulse by placing the pyroelectric detection head on the quartz
window provided for light measurements on the water treatment chamber. The
radiometer was calibrated at 254 nm. The UV-light intensity at 254 nm was determined
by comparing the UV-light intensity obtained using another radiometer
(SED240/ACT5/W detector head, International lights, Newburyport, MA) as per the data
given by Xenon Corporation (Wilmington, MA.)

**Temperature measurement**

The bulk temperature of the water before and after treatment was measured by
placing a thermometer at the center of the container after mixing it well. Several
measurements were taken and average was reported.
6.4. **Results and Discussion**

Method used for spore harvesting played a significant role in getting a higher spore concentration (Table 6.1). Spores grown on agar medium yielded more spores than broth as thin agar plates provide less nutrition over 7 days of incubation. Also greater number of days of incubation resulted in a higher spore concentration as more vegetative cells produce spores because of lack of nutrition and moisture over the period of incubation.

<table>
<thead>
<tr>
<th>Method #</th>
<th>Spore preparation method</th>
<th>Final spore concentration (log_{10} CFU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>950 mM Tris-Hcl/EDTA buffer + 3 days incubation on Tryptic soy agar</td>
<td>1.45</td>
</tr>
<tr>
<td>2</td>
<td>Phosphate buffer saline procedure + 7 days incubation in Tryptic soy broth</td>
<td>4.33</td>
</tr>
<tr>
<td>3</td>
<td>10 mM Tris-Hcl/EDTA buffer + 7 days incubation on tryptic soy agar</td>
<td>5.43</td>
</tr>
<tr>
<td>4</td>
<td>Phosphate buffer saline procedure + 7 days incubation on tryptic soy agar</td>
<td>8.34</td>
</tr>
</tbody>
</table>

The wash solution also played a significant role in getting higher spore concentration as some wash solutions might have injured the spores resulting in lower spore counts. Since spore suspension prepared by method 4 yielded the highest spore concentration (8.34 log_{10} CFU/ml), Method 4 was followed to prepare the inoculum. The pulsed UV treatment results demonstrated the complete inactivation of *B. subtilis* for all the flow rates evaluated (2, 4, 6, 8, 10, and 14 L/min), which yielded 5.5 log_{10} reduction or more (Table 6.2). Initial inoculum concentration for each flow rate was slightly different (ranged from 5.5 to 6.5 log_{10} CFU/ml). Furthermore, there was no
growth observed after enrichment in dark or under light, which indicated that there were no injured cells and no recovery of the spores because of the photorepair mechanism. Therefore, all the spores subjected to pulsed UV-light treatment were completely inactivated under the evaluated conditions.

Table 6.2. Inactivation of *Bacillus subtilis* spores by pulsed UV-light treatment.

<table>
<thead>
<tr>
<th>Flow rate (L/min)</th>
<th>Population ($\log_{10}$ CFU/ml)</th>
<th>Growth after enrichment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>5.5 – 6.5</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>0</td>
<td>No</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>No</td>
</tr>
</tbody>
</table>

*Both under light and no-light conditions.

Absorption at 254 nm of pulsed UV-light treated water was reduced significantly for most of the cases suggesting reduction in turbidity (Table 6.3), which suggested that pulsed UV-light treatment not only disinfects the water, but also disintegrates the organic material by oxidation which results in purer sterile water.

Table 6.3. UV-light absorption at 254 nm.

<table>
<thead>
<tr>
<th>Flow rate (L/min)</th>
<th>UV-light absorption at 254 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>2</td>
<td>0.093±0.023</td>
</tr>
<tr>
<td>4</td>
<td>0.000±0.093*</td>
</tr>
<tr>
<td>6</td>
<td>0.131±0.076</td>
</tr>
<tr>
<td>8</td>
<td>0.136±0.042</td>
</tr>
<tr>
<td>10</td>
<td>0.052±0.039</td>
</tr>
<tr>
<td>14</td>
<td>0.028±0.069</td>
</tr>
</tbody>
</table>

*Negative value replaced with 0.00.
Broadband energy per pulse (J/pulse), power (W), power per area (W/cm²), and estimated power at 254 nm (W/cm²) were reported for each tested flow rate on Table 6.4. The energy delivered by pulsed UV-light was measured using the radiometer suggesting that approximately 0.21 W/cm² (difference between the broadband power with no water (0.56 W/cm²) and average broadband power with water (0.35 W/cm²)) of the energy is absorbed by inoculated water (Table 6.4). Some portion of the UV-light was absorbed directly by the microorganism and some by water resulting in an increase in the bulk temperature, up to 6°C under the evaluated conditions.

Table 6.4. UV-light energy measurements during pulsed UV-light treatment.  

<table>
<thead>
<tr>
<th>Flow rate (No water)</th>
<th>Broadband energy per pulse (J/pulse)</th>
<th>Broadband power² (W)</th>
<th>Broadband power per area³ (W/cm²)</th>
<th>Estimated power at 254 nm⁴ (W/cm²)</th>
<th>Bulk temperature increase (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 L/min</td>
<td>3.35</td>
<td>10.05</td>
<td>0.56</td>
<td>0.0112</td>
<td>N/A</td>
</tr>
<tr>
<td>2 L/min</td>
<td>2.13</td>
<td>6.39</td>
<td>0.35</td>
<td>0.0070</td>
<td>2</td>
</tr>
<tr>
<td>4 L/min</td>
<td>2.12</td>
<td>6.36</td>
<td>0.35</td>
<td>0.0070</td>
<td>3</td>
</tr>
<tr>
<td>6 L/min</td>
<td>2.12</td>
<td>6.36</td>
<td>0.35</td>
<td>0.0070</td>
<td>3</td>
</tr>
<tr>
<td>8 L/min</td>
<td>2.11</td>
<td>6.33</td>
<td>0.35</td>
<td>0.0070</td>
<td>4</td>
</tr>
<tr>
<td>10 L/min</td>
<td>2.10</td>
<td>6.30</td>
<td>0.35</td>
<td>0.0070</td>
<td>5</td>
</tr>
<tr>
<td>14 L/min</td>
<td>2.04</td>
<td>6.12</td>
<td>0.34</td>
<td>0.0068</td>
<td>6</td>
</tr>
</tbody>
</table>

¹The Radiometer was calibrated at 254 nm. Broadband energy is reported throughout.  
²Three pulses were produced per second.  
³Radiometer had a 48 mm diameter area of exposure.  
⁴Based on the comparison of measurements with SED240/ACT5/W radiometer detector head, as suggested by Xenon Corporation, 2% of the broadband energy was at 254 nm based on the comparison studies.
6.5. Conclusions

Pulsed UV-light treatment has shown to be very effective in inactivating *B. subtilis* spores in this study. The results clearly show the potential of pulsed UV-light to be utilized for water disinfection cost-effectively. Testing at higher flow rates is needed for the optimization of the system. In general, vegetative cells need less energy than spores to be inactivated, and hence pulsed UV-light can be used effectively to inactivate pathogens in a short period of time with less energy. Especially pulsed UV-light can inactivate *Cryptosporidium parvum*, a protozoa of major concern in water, effectively as it is less resistant than *Bacillus subtilis* spores. Boeger et al. (1999) reported that one pulse of pulsed UV-light inactivated 1.00 and 4.60 log10 CFU/ml of *Bacillus subtilis* and *Cryptosporidium parvum*, respectively. Therefore, pulsed UV-light has a potential to be utilized for disinfection of vegetative cells, bacterial spores, and protozoa such as *Cryptosporidium parvum*. Also a pulsed UV-light provides a mercury free UV-light treatment which does not produce any hazardous by-products and environmentally friendly.

6.6. References


7. **Infrared Heat Treatment for Inactivation of**

*Staphylococcus aureus* in Milk

7.1. **Abstract**

The efficacy of infrared heating for inactivation of *Staphylococcus aureus*, a pathogenic microorganism, in milk was studied to investigate the potential of this technology for milk pasteurization. *S. aureus* population was reduced from 0.10 to 8.41 log$_{10}$ CFU/ml, depending upon the treatment conditions. The effects of infrared lamp temperature (536, 619°C), volume of the treated milk sample (3, 5, and 7 ml), and treatment time (1, 2, and 4 min) were found to be statistically significant (p<0.05). Complete inactivation of *S. aureus* was obtained in two cases within 4 min at a 619°C lamp temperature, resulting in 8.41 log$_{10}$ CFU/ml reduction. Enrichment resulted in growth as some of the injured cells were able to repair. Further investigation of infrared heat treatment for longer treatment times (> 4 min) indicated that there was no growth observed following enrichment in most cases for treatment at a 619°C lamp temperature. The results demonstrated that infrared heating has an excellent potential for effective inactivation of *S. aureus* in milk. Further optimization of the process may result in a commercially successful milk pasteurization method.
7.2. **Introduction**

Infrared radiation is part of the electromagnetic spectrum in the wavelength range between 0.5 and 1000 μm (Rosenthal, 1996), which is mainly utilized for processing food materials. Far infrared radiation has been used in the industry since the 1950s in United States (Skjoldebrand, 2001) because of the following advantages: 1) higher heat transfer capacity, 2) instant heating because of direct heat penetration, 3) high energy efficiency, 4) faster heat treatment, 5) fast regulation response, 6) better process control, 7) no heating of surrounding air, 8) equipment compactness, 9) uniform heating, 10) preservation of vitamins, and 11) less chance of flavor losses from burning of foods (Dagerskog and Osterstrom, 1979; Afzal et al., 1997; Skjoldebrand, 2002).

Recently, there is an increasing interest in the applicability of infrared radiation for inactivation of pathogens. Food components and microorganisms absorb effectively in far-infrared region (3 to 1000 μm), resulting in heating of food systems and thus inactivation of pathogens. Infrared heating inactivates the pathogen by damaging intracellular components such as DNA, RNA, ribosome, cell envelope, and/or proteins in the cell (Sawai et al., 1995). Absorption of infrared energy by water molecule in microorganisms is one of the important factors for microbial inactivation since water absorbs readily in the infrared region and results in rapid temperature increase (Hamanaka et al., 2006).

Bacterial inactivation is influenced by the peak wavelength of the lamp, temperature of the lamp, physical state of the microorganism, depth of the food, composition, shape and surface characteristics of food, etc. Hamanaka et al. (2006) treated *B. subtilis* spores on stainless steel Petri dishes with varying water activities using
three infrared lamps having peak wavelength of 950, 1100, and 1150 nm. Water activity played a vital role in the inactivation of bacterial spores since the state and amount of water molecule present in the spores affect the effective absorption of infrared energy. The spores treated with 950, 1100, and 1150 nm peak wavelength radiation were most resistant to water activities of 0.9, 0.7, and 0.6, respectively, resulting in maximum D values of 5.7, 21.3, and 32 min, respectively.

*Escherichia coli* O157:H7 suspended in 0.05 M physiological phosphate buffered saline was treated with infrared radiation at radiative power of 0.322 J/s/cm² on the surface of bacterial suspension (Sawai et al., 1995). The temperature of the bacterial suspension increased exponentially from approximately 280ºK to 336ºK during a 10 min infrared irradiation treatment. The inactivation effect of infrared heating was better than convective heating for the same heating time and same bulk temperature rise in medium (Sawai et al., 1995). *E. coli* cells were inactivated even when the cell suspension was cooled down, where the bulk temperature was below lethal level. Although the effect of bulk temperature was negligible, because of the absorption of infrared energy in a thin layer of the bacterial suspension solution, inactivation of *E. coli* occurred. The temperature of the thin film at the surface was much higher than that of bulk temperature. Consequently, the authors suggested that differences in inactivation effects were because of this increased temperature in the thin film in case of infrared heating. They also noted that sensitivity to rifampicin was increased followed by increase in sensitivity of chlaramphenicol during both infrared and convective heating, suggesting that inactivation occurred mainly because of damage to RNA polymerase and ribosome (Sawai et al., 1995).
Giraffa and Bossi (1984) investigated the efficacy of infrared heating on microbial reduction in milk. Infrared heat treatment of milk at 65°C and 1000 L/h flow rate resulted in reductions of standard plate count, Psychotropic bacteria, Coliforms, Enterococci, Lactic acid bacteria (grown in MRS agar), and Lactic acid bacteria (grown in M17 agar), by 63%, 96% 99.9%, 80%, 71%, and 60%, respectively. The surface pasteurization effect of infrared radiation in cottage cheese was investigated by Rosenthal et al. (1996). Yeast and mold cells were reduced by approximately 3 log₁₀ CFU/ml when treated with an infrared lamp with energy 250 J/s at a distance of 2.5 to 3 cm from the cheese surface. Even after 8 weeks of storage at 4°C, less than 100 yeast and mold cells/g was found, suggesting that infrared pasteurization is effective for surface pasteurization.

Hashimoto et al. (1992a) obtained reductions of more than 4.5 log₁₀ CFU/ml of S. aureus and 2 log₁₀ CFU/ml of E. coli 745 with an irradiation power of 7.5x10⁻⁷ J/s-cm² when using selective agar. The FIR effect on the pasteurization of bacteria on or within wet-solid medium was evaluated by Hasimoto et al. (1992b). A complete inactivation of E. coli was obtained with irradiation at 4.36x10⁻¹ J/s-cm² for 6 min., which corresponds to approximately 2 log₁₀ CFU/plate when there is no medium added on top of the bacteria. However, when the medium was added on top of the plated bacteria to a thickness of 1-2 mm, approximately 1 log₁₀ CFU/plate was obtained under the same experimental condition.

Hashimoto et al. (1993) investigated the effect of irradiation power on IR pasteurization below lethal temperature of bacteria. Approximately 4 log₁₀ CFU/ml and 1 log₁₀ CFU/ml reductions of S. aureus were obtained by FIR and NIR, respectively,
when the irradiation power was $7.57 \times 10^{-1}$ J/s-cm$^2$, indicating that the FIR heating is more effective in inactivating the microbial population than NIR heating.

Jun and Irudayaraj (2003) studied the effect of selective infrared heating on inactivation of *Aspergillus niger* and *Fusarium proliferatum* in corn meal. The spectral wavelength was manipulated using a bandpass filter (5.45 to 12.23 $\mu$m). Approximately 2 $\log_{10}$ CFU/g reduction of *Aspergillus niger* was obtained with 5 min treatment time with or without bandpass filter. Reductions of 1.5 and 2 $\log_{10}$ CFU/g of *Fusarium proliferatum* were obtained for 5 min with and without bandpass filter, respectively. The $\log_{10}$ reduction obtained for both *Aspergillus niger* and *Fusarium proliferatum* with filter were slightly higher than that obtained without a filter.

If the food is treated with high power infrared radiation for a short period of time, surface sterilization of food material can be achieved. As infrared heating mainly heats a thin layer from the surface, the food product can be rapidly cooled after infrared treatment and thus provides less change in the quality of food material because of negligible heat conduction (Hamanaka et al., 2000). Surface sterilization of wheat for inactivation of natural spoilage microorganisms was investigated by Hamanaka et al. (2000). Reductions of 0.83, 1.14, 1.18, and 1.90 $\log_{10}$ CFU/g were obtained with 60 s treatment with 0.5, 1, 1.5, and 2 kW infrared heaters, respectively.

Infrared heating has lower energy requirements to achieve the same temperature, leading to lower operational costs. Hebber et al. (2004) reported that the specific energy consumption for drying of potato were 17.1 and 7.60 MJ/kg of water, for hot air drying and infrared drying, respectively. The specific energy consumptions for drying of carrot were 16.15 and 7.15 MJ/kg of water for hot air drying and infrared drying, respectively.
This clearly shows that energy consumptions were reduced to 44% and 38% for potato and carrot drying. Furthermore, infrared heating also resulted in reduction of the total processing time for drying. Therefore, infrared heating has a potential to be utilized instead of conventional heating. The efficacy of infrared heating for inactivation of \textit{Staphylococcus aureus}, a pathogenic microorganism, in milk was studied to investigate the potential of this technology for milk pasteurization.

\section*{7.3. Materials and Methods}

\textbf{Preparation of inoculum}

\textit{Staphylococcus aureus} (ATCC 25923; Penn State Food Microbiology Culture Collection, University Park, PA) cells were grown in 150 ml Tryptic Soy Broth (TSB; Difco, Sparks, MD) at 37\textdegree C for 24 h, and harvested by centrifugation (Sorvall STH750, Kendro Lab Products, Newtown, CT) at 3,300 \textit{x} \textit{g} for 25 min at 4\textdegree C. A 100 ml sample of raw milk (obtained from the University Creamery or dairy farm at the Pennsylvania State University, University Park, PA) was added to the centrifuged \textit{S. aureus} cell pellet from previous step to yield approximately 8 to 9 log\textsubscript{10} CFU/ml.

\textbf{Infrared heating system}

A lab scale, custom-made infrared heating system with a cone-shaped, aluminum waveguide (35 cm diameter at top, 2.5 cm diameter at bottom, and 44 cm high) was used in this study (Figure 7.1 and Figure 7.2). The waveguide was made up of aluminum because of its high emissivity of 0.8.
Figure 7.1. Schematics of the infrared heating system.

Figure 7.2. Lab scale Infrared heating system.
The system and the control program were originally developed by Jun and Irudayaraj (2003) and the existing system was modified to suit the current application. The infrared heating system had six ceramic infrared lamps (Mor Electric Heating Association, Inc., Comstock Park, MI). The infrared lamp is a half trough emitter with a maximum power of 500 W for 120 V input and had a cast-in K type thermocouple. All the lamps were fixed inside the closing on top of the waveguide and arranged symmetric to the central axis of the waveguide. The system was controlled by a digital controller (PC) via a program written using Lab Windows software (ver 4.01, National Instruments, Austin, TX). The control program written in Lab Windows is given in Appendix C. The lamp temperature was continuously measured using K-type thermocouples through a data acquisition system (model 34970A; Hewlett-Packard Co., Englewood, CO) via a 20-channel amplifier/multiplexer and A/D converter. Based on the temperature reading and the control logic used in the software, digital signals were sent through the parallel port to turn on/off the infrared lamps through a solid state relay, in order to maintain the temperature set. The Graphical User Interface (GUI) was used to establish the temperature/wavelength setting and to turn the system on/off.

**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 infrared lamps (536, 619°C), volume of the milk sample (3, 5, and 7 ml), and treatment time (1, 2, and 4 min). Three replications were performed for each condition. Two different temperatures were obtained by allowing the lamps to reach steady state temperature at two conditions. For the first
condition, the program was used to control the temperature of the lamp using solid state relays, and the lamps were allowed to reach a steady state temperature.

The average temperature of the infrared lamps was 536±20°C. In the second condition, the lamps were directly connected to an 120 V power supply rather than controlling the lamp temperature by manipulation. In this way, the lamps reached their maximum temperatures. The average temperature of the infrared lamps was 619±5°C. Also, the effect of longer treatment times (> 4 min) was investigated by treating the microorganisms for 5, 10, and 15 min in addition to the treatments up to 4 min.

**Infrared heat treatment**

Milk, artificially inoculated with *Staphylococcus aureus* cells was treated with infrared heat by placing the sample approximately 1 cm below the bottom opening of the waveguide. The milk sample was kept in an autoclaved polypropylene dish with approximately 3.3 cm diameter and 1.7 cm height.

**Microbiological analysis**

Untreated and infrared heat treated milk samples were analyzed for *Staphylococcus aureus* by serially diluting and plating on Baird-Paker agar base with a spiral plater (Autoplate® 4000; Spiral Biotech, Norwood, MA), followed by incubation at 37°C for 24 h. Finally, surviving fractions of *S. aureus* were enumerated by Q-Count™ (Spiral Biotech). The log₁₀ reduction of *S. aureus* population was calculated by estimating the difference between average log₁₀ value of control samples and infrared heat treated samples. Furthermore, an enrichment procedure was performed for zero or
low counts by transferring 1-ml of sample into 9-ml of TSB and incubated at 37°C for 24-48 h.

**Temperature measurement**

The temperature of the milk during infrared heat treatment was monitored using K-type thermocouples attached to a data-logger (Omegaette HH306, Omega Engineering Inc., Stamford, CT).

**Statistical analysis**

The obtained data were analyzed statistically by ANOVA (Analysis of Variance - general linear model) using MINITAB® (version 13.3; Minitab Inc., State College, PA). A full quadratic model with all the interactions was used for the data analysis with a 95% confidence level. Extreme outliers were removed from the statistical analysis in order to reduce the bias. Because of the variation in log10 reductions of replications, sometimes it is possible to get negative reduction for mild treatments. However, theoretically, there can not be a negative reduction and hence it was replaced with zero reduction.

### 7.4. Results and Discussion

Infrared heating effectively inactivated *S. aureus* in milk within four minutes. Reductions of 0.10 to 8.41 log10 CFU/ml was obtained (Table 7.1). Complete inactivation of *S. aureus* was obtained at 619°C lamp temperature within 4 min, when the sample volume was 3 or 5 ml. However, positive growth during enrichment indicated that some of the cells were injured because of infrared heating and were able to repair.
Table 7.1. Log₁₀ reduction values of *S. aureus* in milk by infrared heat treatment.

<table>
<thead>
<tr>
<th>Volume</th>
<th>Log₁₀ reduction₁,₂</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 min</td>
</tr>
<tr>
<td></td>
<td>536°C</td>
</tr>
<tr>
<td>3 ml</td>
<td>A 0.10 ± 0.17 A,a</td>
</tr>
<tr>
<td>5 ml</td>
<td>A 0.16 ± 0.27 A,a</td>
</tr>
<tr>
<td>7 ml</td>
<td>A 0.16 ± 0.28 A,a</td>
</tr>
</tbody>
</table>

₁Average of three replications is listed with the standard deviation (outliers were not used for calculation of average). Initial inoculum was 8.41 ± 0.09 log₁₀ CFU/ml.

₂Values not preceded by the same upper case letter in the same column are significantly different from each other. Values not followed by the same upper case letter for the specific temperature (536 or 619°C) in the same row are significantly different from each other for that particular temperature. Values not followed by the same lower case letter for the specific time level (1, 2, or 4 min) in the same row are significantly different from each other for that particular time level.

³Extreme outliers were not included for data analysis.

⁴There was growth observed after enrichment indicating injured cells.

All of the main effects (temperature, volume of sample, and treatment time) were statistically significant (p<0.05) (Table 7.2 and Figure 7.3). All the interactions (time*volume, volume*temperature, and temperature*time interactions in analysis of variance) were also statistically significant (p<0.05) (Table 7.2 and Figure 7.4). An R² of 0.967% indicates that the analysis of variance was able to explain about 97% of the variation in the data.
Figure 7.3. Main effects plot (fitted means) for log_{10} reduction

Table 7.2. Analysis of variance for log_{10} reduction of S. aureus.

<table>
<thead>
<tr>
<th>Source</th>
<th>Degrees of freedom</th>
<th>Sequential sum of squares</th>
<th>Adjusted sum of squares</th>
<th>Adjusted mean square</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume</td>
<td>2</td>
<td>34.08</td>
<td>22.77</td>
<td>11.38</td>
<td>30.74</td>
<td>0.000*</td>
</tr>
<tr>
<td>Temperature</td>
<td>1</td>
<td>38.49</td>
<td>38.91</td>
<td>38.91</td>
<td>105.05</td>
<td>0.000</td>
</tr>
<tr>
<td>Time</td>
<td>2</td>
<td>197.58</td>
<td>176.72</td>
<td>88.36</td>
<td>238.56</td>
<td>0.000</td>
</tr>
<tr>
<td>Volume*Temperature</td>
<td>2</td>
<td>7.66</td>
<td>7.92</td>
<td>3.96</td>
<td>10.68</td>
<td>0.000</td>
</tr>
<tr>
<td>Temperature*Time</td>
<td>2</td>
<td>43.35</td>
<td>37.59</td>
<td>18.80</td>
<td>50.75</td>
<td>0.000</td>
</tr>
<tr>
<td>Volume*Time</td>
<td>4</td>
<td>20.65</td>
<td>21.50</td>
<td>5.37</td>
<td>14.51</td>
<td>0.000</td>
</tr>
<tr>
<td>Volume<em>Temperature</em>Time</td>
<td>4</td>
<td>6.28</td>
<td>6.28</td>
<td>1.57</td>
<td>4.24</td>
<td>0.007</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>11.85</td>
<td>11.85</td>
<td>0.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>49</td>
<td>359.36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Bolded values indicate statistically significant terms (p<0.05).
Figure 7.4. Interaction plot (fitted means) for log_{10} reduction.

**Effect of treatment time**

The effect of treatment time on inactivation of *S. aureus* was significant (*p*<0.05) (Table 7.1). As expected, increase in the lamp temperature resulted in increased log_{10} reduction because of increased energy absorption and temperature increase. For instance, reductions of 0.29, 3.43, and 8.41 log_{10} CFU/ml were obtained at 619°C lamp temperature for 1, 2, and 4 min treatment time, respectively, when 3 ml of milk was treated. The rate of inactivation of *S. aureus* was increased rapidly after a 2 min treatment, indicating that the temperature of the milk sample reached the lethal level. As there was growth observed after enrichment for 4 min infrared treatment at 619°C, the effect of longer treatment times was investigated by treating *S. aureus* at the specified conditions for up to 15 min (Table 7.3). Complete inactivation of *S. aureus* was obtained in all the tested conditions, corresponding to 8.41 log_{10} CFU/ml. The enrichment
procedure performed indicated that for treatments longer than 5 min at 619°C lamp
temperature there was no growth observed in most cases. However, infrared treatments
at a lamp temperature of 536°C resulted in growth after enrichment exhibiting that some
of the cells were injured and able to repair themselves.

Table 7.3. Infrared heat treatment of *S. aureus* for longer time.

<table>
<thead>
<tr>
<th>Volume (ml)</th>
<th>Growth after enrichment 5 min</th>
<th>Growth after enrichment 10 min</th>
<th>Growth after enrichment 15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 ml</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>5 ml</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>7 ml</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

The interaction of treatment time with volume and temperature (time*volume and
time*temperature terms in the analysis of variance) were statistically significant (p<0.05),
indicating that there was a close correlation. For instance, lower sample volume and
lower temperatures resulted in lower reductions, while the large volume and higher
temperature combinations resulted in larger inactivation (Figure 7.4).

**Effect of lamp temperature**

Inactivation of *S. aureus* obtained at 536 and 619°C were statistically significant
(p<0.05) (Figure 7.3 and Table 7.2). For instance, reductions of 2.96, 2.96, and 1.66
log$_{10}$ CFU/ml were obtained for treatment of 3, 5, 7 ml milk samples at 4 min at 536°C,
while reductions of 8.41, 8.41, 3.45 log$_{10}$ CFU/ml were obtained at 619°C lamp
temperature for a 4 min treatment. Generally lower temperatures in combination with
shorter treatment times and lesser volume resulted in lower reductions as evident from
the significance of temperature*volume and temperature*time interactions (p<0.05).
Also it is noted that 619°C lamp temperature was very effective in inactivation of *S. aureus* as compared to 536°C. Increase in lamp temperature resulted in increase in available infrared energy for microbial inactivation. The temperature of the milk sample increased rapidly during the infrared heat treatment (Figure 7.5).

![Figure 7.5. Temperature increase during infrared heating.](image)

As seen in Figure 7.5, the temperature of the milk increased significantly higher when there was no relay as the infrared lamp temperature was higher. As expected the rate of temperature increase was higher when lesser volume of milk was treated as more energy is readily available to heat the milk sample. The temperature was raised up to 55 to 105°C within five minutes of infrared heat treatment. Optimizing the temperature of milk during infrared heat treatment could result in less detrimental quality changes.
Effect of volume of milk

Infrared radiation mainly heats a thin layer of milk sample from the surface, because of its poor penetration capacity. Therefore, it is vital to know the effect of volume on inactivation of S. aureus. In general, an increase in the sample milk volume resulted in lower inactivation as infrared radiation can not penetrate deep and heats up only a few millimeters below the surface of the milk sample. Reductions of 3.43, 1.37, and 0.57 log_{10} CFU/ml were obtained when 3, 5, 7 ml volume of milk were treated at 619°C for 4 min, respectively (p<0.05).

7.5. Conclusions

Complete inactivation of S. aureus was obtained in two cases within 4 min at 619°C. The corresponding inactivation was 8.41 log_{10} CFU/ml. However, the enrichment was positive indicating there was cell injury. Further inactivation studies at longer treatment time indicated that, most of the samples treated for more than 5 min at both 536 and 619°C resulted in no detectable colonies. However, few treatments were positive after enrichment procedure indicating that cells were injured because of infrared heat treatment. As expected, S. aureus treated at 536°C was able to grow during enrichment. In case of treatments performed at 619°C, most of the enrichment was negative indicating that higher temperature resulted in complete inactivation of S. aureus with no cell injury. This shows that infrared heating has a potential to be utilized for microbial inactivation. The effects of volume, treatment time, and lamp temperature and their interactions were significant (p<0.05). Generally, lower volume of milk, longer treatment time, and higher lamp temperature resulted in greater inactivation of S. aureus.
Infrared heating requires less energy and reduces the treatment time when compared to conventional heating (Afzal et al., 1999). Spectral manipulation of infrared radiation results in selective heating of food components. Proper spectral manipulation of infrared radiation might result in selective heating of *S. aureus* in milk without heating other food components and result in fewer quality changes in milk as compared to conventional heating and yet produce milk which is safe to consume.

Further studies on optimization of the process, sensory evaluation, and quality changes during infrared heating have to be investigated in detail. Optimization of the infrared heating process to maintain the quality of milk may result in an alternative heating method for milk pasteurization.

### 7.6. References


8. Microscopic and Spectroscopic Analysis of Inactivation of *Staphylococcus aureus* by Pulsed UV-Light and Infrared Heating.

8.1. Abstract

Pulsed UV-light and infrared heat treated *S. aureus* cells were analyzed using transmission electron microscopy to identify the damages caused during the treatment. A five second treatment of *S. aureus* with pulsed UV-light resulted in complete inactivation of *S. aureus* even after enrichment. The temperature increase during the pulsed UV-light treatment was 2°C. *S. aureus* was treated using six ceramic infrared lamps with the power of 500 W. A 5 ml of *S. aureus* cells in phosphate buffer was treated at 700°C lamp temperature for 20 min. The microscopic observation clearly indicated that there was cell wall damage, cytoplasmic membrane shrinkage, cellular content leakage, and mesosome disintegration for both pulsed UV-light and infrared treatments. The structural damage of *S. aureus* during pulsed UV-light treatment might be caused by the constant disturbances of the intermittent pulses. Temperature increase might be the cause of the cellular damage by infrared heat treatment. FTIR microspectrometry was successfully used to classify the pulsed UV-light and infrared heat treated *S. aureus* by discriminant analysis. Further investigation on identification of key absorption bands may result in a better assessment of the chemical and structural changes during pulsed UV-light and infrared heating.
8.2. Introduction

Pulsed UV-light is produced by accumulating the energy in a capacitor and releasing it as a short duration pulse to magnify the power greatly. There is an increased interest in using pulsed UV-light for inactivation of pathogenic microorganisms in recent years because of the very short period of time required. Pulsed UV-light is a broad-band spectrum in the wavelength range of 100-1100 nm, with approximately 54% of energy in the ultraviolet range.

Infrared radiation is part of the electromagnetic spectrum in the wavelength range between 0.5 and 1000 μm (Rosenthal, 1996). Far-infrared radiation can be used for heating of food systems and inactivation of pathogens because of higher absorption of energy in the far-infrared wavelength range (3 to 1000 μm) by microorganism and food components. Therefore, infrared heating has a potential to be used for microbial inactivation in foods.

It is important to know the underlying mechanism of microbial inactivation to optimize the inactivation process. Transmission electron microscopy (TEM) and infrared spectroscopy can be utilized for this purpose. TEM is a high resolution microscope, which uses an electron beam to discriminate cellular level details of the microorganisms. TEM can distinguish points closer than 0.5 nm (Prescott et al., 1999). A thin section of bacterial cell is treated with various chemicals to stabilize the cell structure, cut into a thin slice using a diamond or glass knife, stained to increase the contrast, and exposed to electron beam. As the composition of the cell components differ, the intensity of electron scattering also varies thereby producing an image of the internal structure of the bacteria. Several researchers have used electron microscopy techniques successfully for the
Investigation of inactivation mechanisms of several novel technologies such as pulsed electric field, antimicrobial agents, etc. (Brouillette et al., 2004; Calderon-Miranda et al., 1999; Liu et al., 2004). The effect of pulsed electric field and nisin on *Listeria innocua* in skim milk was investigated using TEM by Calderon-Miranda et al. (1999). They noticed several features of pulsed electric field damaged cells such as lack of cytoplasm, cell wall damage, cytoplasmic clumping, increase in cell membrane thickness, poration of cell wall, and leaching of cellular content. Ruptures of cell wall and cell membranes were observed at selected electric field intensities. Liu et al. (2004) explained that the inactivation of bacteria by chitosan is because of cell wall damage by using TEM. The outer membrane of chitosan treated *E. coli* was altered after chitosan treatment. The cell membrane of chitosan treated *Staphylococcus aureus* was disrupted and the cellular contents were leaked.

Infrared spectroscopy is a chemical analytical method which can be used to determine the chemical and structural information of the target material based on vibration transitions. Various food/microbial components absorb infrared light at specific wavelengths. Thus, infrared spectroscopy produces a fingerprint of spectral absorption characteristics of the biological components by providing absorption/transmission characteristics over time. The spectrum obtained is transformed from the time domain into frequency domain by Fourier transformation, so that absorption with respect to a particular wavelength could be assessed (Wilson and Goodfellow, 1994).

Fourier transform spectroscopy (FTIR) can be used to discriminate pathogenic microorganisms by spatially resolving the structural and compositional information of the microorganisms at molecular level (Yu and Irudayaraj, 2005; Gupta et al., 2004). Liu et
al. (2004) utilized FTIR for determination of the interaction between chitosan and synthetic phospholipids membrane in an effort to understand the basic inactivation mechanism. Several researchers successfully utilized FTIR for the discrimination of microorganisms and to investigate the changes in chemical composition because of several processes. Therefore, the use of TEM and FTIR will be beneficial for the investigation of inactivation mechanisms for pulsed UV-light and infrared treatments.

### 8.3. Materials and Methods

**Inoculum preparation**

*Staphylococcus aureus* (ATCC 25923; Penn State Food microbiology culture collection, University Park, PA) was grown at 37°C for 24 h, followed by centrifugation at 3,300 x g for 25 min. The cells were resuspended in 0.1 M phosphate buffer (pH 7.2; Becton Dickinson microbiology systems, Sparks, MD) to yield about 8-9 log₁₀ CFU/ml.

**Pulsed UV-light treatment**

Phosphate buffer artificially inoculated with *S. aureus* was treated with pulsed UV-light (Steripulse-XL 3000® pulsed light sterilization system, Xenon Corporation, Wilmington, MA). A 12-ml sample of *S. aureus* kept in an aluminum dish of 7-cm diameter was treated at 8-cm below the quartz window of the sterilization system. The distance between the central axis of the UV-strobe and the quartz window was 5.8-cm. *S. aureus* were treated for 1, 2, 4, 8, 16, and 32 s for spectroscopic analysis and 5 s for microscopic analysis.
Infrared heat treatment

A lab scale, custom-made infrared heating system developed by Jun and Irudayaraj (2003) was used for infrared heat treatment. The infrared heating system comprised of six ceramic infrared lamps and the temperature was controlled by a Lab Windows program (ver 4.01, National Instruments, Austin, TX) using solid state relays. The lamps had a maximum power of 500 W for 120 V input. The bacterial sample was treated approximately cm below the infrared lamps. A 5 ml of *S. aureus* cells in phosphate buffer was treated at 700°C for 1, 2, 4, 8, and 16 min for spectroscopic analysis. A 5 ml of phosphate buffer with *S. aureus* were treated at 700°C for 20 min to evaluate the structural damage during infrared heat treatment using microscopic analysis.

TEM analysis

Pulsed UV-light and infrared heat treated *S. aureus* cells were further processed for the TEM imaging as follows:

**Fixation and embedding of bacterial cells:** Steps for this process were as follows: 1) Primary fixation: In order to inactivate the cells, to stop the enzyme activity, and to crosslink proteins, the cells were treated overnight with 2.5% glutaraldehyde in 0.1 M sodium cacodylate solution at 4°C; 2) Buffer wash: The aldehyde residues were removed by washing the cells with 0.1M sodium cacodylate for five minutes. This procedure was repeated three times; 3) Secondary fixation: Secondary fixation was performed with 1% osmium tetroxide in 0.1M sodium cacodylate buffer in order to provide electron density for contrast while TEM imaging by oxidizing the double bonds in unsaturated fatty acids and forming monoesters; 4) Buffer wash: To remove excess
osmium, a buffer wash was performed three times for five minutes each; 5) *En bloc* staining: Buffer washed cells were stained in filtered 2% Uranyl acetate in a dark container for 1 h. This procedure further preserves the membranes and provides electron density for TEM imaging; 6) Dehydration: A dehydration procedure was performed to replace air spaces in the tissues. The samples were washed for 10 min in each of the following solutions: 50% ethanol, 70% ethanol, 90% ethanol, 100% ethanol (3 times), 100% EM grade ethanol (3 times), and 100% acetone (3 times). Initially the air spaces were replaced by increasing concentrations of ethanol and then ethanol was replaced with acetone; 7) Infiltration: The dehydrated samples were treated in a rotor with graded series of increasing concentrations of resin and acetone until the concentration of resin was 100%. The samples were treated for 2 h each in the following solutions: 50:50 acetone: resin, 25:75 acetone: resin, and 100% resin (3 times); and 8) Embedding and polymerization: The specimen in the resin was heated overnight at 60°C to polymerize the resin and to form a solid block of bacterial cells.

**Sectioning and staining:** Excess resin was trimmed off to expose and facilitate easy access to bacterial cells during sectioning. Ultra-thin sections (~30 nm thickness) of bacteria were sliced using a diamond knife (Ultra-45; Diatome, Fort Washington, PA) in an Ultramicrotome (Reichard-Jung, Vienna, Austria). The sections were collected in a grid. A staining procedure was performed as follows; 1) The grids were immersed with the section-side down in filtered 2% Uranyl acetate in 50% ethanol for 16 min, 2) The grids were then immersed quickly in nanopure water (Carbon dioxide removed by boiling for 5 min) and vertically agitate gently for 1 min, 3) Excess water on the grids were removed and they were dried completely, 4) In a staining dish, grids were immersed for
12 min section side down in filtered lead stain (mixture of 1200 µl boiled nanopure water with no carbon dioxide, 4.65 mg lead citrate, and 11.85 µl 10 N sodium hydroxide) for, 5) Grids were placed in a beaker with 40 ml of boiled water and two drops of 10 N sodium hydroxide solution and washed by vertically agitating for 30 s, 6) Grids were placed next in a beaker with 40 ml of boiled water and one drop of 10 N sodium hydroxide solution and washed by vertically agitating for 30 s, 7) Grids were placed then in a beaker with 40 ml of boiled water for 1 min, and 8) Finally excess water was removed and the grid were dried completely.

**TEM imaging:** Stained ultra-thin sections of bacterial cells were imaged using transmission electron microscopy (JEM 1200 EXII; JEOL, Peabody, MA). The specimen (grids with sections of bacterial cells) was placed in a specimen rod and inserted in the TEM. The height of the specimen was adjusted. The brightness of the electron beam was adjusted and the bacterial cells were focused clearly. Images of the sections of bacterial cells were taken at different magnifications using a high resolution camera (F224; Tietz, Gauting, Germany).

**FTIR spectroscopy analysis**

*S. aureus* cells treated with pulsed UV-light or infrared heating were stored at 4°C until other samples were treated, all samples were further processed as described below. Cell wall and cytoplasm were separated from treated/control *S. aureus* cells. The whole cells and cell walls were evaluated with FTIR spectroscopy as follows:

**Sample preparation for spectroscopic evaluations:** 1) *Whole cell:* One milliliter samples of un-treated and treated *S. aureus* cells were transferred into sterile 1.5
ml micro-centrifuge tubes (VWR International, West Chester, PA) and processed in a
mini-centrifuge (Model no. C-1200, National Labnet Corporation, Woodbridge, NJ) for 5
min at 1,000 rpm. The supernatant was decanted and the cell pellet was smeared onto a
gold coated glass slide (~200-nm thickness of gold) and dried for 24 h in a dessicator at
room temperature; 2) **Cell wall:** The cell wall of *S. aureus* was separated by treating cells
in a sonicator (Branson 1510 sonicator) at 40 kHz for 10 min. Ultrasonicated cells were
centrifuged at 30,000 \( x \) \( g \) for 30 min to separate the cytoplasm and cell wall. The cell
wall was deposited at the bottom of the centrifuge as a pellet and the cytoplasm was
suspended in the supernatant. Cell walls were then smeared onto a gold slide and dried
as explained above.

**FTIR measurements:** Mid infrared spectra of the bacterial cell and cell wall
were measured using a Digilab Excalibur FTS 6000 spectrometer fitted with a UMA 600
infrared microscope (Digilab, Randolph, MA). A ceramic air-cooled IR source with a
\( kBr \) beam was used. The mercury-cadmium-telluride detector was cooled with liquid
nitrogen during data collection. The sample chamber was purged with helium to
minimize the interference from water vapor. Three measurements were taken at different
spots from each replication; hence a total of 9 spectra were collected for each treatment.
Averages of 128 scans were collected for each spectrum from 600 to 6000 cm\(^{-1}\) spectral
region at a resolution of 4 cm\(^{-1}\).

**Discriminant Analysis:** Using Partial Least Square (PLS) and Canonical Variate
Analysis (CVA), the raw data were conditioned by baseline correction and area
normalization to reduce the bias. First and second derivative methods were utilized for
spectral analysis to detect the amide I and amide II protein regions. The data were
analyzed using PLS and CVA as follows: i) **Partial Least Square (PLS):** PLS is a well-established evaluation technique and used widely for the identification and classification of data. It decomposes the original matrix into several products of multiplication corresponding to the concentration, loadings and scores that indicate the variation of the data as well as the degree of fit. In this study, PLS was performed in the spectral range between 800-3000 cm\(^{-1}\). The spectral data were subjected to PLS data compression. Then, each spectrum was reconstructed by a linear combination of the product of scores and their weights (loading). The resulting calculations were used for multiple group classification; 2) **Canonical Variate Analysis (CVA):** The second method used for discriminating between groups of observations was the Canonical Variate Analysis. Canonical variate scores have successively maximized differences between group variances and within groups variances, and the CV loadings have been obtained as eigenvectors of a matrix given by

\[
[W^{-1}] [B]
\]

(Equation 1)

where, \(W\) was the within–groups covariance matrix, and \(B\) was the between–groups covariance matrix. The objective of this procedure was to minimize the within–group variance and to maximize the between-groups variance. The goodness of fit was indicated by the % correct classification. Discriminant models were developed based on the calibration data and evaluated separately using the validation data set. The correctly classified samples were expressed as a percentage of the total number of samples in the specific groups. The spectra were normalized by dividing the intensity values corresponding to each wavenumber in the spectrum by its standard deviation before analysis. All the data were converted in a PLS algorithm for classification and
identification after Fourier smoothing of the spectra using GRAMS-32 software. Using 800-3000 cm$^{-1}$, it was able to classify the time course bacteria treated with IR and UV radiation.

8.4. Results and Discussion

Transmission electron microscopy

The effect of pulsed UV-light and infrared heat treatment on S. aureus was investigated using Transmission Electron Microscopy (TEM). Selected images of damages induced by pulsed UV-light and infrared heating have been shown in Figure 8.1 to Figure 8.3.

Microscopic analyses of S. aureus cells indicate that there were damaged cells occurring both during infrared heating (Figure 8.1A) and pulsed UV-light treatments (Figure 8.1B). In the case of pulsed UV-light, cells were treated for only 5 s. However, the results were comparable to those of infrared heat treatment for 20 min at 700$^\circ$C lamp temperature.

Pulsed UV-light treatment: Pulsed UV-light treated S. aureus cells exhibited severe damage though the cells were treated for only 5 s with pulsed UV-light. Figure 8.2B indicates cell wall damage and cell content leakage at several locations. Thus, some cells lacked cell wall because of the disintegration of cell wall (Figure 8.2C). Furthermore, it was evident that the cytoplasmic membranes were shrinking and the internal cellular structures were collapsing.
Cytoplasmic membrane shrinkage results in the loss of semi permeability of the membrane and hence the osmotic equilibrium of the cell is disturbed. This leads to leakage of cellular content from the cytoplasm and cell death. As mentioned earlier, the samples were treated with pulsed UV-light for just five seconds and there were no significant temperature increases during the treatment. No growth was observed after a 5 s treatment with pulsed UV-light even after enrichment, indicating that \textit{S. aureus} was completely inactivated.
Figure 8.2. Evaluation of pulsed UV-light induced damages in *S. aureus* by TEM: A) Control sample, B) Cell wall rupture, C) Lack of cell wall, D) Cytoplasm shrinkage and cell wall damage, E) Cytoplasm shrinkage and membrane damage, and F) Cell wall damage and cellular content leakage.
Traditionally, it is believed that the inactivation mechanism for pulsed UV-light was mainly because of thymine dimer formation in the bacterial cell. However, microscopic observation indicated that damage to cellular structure occurred with pulsed UV-light treatment. Therefore, it was evident that some other mechanism could contribute to the inactivation of pulsed UV-light in addition to thymine dimer formation. It was evident that some *S. aureus* cells were inactivated by thymine dimer formation since a majority of the pulsed UV-light treated cells were intact without any structural damage. This fact indicated that the cells might have been inactivated by thymine dimer formation because microbiological studies indicated that there was no growth and all the cells were completely inactivated.

It is clear that the temperature of the sample increased rapidly during pulsed UV-light treatment (chapter 3). Therefore, some researchers suggested that pulsed UV-light may also have a thermal effect on the bacteria (Fine and Gervais, 2004; Takeshita et al., 2003; Wekhof, 2000). Because of the difference in absorption of pulsed light energy by bacteria and surrounding media, vaporization of water in the bacteria occurred, leading to a small steam flow in the bacterial cells causing cell disruption. Though the thermal effect plays a vital role at longer treatments with pulsed UV-light (>5 s treatment time), it is negligible for shorter treatments. In this study, *S. aureus* cells were inactivated for 5 s, and there was no significant temperature increase observed. Therefore, the damage occurred to *S. aureus* by pulsed UV-light in this study was not attributed to thermal damage.

The photochemical transformation, thymine dimer formation, did not damage the cellular structure of the bacteria and there was no thermal damage under the tested
conditions. Therefore, there should be some other effect than photochemical or photothermal. The inactivation mechanisms observed in this study were similar to those of cells treated with pulsed electric field (Barbosa-Canovas et al., 1999; Calderon-Miranda et al., 1999; Dutreux et al., 2000). Thus, it can be hypothesized that cellular damage could be because of the pulsing effect. In pulsed UV-light, the energy is stored in a capacitor and released as intermittent pulses with high energy in several MW ranges. The pulse duration ranges from several nano seconds to micro seconds, and there are several pulses produced per second. The pulsed UV-light system utilized in this study produced 3 pulses/s with pulse duration of 360 µs. Because of constant disturbance caused by the pulses, the bacterial cells may undergo stress and hence result in structural damage. Therefore, the inactivation mechanisms of pulsed UV-light can be divided into the following:

a. Photochemical effect: The inactivation is mainly caused by chemical changes in the DNA and RNA. Thymine dimer formation is the major photochemical change attributed to microbial inactivation. There may also be other minor chemical bond formations and/or breakages in bacteria.

b. Photothermal effect: There is a significant temperature increase during longer duration pulsed UV-light treatment. As the heating rate of the bacterial cell and surrounding media are different, localized heating of bacteria occur, which leads to cell death.

c. Photophysical effect: Because of constant disturbance caused by the high energy pulses, structural damages to bacteria may occur. Therefore, the
effectiveness of pulsed UV-light treatment can be improved by optimizing the pulse width and number of pulses.

These results clearly indicate that the inactivation mechanism of pulsed UV-light is different from that of continuous UV-light. Several researchers have suggested that pulsed UV-light is up to four times more effective in inactivation of microorganisms. This study suggests that this increased effectiveness can be attributed to photophysical and photothermal effects of pulsed UV-light.

**Infrared heat treatment:** The cell wall, cytoplasm, and mesosome of the untreated *S. aureus* cells were intact as seen in Figure 8.3A. However, these structures were damaged in infrared heat treated *S. aureus* cells (Figure 8.3). The microscopic observations indicate that there was cell wall damage leading to absence of cell wall (Figure 8.3B). This image clearly indicated that the cell wall was not present for the infrared heat treated sample. Cell wall damage lead to leakage of cellular content including genetic material (Figure 8.3C). It was also noted that the cytoplasmic membrane shrunk upon infrared heat treatment, and damage to the cytoplasmic membrane was also observed (Figure 8.3D and Figure 8.3F). The internal cellular structure, the mesosome was also damaged during infrared heat treatment (Figure 8.3E). The control sample had an intact mesosome in the cytoplasm (Figure 8.3A).
Figure 8.3. Microscopic evaluation of damages to *S. aureus* because of infrared heat treatment: A) Control sample, B) Lack of cell wall, C) Cell wall breakage and cytoplasm content leakage, D) Cytoplasm shrinkage, E) breakage in mesosome, and F) Cytoplasm damage.
FTIR spectroscopy

The pulsed UV-light treated and infrared heat treated cells and cell walls of *S. aureus* were successfully classified by using FTIR spectroscopy. Discriminant analyses of the spectroscopic data are given in Figure 8.4 to Figure 8.6. Distinct clusters were formed for different treatments and treatment times indicating that the FTIR absorption spectra of individual treatments were different. Thus, the treatment time and treatment had a significant impact on the changes in chemical and/or structural changes in the cells. In general, pulsed UV-light treated cells had a better discriminate power than infrared heat treated cells. Also, the characteristics of the spectra for the whole cells and cell walls were significantly different. Hence, the discriminant analysis was able to differentiate this.

Since the composition of cell walls and whole cells differ significantly, differences in the spectra are expected. In this study, cell walls were also used because, during pulsed UV-light and infrared heating, cell wall damage was noticed. A cell wall is made up of polysachharides and proteins, therefore one can expect their contribution to be prominent in the FTIR spectra (Chenxu and Irudayaraj, 2005). However, DNA and RNA are the major contributors for the cytoplasm extract (Chenxu and Irudayaraj, 2005) and hence, in the whole cell, one can expect the contributions from DNA, RNA, proteins, polysachharides. The data are in agreement with this observation. The result shows that the data from the whole cell and cell wall were classified in different clusters (Figure 8.6) for both pulsed UV-light and infrared heat treatments. The infrared heat treated whole cells were spread out indicating that there was significant difference within the infrared heat treated samples at different treatment times.
Figure 8.4. Classification of pulsed UV-light treated *Staphylococcus aureus* by PLS-CVA (PLS Factor- 4): A) whole cell, B) cell wall.
Figure 8.5. Classification of infrared heat treated *Staphylococcus aurues* by PLS-CVA (PLS Factor- 4): A) whole cell, B) cell wall.
Figure 8.6. Differentiation of cells and cell walls of *Staphylococcus aureus* by PLS technique: A) pulsed UV-light treated cells, B) Infrared heat treated cells.
As FTIR was able to differentiate cells treated for different times, it can be utilized for rapid measurement of dose received. FTIR measurements may provide an easier and faster way to determine the adequacy of the pulsed UV-light or infrared heat treatment by classification of the spectral information. For commercial success of an emerging technology, it is crucial to have a rapid measurement technique for the verification of the effective dose absorbed by the food material. For instance, the effectiveness of pasteurization is tested by testing the activity of the enzyme alkaline phosphatase. The results showed that FTIR was successfully able to differentiate *S. aureus* treated for different time and thus different dosage. Thus, FTIR may be used to validate the adequacy of the pulsed UV-light and infrared heat treatment.

Further detailed studies have to be done for the assignment of absorption bands to investigate the chemical and structural changes during pulsed UV-light or infrared heating. The tentative assignment of absorption bands in the infrared region of microbial cells is given in Table 8.1.
Table 8.1. Absorption bands of microbial cells in the infrared region*

<table>
<thead>
<tr>
<th>Wavenumber (cm(^{-1}))</th>
<th>Functional group assignment</th>
<th>Possible biomolecule contributors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2957</td>
<td>(\gamma) (CH(_3)) asymmetric</td>
<td>Fatty acids</td>
<td>Mordechai et al., 2000</td>
</tr>
<tr>
<td>2919</td>
<td>(\gamma) (CH(_2)) asymmetric</td>
<td>Fatty acids</td>
<td>Mordechai et al., 2000</td>
</tr>
<tr>
<td>2872</td>
<td>(\gamma) (CH(_3)) symmetric</td>
<td>Fatty acids</td>
<td>Naumann, 2000</td>
</tr>
<tr>
<td>2852</td>
<td>(\gamma) (CH(_2)) symmetric</td>
<td>Fatty acids</td>
<td>Naumann, 2000</td>
</tr>
<tr>
<td>1790-1750</td>
<td>(\gamma) (C=O) affected by Cl, etc</td>
<td>Not clear</td>
<td>Socrates, 2001</td>
</tr>
<tr>
<td>1741</td>
<td>(\gamma) (C=O)</td>
<td>Lipid esters</td>
<td>Naumann, 2000</td>
</tr>
<tr>
<td>1708</td>
<td>(\gamma) (C=O), H-bonded</td>
<td>RNA, DNA</td>
<td>Mordechai et al., 2000; Naumann, 2000</td>
</tr>
<tr>
<td>~1695</td>
<td>Amide I band components resulting from anti parallel plated sheets and (\beta) turns</td>
<td>Proteins</td>
<td>Naumann, 2000</td>
</tr>
<tr>
<td>~1670</td>
<td>(\gamma) (C==N)</td>
<td>RNA/DNA bases</td>
<td>Socrates, 2001</td>
</tr>
<tr>
<td>~1655</td>
<td>Amide I of (\alpha)-helical structure</td>
<td>Proteins</td>
<td>Naumann, 2000</td>
</tr>
<tr>
<td>~1637</td>
<td>Amide I of (\beta)-sheets</td>
<td>Proteins</td>
<td>Naumann, 2000</td>
</tr>
<tr>
<td>1548</td>
<td>Amide II</td>
<td>Proteins</td>
<td>Naumann, 2000</td>
</tr>
<tr>
<td>1515</td>
<td>Shoulder</td>
<td>Proteins</td>
<td>Naumann, 2000</td>
</tr>
<tr>
<td>1457</td>
<td>(\delta) (CH(_2))</td>
<td>Lipids, Proteins</td>
<td>Mordechai et al., 2000</td>
</tr>
<tr>
<td>1415</td>
<td>C-O-H in plane bending</td>
<td>Carbohydrates, DNA/RNA backbone, proteins</td>
<td>Socrates, 2001</td>
</tr>
<tr>
<td>1402</td>
<td>(\delta) C(CH(_3))(_2) symmetric</td>
<td>Lipids, carbohydrates, proteins</td>
<td>Lucassen et al., 1998</td>
</tr>
<tr>
<td>1312</td>
<td>Amide III</td>
<td>Proteins</td>
<td>Lucassen et al., 1998</td>
</tr>
<tr>
<td>1284</td>
<td>Amide III</td>
<td>Proteins</td>
<td>Lucassen et al., 1998</td>
</tr>
<tr>
<td>1240</td>
<td>(P=O) asymmetric</td>
<td>Phospholipids</td>
<td>Lucassen et al., 1998</td>
</tr>
<tr>
<td>~1160</td>
<td>(\delta) (COP), (CC), (COH)</td>
<td>DNA and RNA backbone</td>
<td>Naumann, 2000; Lucassen et al., 1998</td>
</tr>
<tr>
<td>~1120</td>
<td>(CC) skeletal trans conformation</td>
<td>DNA and RNA backbone</td>
<td>Mordechai et al., 2000; Lucassen et al., 1998</td>
</tr>
<tr>
<td>1200-1000</td>
<td>C-O-C, C-O dominated by ring vibrations</td>
<td>Carbohydrates</td>
<td>Naumann, 2000</td>
</tr>
<tr>
<td>1085</td>
<td>(\gamma) (P==O) symmetric</td>
<td>DNA and RNA, phospholipids</td>
<td>Naumann, 2000; Lucassen et al., 1998</td>
</tr>
<tr>
<td>1076</td>
<td>(\gamma) (CC) skeletal cis conformation</td>
<td>DNA and RNA backbones</td>
<td>Mordechai et al., 2000; Lucassen et al., 1998</td>
</tr>
<tr>
<td>900-800</td>
<td>C==C, C==N, C-H in ring structure</td>
<td>Nucleotides</td>
<td>Socrates, 2001</td>
</tr>
</tbody>
</table>

\(\delta\) – Deformation and \(\gamma\) - Stretch

*Yu and Irudayaraj, 2005.
Possible assignment of absorbance bands to the pulsed UV-light and infrared
heat-treated cells may result in identification of chemical and structural changes in S.
aureus cells during the pulsed UV-light and infrared heat treatment. Yu and Irudayaraj
(2005) reported that because of overlapping absorbance of a multitude of cellular
compounds in the infrared range, the bands are not highly resolved and difficult to apply.
For assignment of absorption bands, only the cells which were treated for the longest
time were used to observe the maximum changes in the whole cells. Therefore, S. aureus
cells treated with infrared heat for 16-min or treated with pulsed UV-light for 32-s, were
used for the assignment of absorption bands.

Pulsed UV-light treated cells has a strong absorption around 1100 cm\(^{-1}\) indicating
that contribution from 1085 cm\(^{-1}\) (DNA, RNA, phospholipids) and 1076 cm\(^{-1}\) (DNA and
RNA backbones) were there. Microscopic observations indicated that there were cell
wall damage and cellular content leakage, even after 5 sec treatment (Figure 8.2). Since,
the pulsed UV-light sample was treated for 16 s, one can expect more damage to the cell
wall and cell lysis. This resulted in leakage of DNA, RNA, and other internal content of
the cytoplasm. Therefore, there was a strong absorption in the RNA/DNA range.
Absorption by carbohydrates (1200 to 1000 cm\(^{-1}\)) might also have contributed to the
strong absorption at 1100 cm\(^{-1}\) (Figure 8.7). Pulsed UV-light treated cells also had an
absorption band at 1160 cm\(^{-1}\), corresponding to DNA and RNA backbone absorption,
indicating that there was significant leakage of genetic material from the treated cells.
Furthermore, absorption in the protein band was also observed, especially at 1284, 1312,
and 1548 cm\(^{-1}\). This clearly suggested significant cell wall damage during pulsed UV-
light treatment.
Possible finger print areas for pulsed UV-light treated whole cells (32 sec):

- DNA and RNA backbones: 1160, and 1120 cm\(^{-1}\)
- DNA, RNA, phospholipids: 1085, and 1076 cm\(^{-1}\)
- Carbohydrates 1200-1000 cm\(^{-1}\)
- Protein (Amide III): 1284, 1312, and 1548 cm\(^{-1}\)

**Figure 8.7. Assignment of absorption bands for pulsed UV-light treated S. aureus.**

Unlike, pulsed UV-light treatment, infrared heat treated *S. aureus* cells had significant absorption at possible fatty acids bands of 2957, 2919, 2872, and 2852 cm\(^{-1}\) (Figure 8.8). Furthermore, bands because of possible lipid ester absorption at 1741 cm\(^{-1}\) could be also noted. Infrared heat treated cells had very strong absorption at 1708 (RNA, DNA), 1402 (lipids, carbohydrates, proteins), and 1120 cm\(^{-1}\) (DNA and RNA backbones). These absorptions strongly suggest that the infrared heat treated cells were damaged significantly and the cellular contents leaked. This was verified with TEM pictures, wherein cell wall and cytoplasmic membrane damage were observed (Figure 8.1). Absorption at the above bands indicated that there was cytoplasmic cell membrane damage and leakage of the cellular contents.
Possible finger print areas for Infrared heat treated whole cells (16 min):
Fatty acids: 2957, 2919, 2872, and 2852 cm$^{-1}$
Lipid esters: 1741 cm$^{-1}$
DNA and RNA backbones: 1120 cm$^{-1}$
Lipids, carbohydrates, and proteins: 1402 cm$^{-1}$
RNA and DNA: 1708 cm$^{-1}$
Protein (Amide I): 1695 cm$^{-1}$

Figure 8.8. Assignment of absorption bands for infrared heat treated \textit{S. aureus}.

### 8.5. Conclusions

The microscopic observations indicated a severe cellular damage after a 5-s pulsed UV-light treatment. There was no significant temperature increase during this treatment indicating that the damage might be because of the pulse effect. Cell wall damage, cytoplasmic membrane shrinkage, cell content leakage, mesosome rupture, and genetic material leakage are some of the phenomenon observed by microscopy. Similarly, infrared heat treated samples also exhibited cell wall damage, condensation of cytoplasm, and cellular content leakage. Results indicated that these emerging technologies have a potential to be used for inactivation of pathogenic microorganisms.
Further detailed investigation on possible inactivation mechanism may result in some useful information.

FTIR was used to classify the pulsed UV-light and infrared heat treated *S. aureus* cells successfully. Therefore, FTIR has a potential to be used as a rapid assessment tool for determination of sufficient dosage absorbed by the microbial cells, since the treatment times correspond to the dosage received during the treatment. Tentative absorption bands were assigned for the FTIR spectral data. These band assignments, strongly suggested that there was damage to cell walls, cytoplasmic membranes and leakage of cellular content. This was in agreement with the microscopic observations. Further detailed studies could result in successful band assignment and information on the chemical and structural changes in *S. aureus* cells induced by pulsed UV-light or infrared heat treatment.

### 8.6. References


9. Conclusions and Scope for Future Research

Increased consumer awareness about minimally processed foods and industries’ thirst to reduce the total cost of food processing, propel researchers to investigate alternative food processing technologies. This research was a step in the direction of identifying two novel food processing technologies - namely pulsed UV-light and infrared heating - as potential food processing technologies, especially for microbial decontamination. Consumption of food contaminated with pathogenic microorganisms cause illnesses and deaths resulting in several billion dollars loss. Because of rigorous Governmental regulations and potential risk of costly recalls, food industries are forced to ensure that their food products are free from pathogenic microorganisms.

The main focus of this study was to investigate the efficacy of pulsed UV-light on inactivation of *Staphylococcus aureus* and *Bacillus subtilis*. The effects of pulsed UV-light on inactivation of *S. aureus* on an agar surface (to represent solid food) and a 0.1M phosphate buffer (to represent liquid food) were investigated. Complete inactivation of *S. aureus* on agar seeded cells was achieved within 5 s of pulsed UV-light treatment resulting in 8.5log$_{10}$ CFU/ml. Reductions of 7.5 log$_{10}$ CFU/ml were achieved at 1, 2, and 5 s treatment of *S. aureus* in a 0.1M phosphate buffer, when the volume of buffer was 12, 24, and 48 ml, respectively. As the volume of the sample increases, the inactivation decreases because of poor penetration of UV-light. There was no significant temperature increase during the pulsed UV-light treatment indicating that inactivation was achieved by pulsed UV-light alone. Following an enrichment procedure, no growth was observed, indicating that there were no injured cells. These results indicated that pulsed UV-light has a potential to be used for surface sterilization of food products and to treat liquid...
foods with high transparency. UV-light, however has poor penetration capacity. Pulsed
UV-light might have better penetration capacity than continuous UV-light. However,
only a small portion of the germicidal UV-light might be available for inactivation of
pathogenic microorganisms as several other components may absorb UV-light and other
radiation energy. Furthermore, the food components may provide protection for the
microorganisms. Hence, it is crucial to investigate the effect of several parameters on
inactivation of pathogenic microorganisms in a real food matrix. Therefore, the efficacy
of pulsed UV-light on inactivation of S. aureus in milk was investigated.

The effects of sample volume, treatment time, and distance of the milk sample
from the quartz window were investigated for inactivation of S. aureus in milk.
Temperature of the milk was increased gradually during a pulsed UV-light treatment,
resulting in temperatures up to 91°C after a 3 min treatment. This clearly indicated that
there might be a synergistic effect of temperature increase on microbial inactivation.
Complete inactivation of S. aureus was achieved when 30 mL of inoculated milk was
treated for 180 s at 8 cm distance from the quartz window and when 12 mL of inoculated
milk was treated for 180 s at 10.5 cm distance from the quartz window. The
corresponding reduction was 8.55 log_{10} CFU/ml. A surface response model predicted the
log_{10} reduction reasonably with an R^2 of 0.87. The model can be further improved, if the
experimental design can incorporate the effect of other variables such as blocking of UV-
light by food components, temperature increases etc. Results clearly demonstrated that
pulsed UV-light can be utilized for milk treatment. However, to test the feasibility of
pulsed UV-light for industrial milk treatment application, one has to investigate the
efficacy of pulsed UV-light for continuous treatment of milk. Furthermore, milk quality
may change because of pulsed UV-light treatments have to be monitored to ensure there is no adverse quality effect. To investigate this possibility, milk inoculated with *S. aureus* was pumped using a peristaltic pump and treated with pulsed UV-light.

*S. aureus* was artificially inoculated to contaminate raw milk, which was then and pumped through a quartz tube placed in the pulsed UV-light chamber and treated. The effects of distance from the quartz window, number of passes, and flow rate were investigated. Reductions obtained from the treatments varied from 0.55 to 7.26 $\log_{10}$ CFU/ml. Complete inactivation of *S. aureus* was obtained at 1) 8 cm sample distance from the quartz window, single pass, and 20 ml/min flow rate, and 2) 11 cm sample distance from the quartz window, 2 passes, and 20 ml/min flow rate combinations. The reduction corresponding to complete inactivation was more than 7 $\log_{10}$ CFU/ml. The temperature of the milk sample rose up to 43°C during the treatment indicating that there might be a synergistic effect of temperature increase. A 29 panelists consumer panel evaluated the pulsed UV-light treated skim and 1% milk samples. There was some perceivable change in the milk flavor. A 9-point hedonic scale with 1 being ‘dislike extremely’ and 9 being ‘like extremely’ was used. Generally, the pulsed UV-light treated sample received 3 to 4 points less than the untreated pasteurized milk samples. The pulsed UV-light treated whole milk had a mushroom like or burnt flavor, and thus was not used in the evaluations. These flavor changes occurred mainly because of prolonged pulsed UV-light exposure for treating a thick layer of milk.

Treating the milk as a thin film will ensure shorter treatment duration, which in turn will reduce the quality changes. Pulsed UV-light can also be used in conjunction
with a thermal pasteurization system since it can inactivate pathogens which are resistant to thermal treatment.

These studies clearly indicated that pulsed UV-light was effective in inactivating vegetative cells of pathogenic microorganisms in agar seeded cells, phosphate buffer, and milk. Treatments which inactivate vegetative cells might not inactivate spores of pathogenic microorganisms. Spores are normally more resistant than vegetative cells and hence very difficult to inactivate by current pasteurization methods. Therefore, the efficacy of pulsed UV-light on inactivation of *B. subtilis* spores in water was investigated.

An annular pulsed UV-light chamber with the lamp at the center was used in this experiment. This design ensured that all the pulsed UV-light energy was utilized for inactivation of bacterial spores because the energy was absorbed from 360° and reflected back by the polished surface. To test the feasibility of the pulsed UV-light process for industrial scale, higher flow rates were tested (up to 14 L/min). Complete inactivation of *B. subtilis* spores was obtained at all tested flow rates up to 14 LPM resulting in reductions up to 6.5 log₁₀ CFU/ml. Enrichment in no-light and light proved that there was no growth indicating that the spores were not able to recover by photorepair mechanisms. Therefore, pulsed UV-light completely inactivated the bacterial spores by not providing a chance to recover. Hence, pulsed UV-light has a potential to be used for inactivation of bacterial spores. As *B. subtilis* is a surrogate of pathogenic strains such as *B. anthracis* and *B. cereus*, these results suggest that pulsed UV-light potentially can inactivate these pathogenic strains also.
The efficacy of infrared heating on inactivation of *S. aureus* was determined as a comparison. Infrared heating has several advantages such as low energy costs, faster heating rate, etc. Reductions of 0.10 to 8.41 log$_{10}$ CFU/ml were obtained under the tested conditions. Complete inactivation of *S. aureus* was achieved at two conditions. Further enrichment indicated that some injured cells repaired themselves. Consequently, infrared heating can likely be used to replace conventional heating systems, resulting in cost reductions of the processing. Another advantage of infrared heating is that one can selectively heat the target material, such as microorganism in the food material without heating other food components. This achievement will ensure the quality of the food material by not inducing any changes because of heat treatments.

When compared to pulsed UV-light treatment, infrared heating could be readily applicable for the treatment of milk at the first glance since quality changes in pulsed UV-light treatment were significant. However, pulsed UV-light has a potential to be used for milk pasteurization provided detrimental wavelengths are filtered to maintain the quality of milk. A further design of infrared heating chamber for continuous milk treatment will result in better quality milk as it reduces the burnt flavor induced mainly due to static milk treatment for a prolonged time at an elevated temperature.

As the flavor changes induced by infrared heat treatment might possibly similar to the changes due to thermal pasteurization, infrared heat treatment might be easily accepted by the consumers. However, light induced flavor changes during pulsed UV-light treatment could cause unacceptability in consumers as indicated by the sensory studies (Appendix B). Nevertheless, it is possible to filter out the wavelengths inducing
light induced flavors by proper treatment chamber and UV-lamp design which could result in acceptable milk quality.

To optimize a disinfection process, it is crucial to understand the pathogen inactivation mechanisms of novel technologies such as pulsed UV-light and infrared heating. Therefore, the inactivation mechanism of pulsed UV-light and infrared heating was investigated by Transmission Electron Microscopy (TEM) and Fourier transform spectroscopy. The pulsed UV-light results showed that the inactivation mechanism is different from continuous UV-light treatment, where inactivation is mainly because of thymine dimer in the DNA. However, a 5-second treatment with pulsed UV-light treatment resulted in cell wall breakage, cytoplasm leakage, damage in the cellular membrane structure, and leakage of the cell content. The damage in the structure of the cell might be caused because of the pulsing effect of pulsed UV-light since many of the damage characteristics were analogous to pulsed electric field damage. Damages caused by pulsed UV-light can be grouped into 1) photochemical damage: Thymine dimer formation and other photochemical changes in DNA; 2) photothermal damage: Damage caused to bacterial cell because of the differences in the heating rates of bacteria and the surrounding media resulting in localized heating of bacterial cell; 3) pulsed effect damage: Damage because of disturbances caused by intermittent pulses. In case of infrared heating, condensation of cytoplasm, shrinkage of cytoplasmic membrane, cell wall damage, and cellular content leakage occurred after an infrared heating treatment. The pulsed UV-light and infrared heat treated samples were successfully classified by FTIR micro-spectrometry and discriminate analysis. Absorption bands in the infrared region were also successfully assigned to the treated cells.
Results of these studies are limited to the conditions tested in the study. As novel technologies, pulsed UV-light and infrared heating are still in developing stages; hence several improvements can be made. For pulsed UV-light treatment, monitoring of the actual energy absorbed by the food sample at different depth levels will be beneficial for model development and process validation. During prolonged pulsed UV-light treatment, temperature of the sample increased. The effects of thermal inactivation should be monitored and recognized. For certain products, temperature build-up can be detrimental to quality. Hence, filtering out the wavelengths causing temperature increases could help preserve the quality of the food. As a broadband spectrum light, pulsed UV-light also includes the wavelength range from 330 to 480 nm which are responsible for photoreactivation, a mechanism to repair the DNA damage caused by UV-light. It will be crucial to filter out this wavelength region in order to ensure the complete inactivation of pathogenic microorganisms. For continuous treatment of liquid food by pulsed UV-light, a mechanism has to be developed to treat a food material as a thin film for reducing treatment time and enhancing quality. For opaque food materials, treatment with some photocatalyst may enhance effectiveness of pulsed UV-light treatment. Provision of a heat sink such as cold air can be beneficial to preserve the quality of the food material by avoiding quality changes caused by temperature build-up. The annular pulsed UV-light system can be modified for treating clear food materials such as apple juice by reducing the annular space. Extensive studies on pathogen inactivation have to be continued in order to identify the most resistant pathogen. An easier and faster way to determine the adequacy of the pulsed UV-light treatments by measuring absorbed UV-light energy has to be developed.
A user friendly ‘Graphical User Interface (GUI)’ with an option for precise control would be beneficial for controlling the infrared lamps. To cool the filter for selective heating, an integrated fan control should be included. Since there are very limited studies on microbial inactivation by infrared heating, the effect of infrared heating on inactivation of pathogenic microorganisms has to be investigated. Extensive sensory evaluation studies have to be done to investigate the possible off-flavors produced during infrared heating and effective elimination of those off-flavors. A predictive model for microbial inactivation has to be developed. Effect of parameters such as composition of food (level of fat content, moisture content, etc), number of infrared lamps, orientation of lamps, type of lamps, and distance of sample from the lamps has to be investigated.

Further detailed study on inactivation mechanism can be tested for pulsed UV-light and infrared heating. Furthermore, the efficacy of the pulsed UV-light can be compared to equivalent energy continuous UV-light and infrared heating to conventional heating in order to investigate potential benefits of these techniques. Detailed study of inactivation of other pathogenic microorganisms and spores by these techniques for different food products could be helpful in designing sterilization equipment. Development of precise prediction models would be helpful for better estimation of processing parameters.
References


Appendix A. Inactivation Kinetics of Pulsed UV-light
Treatment of *Staphylococcus aureus*

UV dose-response curves for dispersed or free-floating microorganisms can be represented by first-order kinetics (EPA, 2003; Severin et al., 1984; Liu, 2005). Generally, an UV-light inactivation curve is sigmoidal (CFSAN-FDA, 2000) with a shoulder and tail. Shoulder effect is due to delayed response of a microorganism to UV-light because of injury (EPA, 2003; CFSAN-FDA, 2000). Photo-reactivation, dark repair, resistance of bacteria are factors influencing shoulder effect. Tailing effect occurs because of shielding of external particles, clumping of bacteria, and resistant microorganisms. Pulsed UV-light has very high instantaneous energy as compared to continuous UV-light. Even a second treatment results in significant inactivation of pathogenic microorganism. Several researchers suggested that inactivation of pathogens occur within seconds because of increased energy availability. Therefore, pulsed UV-light inactivation curves may not have a shoulder because of high instantaneous energy available for microbial inactivation. Pulsed UV-light may also not have tail effect for inactivation of pathogens in a clear solution. However, while treating a complex food matrix with particles, one may need to take into account these effects. Inactivation models developed for UV-light can be utilized for pulsed UV-light modeling since a significant portion of the energy delivered in a pulsed UV-light system is in the UV range (56% of the total energy was in UV range for the germicidal pulsed UV-light lamp used in this study).
The first order inactivation equation (Severin et al., 1984) can be represented as

$$N = N_o \exp^{- (k*I*t)}$$

(Equation 1)

where,

- $N =$ Concentration of viable microorganisms after UV-light treatment (CFU/ml)
- $N_o =$ Concentration of viable microorganisms before UV-light treatment (CFU/ml)
- $k =$ First order inactivation coefficient (cm$^2$/J)
- $I =$ Intensity of UV-light energy applied (J/cm$^2$)
- $t =$ Treatment time (s)

Equation 1 can also be represented as (EPA, 2003)

$$N = N_o \exp^{(-kD)} = 10^{- \left( \frac{D}{D_{10}} \right)}$$

(Equation 2)

where,

- $D = I*t =$ UV dose delivered or fluence rate (J/cm$^2$).
- $D_{10} =$ UV dose required to achieve 90% reduction in microbial population.

Therefore,

$$\log \left( \frac{N_o}{N} \right) = \frac{D}{D_{10}}$$

(Equation 3)

It is important to know the $D_{10}$ value of a microorganism to evaluate the extent of the resistance of the microorganism to UV-light. Higher $D_{10}$ values indicate that the microorganism is very resistant to UV-light and require more energy for inactivation.
From equation 2,

\[
\frac{N}{N_o} = \exp(-kD)
\]

Therefore,

\[
\log\left(\frac{N_o}{N}\right) = kD \cdot \frac{1}{\ln(10)} = \left(\frac{k}{2.303}\right) \cdot D
\]  (Equation 4)

Where, \(\log\left(\frac{N_o}{N}\right)\) is the log_{10} reduction of microbial population and \(\left(\frac{k}{2.303}\right)\) is the slope of the fitted straight line for the plot of log_{10} reduction vs. available UV dosage.

The pulsed UV-light doses received and the corresponding log_{10} reductions of \(S. aureus\) in 0.1M phosphate buffer and agar seeded cell treatment were presented in Figure 1 (chapter 3). The samples were treated with pulsed UV-light for up to 30 s at 8 cm distance from the quartz window (The centre axis of the lamp was located 5.8 cm above the quartz window). However, only data from the first five seconds of treatment were used for model development since complete inactivation was obtained after a 5-s treatment (Figure A1). The broadband energy intensity available at this location was measured to be 0.33 J/cm²/pulse using a radiometer (Ophir PE50, Ophir Optronics Inc., Wilmington, MA). In the case of phosphate buffer treatment, a 48 ml sample was treated with pulsed UV-light.
Figure A1. Inactivation of *S. aureus* by pulsed UV-light treatment.

The log_{10} reduction was plotted against estimated irradiation dosage available to the microorganism and a linear and quadratic equation was fitted. The D_{10} values and rate constants (first order inactivation coefficient, k) were estimated using a linear fit with Equations 2 and 4, respectively (Table A1).

Table A1. Estimation of inactivation model parameters.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>D_{10} value (J/cm^2)</th>
<th>k (cm^2/J)</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate buffer treatment</td>
<td>0.68</td>
<td>2.15</td>
<td>0.95</td>
</tr>
<tr>
<td>Agar seed cells</td>
<td>1.07</td>
<td>3.38</td>
<td>0.90</td>
</tr>
</tbody>
</table>

The D_{10} value of the agar seeded cells was higher because of ~5 log_{10} reduction obtained within 1 s, resulting in reduction in the slope of the equation. The reduction in microbial population after 1 s treatment was slower for additional UV dose supplied as
compared to the phosphate buffer treatment. The first order inactivation coefficient (k) values for *S. aureus* treated in phosphate buffer and as agar seeded cells were 3.38, and 2.15 cm²/J, respectively (Table A1). The reduction was gradual in case of phosphate buffer treatment. Though the linear curve fitting explained more than 90% of the variation in data, quadratic curve fitting resulted in R² of approximately 1.00, indicating that dose response of pulsed UV-light was quadratic (Table A1).

When the microorganisms exhibit a shoulder effect, Equation 2 can be modified as:

\[ N = N_o \left(1 - \left(1 - \exp\left(-kD\right)\right)^d\right), \]

where, d is the intercept of the exponential phase of the dose-response curve with the y-axis (EPA, 2003). Similarly, equation 2 can be modified to take into account the tailing effect as follows:

\[ N = N_o e^{-kd} + N_p e^{(-kpD)} , \]

where, \(N_o\) is the concentration of dispersed microorganisms present, \(N_p\) is the concentration of particles containing microorganisms, and \(k_p\) is the inactivation constant for microorganisms associated with particles (EPA, 2003). In this study, the shoulder and tail effects are not applicable as there was no indication of either a shoulder or a tail in the dose-response curve. Because of availability of sufficient energy, there was no shoulder effect as indicated by 4.91 and 1.55 log₁₀ CFU/ml reduction of *S. aureus* within an 1-second treatment. Furthermore, there was no growth observed after a 5-s treatment, indicating that there was no resistant microbial population surviving. Even following the enrichment procedure, no growth was observed, suggesting that there were no injured cells.


Appendix B. Sensory Evaluation of Pulsed UV-light Treated Milk

It is crucial to monitor the changes in sensory attributes of milk during pulsed UV-light treatment to ensure the commercial applicability. Therefore, the milk treated with pulsed UV-light was evaluated by consumer panelists. The panelists rated the overall liking on a 9-point hedonic scale.

Materials and methods

Pasteurized milk was used instead of raw milk for pulsed UV-light treatments in order to ensure the safety of the consumers. Therefore, pasteurized milk was further treated with pulsed UV-light to test the effect of UV-light on perceivable changes in the overall acceptability of the product. In order to demonstrate the effect of fat content, skim milk and 1% milk were used. Both treated and untreated samples were stored in the refrigerator (4°C) overnight. In order to determine overall acceptability on a 9-point hedonic scale (9 being like extremely and 1 being dislike extremely), about 15 ml of each refrigerated samples were served in sample cups with 3-digit blinding code and served to the panelist in a randomized serving order generated by the Compusense software (Compusense®, Ontario, Canada). Panelists were selected based on the criteria that they are consumers of milk.

A 9-point hedonic scale (1 being ‘dislike extremely’ and 9 being ‘like extremely’) was used to evaluate the overall acceptability of the milk products before and after pulsed UV-light treatment. A consumer panel of 29 panelists evaluated the products. Panelists
received the samples with 3-digit blind codes in a randomized serving order to reduce biases. Compusense® software was used to design and analyze the consumer panel test.

The results clearly show that the UV-light treated milk induce some perceivable change in the flavor of the milk and thus makes the product comparatively less acceptable. Preliminary sensory evaluation studies indicated that the pulsed UV-light treated whole milk had a distinct burnt flavor and/or mushroom soup flavor based on the input from trained sensory panelists. Therefore, only 1% and skim milk were used for further sensory studies. In general, the pulsed UV-light treated milk samples were rated 3 to 4 points less than the untreated samples (Table A2).

Table A2. Sensory evaluation of UV treated milk (n=29).

<table>
<thead>
<tr>
<th>Overall acceptability&lt;sup&gt;1,2&lt;/sup&gt;</th>
<th>Skim milk</th>
<th>1% fat milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (pasteurized)</td>
<td>5.76 ± 1.83A</td>
<td>6.24 ± 2.10A</td>
</tr>
<tr>
<td>UV-light treated (pasteurization followed by UV-light treatment)</td>
<td>1.79 ± 1.29B</td>
<td>2.10 ± 1.52B</td>
</tr>
</tbody>
</table>

<sup>1</sup>A 9-point hedonic scale was used, where 1 = dislike extremely, and 9 = like extremely.  
<sup>2</sup>Mean of 29 observations are given with the standard deviation.  
<sup>3</sup>Means followed by different letter are significantly different from each other in the same column (p ≤ 0.05).

The variation between the samples and judges were statistically significant (p<0.05). However, it is interesting to note that the majority of the consumers rated the control (pasteurized milk) as mostly ‘neither like nor dislike’ or ‘like slightly’. This clearly indicates that there are some flavor changes associated with the pasteurization process which influenced the acceptability rating for the UV-light treated milk samples as the pasteurized milk was further processed with pulsed UV-light. Flavor changes induced by pasteurization also contribute to the lower acceptability rating. The ratings
for pulsed UV-light treated skim milk and 1% milk were $1.79 \pm 1.29$ and $2.10 \pm 1.52$, respectively, while the control samples had a rating of $5.76 \pm 1.83$ and $6.24 \pm 2.10$, respectively.

Previous research shows that pulsed UV-light can inactivate pathogens in a very short time (up to several seconds exposure) if the thickness of the food product is minimal. A setup which will allow milk to flow as a thin (1-3 mm thickness) film will be helpful in reducing the exposure time as the penetration efficiency increases exponentially, which will in turn reduce the treatment time, which will also reduce the changes in the sensory quality of the milk.

In conclusion, sensory evaluation of pulsed UV-light treated and untreated milk was performed. The treated milk received slightly lower rating than the untreated milk indicating that there was some change to the sensory attributes. However, by optimizing the process parameters, one can further improve the quality of milk.
Appendix C. Source Code: Infrared Heating Control Logic

#include <utility.h>
#include <ansi_c.h>
#include <formatio.h>
#include <rs232.h>
#include <cvirte.h>  /* Needed if linking in external compiler; harmless otherwise */
#include <userint.h>
#include "6lamps.h"
#define OFF 0
#define ON 1
#define LPT1 0x378
char word;
char set[10];
double sum=0;
double save=0;
int counter=0;
int sign;
int end=0;
int dim1=1,dim2=1,dim3=1;dim4=1;dim5=1;dim6=1;
double save1=0;
//char TEMPSTR[7];
char buf[260];
int index_num;
char sendword[30];
static int panelHandle;
static int configHandle;
static int choiceHandle;
static int setHandle;
static int graphHandle;
char proj_dir[256];
char file_name[300];
static int go_num1,go_num2,go_num3,go_num4,go_num5,go_num6;
int read;
int choice1;
int choice2;
int loop1=0;
int loop2=0;
int loop3=0;
int fan;
int onandoff1, onandoff2, onandoff3, onandoff4,onandoff5, onandoff6;
double readings1,readings2,readings3,readings4,readings5,readings6;
int comport = 1,
    baudrate = 9600,
    parity = 0,
databits = 8,
stopbits = 1;
char Rsbuf[1000];
int datanum=0;
int goflag=0;
int goflag1=0;
int goflag2=0;
int temp;
int stringsize,bytes_sent;
double datapoints[15][20000];
double graphdata1[3],graphdata2[3],graphdata3[3],graphdata4[3]
,graphdata5[4],graphdata6[2],graphdata7[6];
typedef struct {
    int channel;
    double data[15];
    int unit;
} DataType;
DataType Get;
double calib(double x);
void send_data(void);
void receive_data(void);
void SetConfigParms1 (void);
void GetConfigParms1 (void);
void Control_GK(void);
void Control_New(void);
void Control_New1(void);
int ComRdStr(int port, char *s, int term)
{
    int temp, ret = 0;
    while ((temp = ComRdByte(port)) != term)
    {
        if (temp == -99) break;
        s[ret++] = (char)temp;
    }
    s[ret] = '\0';
    return ret;
}
int main (int argc, char *argv[])
{
    if (InitCVIRTE (0, argv, 0) == 0) /* Needed if linking in external compiler;
harmless otherwise */
    return -1; /* out of memory */
    if ((panelHandle = LoadPanel (0, "6lamps0228.uir", PANEL)) < 0)
        return -1;
    graphHandle = LoadPanel (0, "6lamps0228.uir",GRAPH);
    DisplayPanel (panelHandle);
SetCtrlAttribute(panelHandle,PANEL_STOPBUTTON,ATTR_VISIBLE,0);
RunUserInterface();
return 0;
}

int CVICALLBACK ProceedCall (int panel, int control, int event,
void *callbackData, int eventData1, int eventData2)
{
int i,ret=0;
char Temp[10];
switch (event) {
    case EVENT_COMMIT:
        SetCtrlAttribute(panelHandle,PANEL_STOPBUTTON,ATTR_VISIBLE,1);
        SetCtrlAttribute(panelHandle,PANEL_GOBUTTON,ATTR_VISIBLE,0);
        DisplayPanel(graphHandle);
        SetCtrlAttribute(graphHandle,GRAPH_TEMP_GRAPH1_1,ATTR_VISIBLE,0);
        SetCtrlAttribute(graphHandle,GRAPH_TEMP_GRAPH2_1,ATTR_VISIBLE,0);
        SetCtrlAttribute(graphHandle,GRAPH_TEMP_GRAPH3_1,ATTR_VISIBLE,0);
        SetCtrlAttribute(graphHandle,GRAPH_TEMP_GRAPH4_1,ATTR_VISIBLE,0);
        SetCtrlAttribute(graphHandle,GRAPH_TEMP_GRAPH5_1,ATTR_VISIBLE,0);
        SetCtrlAttribute(graphHandle,GRAPH_TEMP_GRAPH6_1,ATTR_VISIBLE,0);
        OpenComConfig(comport, "", baudrate, parity, databits,
stopbits, 1024, 512);
        strcpy(sendword, "INIT\n");
        stringsize = StringLength(sendword);
        bytes_sent = ComWrt(comport, sendword, stringsize);
        strcpy(sendword, "FETCh?\n");
        stringsize = StringLength(sendword);
        bytes_sent = ComWrt (comport, sendword, stringsize);  */
goflag =1;
    break;
}
return 0;
}

void send_data(void)
{
    int j;
    int ratio=0;
    /*
    strcpy(sendword, "READ?\n");
    stringsize = StringLength(sendword);
    bytes_sent = ComWrt (comport, sendword, stringsize); */
    strcpy(sendword, "INIT\n");
    stringsize = StringLength(sendword);
    bytes_sent = ComWrt (comport, sendword, stringsize);
    strcpy(sendword, "FETCh?\n");
    stringsize = StringLength(sendword);
    bytes_sent = ComWrt (comport, sendword, stringsize);
    strcpy(sendword, "READ?\n");
    stringsize = StringLength(sendword);
    bytes_sent = ComWrt (comport, sendword, stringsize);
    strcpy(sendword, "INIT\n");
    stringsize = StringLength(sendword);
    bytes_sent = ComWrt (comport, sendword, stringsize);
    strcpy(sendword, "FETCh?\n");
    stringsize = StringLength(sendword);
    bytes_sent = ComWrt (comport, sendword, stringsize);
    break;
}
void receive_data(void)
{
    int j=0;
    int k=0;
    int l=0;
    int ratio=0;
    //ComRd (comport, Rsbuf, 200);
    if (choice1)
    {
        Scan(Rsbuf, "%s[i14w1]>%i", &(Get.unit));
        if (Get.unit==1) ratio=10;
        if (Get.unit==2) ratio=100;
        Scan(Rsbuf, "%s[i1w6]>%f[p4]", &(Get.data[0]));
        Get.data[0]= Get.data[0]*ratio;
        Scan(Rsbuf, "%s[i30w1]>%i", &(Get.unit));
        if (Get.unit==1) ratio=10;
        if (Get.unit==2) ratio=100;
        Scan(Rsbuf, "%s[i17w6]>%f[p4]", &(Get.data[1]));
        Get.data[1]= Get.data[1]*ratio;
        Scan(Rsbuf, "%s[i46w1]>%i", &(Get.unit));
        if (Get.unit==1) ratio=10;
        if (Get.unit==2) ratio=100;
        Scan(Rsbuf, "%s[i33w6]>%f[p4]", &(Get.data[2]));
        Scan(Rsbuf, "%s[i62w1]>%i", &(Get.unit));
        if (Get.unit==1) ratio=10;
        if (Get.unit==2) ratio=100;
        Scan(Rsbuf, "%s[i49w6]>%f[p4]", &(Get.data[3]));
        Scan(Rsbuf, "%s[i110w1]>%i", &(Get.unit));
        if (Get.unit==1) ratio=10;
        if (Get.unit==2) ratio=100;
        Scan(Rsbuf, "%s[i97w6]>%f[p4]", &(Get.data[4]));
        Scan(Rsbuf, "%s[i174w1]>%i", &(Get.unit));
        if (Get.unit==1) ratio=10;
        if (Get.unit==2) ratio=100;
        Scan(Rsbuf, "%s[i161w6]>%f[p4]", &(Get.data[5]));
        Scan(Rsbuf, "%s[i190w1]>%i", &(Get.unit));
        if (Get.unit==1) ratio=10;
        if (Get.unit==2) ratio=100;
        Scan(Rsbuf, "%s[i177w6]>%f[p4]", &(Get.data[6]));
    }
}
if (choice2)
{
    Scan(Rsbuf, "\%s>\%15f[x]", Get.data);
}

int CVICALLBACK CloseMainCall (int panel, int control, int event, void *callbackData, int eventData1, int eventData2)
{
    switch (event) {
    case EVENT_COMMIT:
        SetCtrlVal (panelHandle, PANEL_LED, OFF);
        CloseCom (comport);
        outp(LPT1,0x00);
        QuitUserInterface (0);
        go_num1 =0;
        go_num2 =0;
        go_num3 =0;
        go_num4 =0;
        break;
    }
    return 0;
}

int CVICALLBACK timer_control (int panel, int control, int event, void *callbackData, int eventData1, int eventData2)
{
    int i;
    switch (event) {
        case EVENT TIMER TICK:
            if (goflag ==1)
            {
                // SetSystemAttribute (ATTR ALLOW UNSAFE TIMER EVENTS, 1);
                SetCtrlVal (panelHandle, PANEL_LED, ON);
                send_data();
                SetCtrlVal (panelHandle, PANEL_LED, OFF);
                oflag1=1;
            }
            break;
    }
    return 0;
}

int CVICALLBACK timer2 control (int panel, int control, int event, void *callbackData, int eventData1, int eventData2)
{
    switch (event) {

case EVENT_TIMER_TICK:
    // SetSystemAttribute
    (ATTR_ALLOW_UNSAFE_TIMER_EVENTS, 1);
    if (goflag1 == 1)
    {
        receive_data();
        goflag2 = 1;
        Control_New();
    }
break;

return 0;
}

int CVICALLBACK end_job (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2)
{
    switch (event) {
    case EVENT_COMMIT:
        SetCtrlAttribute(panelHandle, PANEL_STOPBUTTON, ATTR_VISIBLE, 0);
        SetCtrlAttribute(panelHandle, PANEL_GOBUTTON, ATTR_VISIBLE, 1);
        SetCtrlVal (panelHandle, PANEL_LED, OFF);
        SetCtrlVal (panelHandle, PANEL_LED1, OFF);
        SetCtrlVal (panelHandle, PANEL_LED2, OFF);
        SetCtrlVal (panelHandle, PANEL_LED3, OFF);
        SetCtrlVal (panelHandle, PANEL_LED4, OFF);
        SetCtrlVal (panelHandle, PANEL_LED5, OFF);
        SetCtrlVal (panelHandle, PANEL_LED6, OFF);
        CloseCom (comport);
        SetCtrlVal (panelHandle, PANEL_FAN, OFF);
        outp(LPT1, 0x00);
        goflag = 0;
        goflag1 = 0;
        goflag2 = 0;
        go_num1 = 0;
        go_num2 = 0;
        go_num3 = 0;
        go_num4 = 0;
        sum = 0; save = 0;
        end = 1;
        break;
    }
    return 0;
}

int Clear (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2)
{  
int i,j, readings;
switch (event) {
  case EVENT_COMMIT:
    /* DeleteListItem (panelHandle, PANEL_TEMP_LIST, 0, 0);*/
    ClearListCtrl (panelHandle, PANEL_TEMP_LIST);
    datanum=0;
    ClearStripChart (graphHandle, GRAPH_TEMP_GRAPH1);
    ClearStripChart (graphHandle, GRAPH_TEMP_GRAPH2);
    ClearStripChart (graphHandle, GRAPH_TEMP_GRAPH3);
    ClearStripChart (graphHandle, GRAPH_TEMP_GRAPH4);
    ClearStripChart (graphHandle, GRAPH_TEMP_GRAPH5);
    ClearStripChart (graphHandle, GRAPH_TEMP_GRAPH6);
    ClearStripChart (graphHandle, PANEL_TEMP_GRAPH7);
    /* DeleteGraphPlot (panelHandle, PANEL_TEMP_GRAPH, -1,
     VAL_DELAYED_DRAW); */
    SetCtrlVal (panelHandle, PANEL_LED1, OFF);
    SetCtrlVal (panelHandle, PANEL_LED2, OFF);
    SetCtrlVal (panelHandle, PANEL_LED3, OFF);
    SetCtrlVal (panelHandle, PANEL_LED4, OFF);
    SetCtrlVal (panelHandle, PANEL_LED5, OFF);
    SetCtrlVal (panelHandle, PANEL_LED6, OFF);
    //  goflag=0;
    //  goflag1=0;
    SetCtrlVal (panelHandle, PANEL_TEST2, " ");
    for( i =0;i<1000;i++)
    {
      j + =1;
    }
    SetCtrlVal (panelHandle, PANEL_CLEAR, OFF);
    SetCtrlVal (panelHandle, PANEL_LED, OFF);
    outp(LPT1,(0x00|word));
    go_num1 =0;
    go_num2 =0;
    go_num3 =0;
    go_num4 =0;
    end =1;
    break;
}
return 0;
}
int  Save(int panel, int control, int event, void *callbackData, int eventData1, int eventData2)
{
  FILE *filehandle;
  int i;
  switch ( event) {

}
case EVENT_COMMIT:

    FileSelectPopup (proj_dir, ".txt", ".txt", "Save As",
    VAL_OK_BUTTON, 0, 1, 0, file_name);
    filehandle = fopen (file_name, "w+");
    fprintf(filehandle, "RS232C OPERATING SYSTEM 

    ");
    fprintf(filehandle, "*********< Information >********* 
    ");
    fprintf(filehandle, "Channel Acquired: LAMP1 LAMP2 LAMP3 LAMP4 LAMP5 LAMP6 SAMP1 SAMP2 
    ");
    fprintf(filehandle, "Date %s   Time: %s \n", DateStr(), TimeStr());
    fprintf (filehandle, "----------------------------------
    ");
    for (i=0;i<datanum;i++)
    {
        fprintf (filehandle, "%d : %4.1f  %4.1f  %4.1f   %4.1f   %4.1f   %4.1f  %4.1f  %4.1f  %4.1f  %4.1f  %4.1f  %4.1f
    ");
    }
    fclose (filehandle);
}

int CVICALLBACK ConfigCall (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2)
{
    switch (event) {
    case EVENT_COMMIT:
        configHandle = LoadPanel (panelHandle, 
        "6lamps0228.uir",CONFIG);
        //   SetCtrlVal(configHandle, CONFIG_SETWHAT, "Setting Hydro Thermometer");
        InstallPopup (configHandle);
        SetConfigParms1();
        break;
    }
    return 0;
}

void SetConfigParms1 (void)
{
    SetCtrlVal (configHandle, CONFIG_COMPORT, comport);
    SetCtrlVal (configHandle, CONFIG_BAUDRATE, baudrate);
    SetCtrlVal (configHandle, CONFIG_PARITY, parity);
    SetCtrlVal (configHandle, CONFIG.databits, databits);
    SetCtrlVal (configHandle, CONFIG_STOPBITS, stopbits);
}
/*********************************************************************/
void GetConfigParms1 (void)
{
    GetCtrlVal (configHandle, CONFIG_COMPORT, &comport);
    GetCtrlVal (configHandle, CONFIG_BAUDRATE, &baudrate);
    GetCtrlVal (configHandle, CONFIG_PARITY, &parity);
    GetCtrlVal (configHandle, CONFIG_DATABITS, &databits);
    GetCtrlVal (configHandle, CONFIG_STOPBITS, &stopbits);
}
int CloseConfigCallback (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2)
{
    switch (event) {
        case EVENT_COMMIT:
            choiceHandle = LoadPanel (panelHandle, "6lamps0228.uir",CHOICE);
            GetConfigParms1();
            DiscardPanel (configHandle);
            InstallPopup (choiceHandle);
            break;
    }
    return 0;
}
int CVICALLBACK Closechoice (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2)
{
    switch (event) {
        case EVENT_COMMIT:
            setHandle = LoadPanel (panelHandle, "6lamps0228.uir",SET);
            GetCtrlVal (choiceHandle, CHOICE_CHECKBOX1, &choice1);
            GetCtrlVal (choiceHandle, CHOICE_CHECKBOX2, &choice2);
            if (choice1 == choice2)
                MessagePopup ("Error Message", "You must choose either of
two systems");
            else
                DiscardPanel (choiceHandle);
            if (choice1)
            {
                SetCtrlAttribute(setHandle,SET_SETPOINT_5,ATTR_VISIBLE,0);
                SetCtrlAttribute(setHandle,SET_BINARYSWITCH5,ATTR_VISIBLE,0);
                SetCtrlAttribute(setHandle,SET_RING5,ATTR_VISIBLE,0);
                SetCtrlAttribute(setHandle,SET_SETPOINT_6,ATTR_VISIBLE,0);
                SetCtrlAttribute(setHandle,SET_BINARYSWITCH6,ATTR_VISIBLE,0)
            }
SetCtrlAttribute(setHandle, SET_RING6, ATTR_VISIBLE, 0);
}
InstallPopup(setHandle);
{
    break;
}
return 0;

int CVICALLBACK CloseConfigCallback1 (int panel, int control, int event, void *callbackData, int eventData1, int eventData2)
{
    int i;
    double j;
    char set[20];
    switch (event) {
        case EVENT_COMMIT:
            GetCtrlVal(setHandle, SET_SETPOINT, &readings1);
            graphdata1[1] = readings1;
            GetCtrlVal(setHandle, SET_SETPOINT_2, &readings2);
            graphdata2[1] = readings2;
            GetCtrlVal(setHandle, SET_SETPOINT_3, &readings3);
            graphdata3[1] = readings3;
            GetCtrlVal(setHandle, SET_SETPOINT_4, &readings4);
            graphdata4[1] = readings4;
            GetCtrlVal(setHandle, SET_SETPOINT_5, &readings5);
            graphdata5[1] = readings5;
            GetCtrlVal(setHandle, SET_SETPOINT_6, &readings6);
            graphdata6[1] = readings6;
            GetCtrlVal(setHandle, SET_BINARYSWITCH1, &onandoff1);
            GetCtrlVal(setHandle, SET_BINARYSWITCH2, &onandoff2);
            GetCtrlVal(setHandle, SET_BINARYSWITCH3, &onandoff3);
            GetCtrlVal(setHandle, SET_BINARYSWITCH4, &onandoff4);
            GetCtrlVal(setHandle, SET_BINARYSWITCH5, &onandoff5);
            GetCtrlVal(setHandle, SET_BINARYSWITCH6, &onandoff6);
            DiscardPanel(setHandle);
            Fmt(set, "%s<%f[p2]", readings1);
            SetCtrlVal(panelHandle, PANEL_STRING1, set);
            Fmt(set, "%s<%f[p2]", readings2);
            SetCtrlVal(panelHandle, PANEL_STRING2, set);
            Fmt(set, "%s<%f[p2]", readings3);
            SetCtrlVal(panelHandle, PANEL_STRING3, set);
            Fmt(set, "%s<%f[p2]", readings4);
            SetCtrlVal(panelHandle, PANEL_STRING4, set);
            Fmt(set, "%s<%f[p2]", readings5);
            SetCtrlVal(panelHandle, PANEL_STRING5, set);
            Fmt(set, "%s<%f[p2]", readings6);
SetCtrlVal (panelHandle, PANEL_STRING6, set);
SetCtrlAttribute(panelHandle, PANEL_GOBUTTON, ATTR_DIMMED, 0);
SetCtrlAttribute(panelHandle, PANEL_CONFIG_CALL, ATTR_DIMMED, 1);
if (choice2)
{
    SetCtrlVal (panelHandle, PANEL_TEST1, "New Heating System");
    SetCtrlAttribute(panelHandle, PANEL_FAN, ATTR_DIMMED, 0);
}
if (choice1) SetCtrlVal (panelHandle, PANEL_TEST1, "Old Heating System");
    if (onandoff1 == 0)
    {
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH1, ATTR_DIMMED, 1);
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH1_1, ATTR_DIMMED, 1);
        dim1 = 0;
    }
    if (onandoff2 == 0)
    {
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH2, ATTR_DIMMED, 1);
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH2_1, ATTR_DIMMED, 1);
        dim2 = 0;
    }
    if (onandoff3 == 0)
    {
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH3, ATTR_DIMMED, 1);
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH3_1, ATTR_DIMMED, 1);
        dim3 = 0;
    }
    if (onandoff4 == 0)
    {
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH4, ATTR_DIMMED, 1);
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH4_1, ATTR_DIMMED, 1);
        dim4 = 0;
    }
    if (onandoff5 == 0)
    {
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH5, ATTR_DIMMED, 1);
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH5_1, ATTR_DIMMED, 1);
        dim5 = 0;
    }
    if (onandoff6 == 0)
    {
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH6, ATTR_DIMMED, 1);
        SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH6_1, ATTR_DIMMED, 1);
        dim6 = 0;
    }
double calib(double x) {
    return (2898/(x+273));
}

double decalib(double x) {
    return (2898/x -273);
}

int CVICALLBACK setpoint1 (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2) {
    char TEMPSTR[7];
    switch (event) {
        case EVENT_COMMIT:
            GetCtrlVal (setHandle, SET_SETPOINT, &readings1);
            SetCtrlVal(setHandle, SET_RING1, calib(readings1));
            break;
    }
    return 0;
}

int CVICALLBACK setpoint2 (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2) {
    char TEMPSTR[7];
    switch (event) {
        case EVENT_COMMIT:
            GetCtrlVal (setHandle, SET_SETPOINT_2, &readings2);
            SetCtrlVal(setHandle, SET_RING2, calib(readings2));
            break;
    }
    return 0;
}

int CVICALLBACK setpoint3 (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2) {
    char TEMPSTR[7];
    switch (event) {
        case EVENT_COMMIT:
            GetCtrlVal (setHandle, SET_SETPOINT_3, &readings3);
            SetCtrlVal(setHandle, SET_RING3, calib(readings3));
            break;
    }
}
return 0;
}

int CVICALLBACK setpoint4 (int panel, int control, int event,
        void *callbackData, int eventData1, int eventData2)
{
        switch (event) {
                case EVENT_COMMIT:
                        GetCtrlVal (setHandle, SET_SETPOINT_4, &readings4);
                        SetCtrlVal(setHandle, SET_RING4, calib(readings4));
                        break;
        }
        return 0;
}

int CVICALLBACK setpoint5 (int panel, int control, int event,
        void *callbackData, int eventData1, int eventData2)
{
        switch (event) {
                case EVENT_COMMIT:
                        GetCtrlVal (setHandle, SET_SETPOINT_5, &readings5);
                        SetCtrlVal(setHandle, SET_RING5, calib(readings5));
                        break;
        }
        return 0;
}

int CVICALLBACK setpoint6 (int panel, int control, int event,
        void *callbackData, int eventData1, int eventData2)
{
        switch (event) {
                case EVENT_COMMIT:
                        GetCtrlVal (setHandle, SET_SETPOINT_6, &readings6);
                        SetCtrlVal(setHandle, SET_RING6, calib(readings6));
                        break;
        }
        return 0;
}

void Control_New(void)
{
        int on_num1,on_num2,on_num3,on_num4,on_num5,on_num6;
        // char TEMPSTR[7];
        double dty1,dty2,dty3,dty4,dty5,dty6,dy1,dy2,dy3,dy4,dy5,dy6;
        char word1,word2, word3, word4, word5, word6,word7;
        char sent_word1[30];
        char sent_word;
        dy1 = readings1 - Get.data[0];
        /*dy1 is the difference between set temperature
        (readings1) and the actual temperature (Get.data[0])- Kadir */
dy2 = readings2 - Get.data[1];
dy3 = readings3 - Get.data[2];
dy4 = readings4 - Get.data[3];
dy5 = readings5 - Get.data[4];
dy6 = readings6 - Get.data[5];
dty1 = ((datanum > 1) ?
    datapoints[0][datanum - 1] - datapoints[0][datanum - 2] : 0.0);
/* dty1 tries to check there is a difference between the temperature
   collected in the previous time step and the current time step. If there is a difference
   exist it will adjust the on_num value to compensate for that - Kadir*/
dty2 = ((datanum > 1) ?
    datapoints[1][datanum - 1] - datapoints[1][datanum - 2] : 0.0);
dty3 = ((datanum > 1) ?
    datapoints[2][datanum - 1] - datapoints[2][datanum - 2] : 0.0);
dty4 = ((datanum > 1) ?
    datapoints[3][datanum - 1] - datapoints[3][datanum - 2] : 0.0);
dty5 = ((datanum > 1) ?
    datapoints[4][datanum - 1] - datapoints[4][datanum - 2] : 0.0);
dty6 = ((datanum > 1) ?
    datapoints[5][datanum - 1] - datapoints[5][datanum - 2] : 0.0);

//number 1
if(readings1 > 500)
{
    if (dy1 < 0 ) on_num1 = 0;
    else if (Get.data[0] > (0.999*readings1)) on_num1 = 1;
    else if (Get.data[0] > (0.997*readings1)) on_num1 = 3;
    //
    else if (Get.data[0] > (0.996*readings1)) on_num1 = 5;
    //
    else if (Get.data[0] > (0.994*readings1)) on_num1 = 7;
    //
    else if (Get.data[0] > (0.992*readings1)) on_num1 = 9;
    //
    else if (Get.data[0] > (0.99*readings1)) on_num1 = 10;
    else if (Get.data[0] > (0.95*readings1)) on_num1 = 11; /*
    else if (Get.data[0] > (0.9*readings1)) on_num1 = 14;
    else if (Get.data[0] > (0.8*readings1)) on_num1 = 15; */
    else on_num1 = 16;
    if((dy1 < 10.0) && (dy1 > -5)) //Kadir changed it to 10 (initially it was 80
    (dy1)
    { if (dty1 < -1.0)
      on_num1 += 16;
      else if (dty1 < -0.7)
        on_num1 += 12;
      else if (dty1 < -0.2)
        on_num1 += 8;
    }
}
else if(readings1 > 300)
{
    if (dy1 < 0 )   on_num1 = 0;
    else if (Get.data[0] > (0.995*readings1)) on_num1 = 2;
    else if (Get.data[0] > (0.99*readings1)) on_num1 = 3;
    else if (Get.data[0] > (0.96*readings1)) on_num1 = 3;
    else if (Get.data[0] > (0.9*readings1)) on_num1 = 4;
    else if (Get.data[0] > (0.85*readings1)) on_num1 = 4;
    else if (Get.data[0] > (0.8*readings1)) on_num1 = 5;
    else if (Get.data[0] > (0.7*readings1)) on_num1 = 6;
    else if (Get.data[0] > (0.6*readings1)) on_num1 = 7;
    else on_num1 = 8;
    if((dy1 < 80.0) && (dy1 > -10))
    {
        if (dty1 < -2.0)
            on_num1 += 6;
        else if (dty1 < -0.7)
            on_num1 += 4;
        else if (dty1 < -0.2)
            on_num1 += 3;
        else if (dty1 < 0)
            on_num1 += 2;
    }
}
else if ( readings1 > 150)
{
    if (dy1 < 0 )   on_num1 = 0;
    else if (Get.data[0] > (0.99*readings1)) on_num1 = 0;
    else if (Get.data[0] > (0.96*readings1)) on_num1 = 2;
    else if (Get.data[0] > (0.9*readings1)) on_num1 = 3;
    else if (Get.data[0] > (0.85*readings1)) on_num1 = 4;
    else if (Get.data[0] > (0.8*readings1)) on_num1 = 5;
    else if (Get.data[0] > (0.7*readings1)) on_num1 = 6;
    else if (Get.data[0] > (0.6*readings1)) on_num1 = 7;
    else on_num1 = 8;
    if((dy1 > -1) && (dty1 < 0))
        on_num1 += 2;
}
else
{
    if (dy1 < 0 )   on_num1 = 0;
    else if (Get.data[0] > (0.99*readings1)) on_num1 = 0;
    else if (Get.data[0] > (0.96*readings1)) on_num1 = 0;
    else if (Get.data[0] > (0.9*readings1)) on_num1 = 1;
    else if (Get.data[0] > (0.85*readings1)) on_num1 = 2;
    else if (Get.data[0] > (0.8*readings1)) on_num1 = 3;
    else if (Get.data[0] > (0.7*readings1)) on_num1 = 4;
else if (Get.data[0] > (0.6*readings1))   on_num1 = 5;
else on_num1 = 7;
if((dy1 > -1)&& (dty1 < 0))
on_num1 += 2;
}
//on_num1 = 16;
go_num1++;
go_num1 = go_num1 % 16;
if (((go_num1 < on_num1) &&(onandoff1))
// if (onandoff1)
{
    word1=0x01;
    SetCtrlVal (panelHandle, PANEL_LED1, ON);
}
else
{
    word1=0x00;
    SetCtrlVal (panelHandle, PANEL_LED1, OFF);
}
// number 2
if(readings2 > 500)
{
    if (dy2 < 0 )   on_num2 = 0;
    else if (Get.data[1] > (0.999*readings2)) on_num2 = 1;
    else if (Get.data[1] > (0.998*readings2)) on_num2 = 3;
    else if (Get.data[1] > (0.997*readings2)) on_num2 = 5;
    else if (Get.data[1] > (0.996*readings2)) on_num2 = 7;
    else if (Get.data[1] > (0.994*readings2)) on_num2 = 9;
    else if (Get.data[1] > (0.992*readings2)) on_num2 = 10;
    else if (Get.data[1] > (0.99*readings2)) on_num2 = 13;*/
    else if (Get.data[1] > (0.95*readings2)) on_num2 = 10;*/
    else if (Get.data[1] > (0.9*readings2)) on_num2 = 15;*/
else on_num2 = 16;
if((dy2 < 10.0) && (dy2 > -5))  //Kadir - Changed dy2<80.0 to dy2<10.0
    if (dty2 < -1.0)
on_num2 += 16;
    else if (dty2 < -0.7)
on_num2 += 12;
    else if (dty2 < -0.2)
on_num2 += 8;
}
else if(readings2 > 300)
{
    if (dy2 < 0 )   on_num2 = 0;
}
else if (Get.data[1] > (0.995*readings2)) on_num2 = 2;
else if (Get.data[1] > (0.99*readings2)) on_num2 = 3;
else if (Get.data[1] > (0.96*readings2)) on_num2 = 3;
else if (Get.data[1] > (0.9*readings2)) on_num2 = 4;
else if (Get.data[1] > (0.85*readings2)) on_num2 = 4;
else if (Get.data[1] > (0.8*readings2)) on_num2 = 5;
else if (Get.data[1] > (0.7*readings2)) on_num2 = 6;
else if (Get.data[1] > (0.6*readings2)) on_num2 = 7;
else on_num2 = 8;
if((dy2 < 80.0) && (dy2 > -10))
  { if (dty2 < -2.0)
    on_num2 += 6;
  else if (dty2 < -0.7)
    on_num2 += 4;
  else if (dty2 < -0.2)
    on_num2 += 3;
  else if (dty2 < 0)
    on_num2 += 2;
  }
else if ( readings2 > 150)
  {
    if (dy2 < 0 )   on_num2 = 0;
    else if (Get.data[1] > (0.99*readings2)) on_num2 = 0;
    else if (Get.data[1] > (0.96*readings2)) on_num2 = 2;
    else if (Get.data[1] > (0.9*readings2)) on_num2 = 3;
    else if (Get.data[1] > (0.85*readings2)) on_num2 = 4;
    else if (Get.data[1] > (0.8*readings2)) on_num2 = 5;
    else if (Get.data[1] > (0.7*readings2)) on_num2 = 6;
    else if (Get.data[1] > (0.6*readings2)) on_num2 = 7;
    else on_num2 = 8;
    if((dy2 > -1)&& (dty2 < 0))
      on_num2 += 2;
  }
else
  {
    if (dy2 < 0 )   on_num2 = 0;
    else if (Get.data[1] > (0.99*readings2)) on_num2 = 0;
    else if (Get.data[1] > (0.96*readings2)) on_num2 = 0;
    else if (Get.data[1] > (0.9*readings2)) on_num2 = 1;
    else if (Get.data[1] > (0.85*readings2)) on_num2 = 2;
    else if (Get.data[1] > (0.8*readings2)) on_num2 = 3;
    else if (Get.data[1] > (0.7*readings2)) on_num2 = 4;
    else if (Get.data[1] > (0.6*readings2)) on_num2 = 5;
    else on_num2 = 7;
    if((dy2 > -1)&& (dty2 < 0))
      on_num2 += 2;
on_num2 += 2;

//on_num2 = 16;
go_num2++;
go_num2 = go_num2 % 16;
if ((go_num2 < on_num2) && (onandoff2))
  // if (onandoff2)
  {
    word2 = 0x02;
    SetCtrlVal (panelHandle, PANEL_LED2, ON);
  }
else
  {
    word2 = 0x00;
    SetCtrlVal (panelHandle, PANEL_LED2, OFF);
  }

// number 3
if (readings3 > 500)
{
  if (dy3 < 0) on_num3 = 0;
  else if (Get.data[2] > (0.999*readings3)) on_num3 = 1;
  else if (Get.data[2] > (0.998*readings3)) on_num3 = 3;
  /*
   else if (Get.data[2] > (0.997*readings3)) on_num3 = 5;
   else if (Get.data[2] > (0.996*readings3)) on_num3 = 7;
   else if (Get.data[2] > (0.994*readings3)) on_num3 = 9;
   */
  else if (Get.data[2] > (0.992*readings3)) on_num3 = 10;
  else if (Get.data[2] > (0.99*readings3)) on_num3 = 13; /*
  */
  else if (Get.data[2] > (0.96*readings3)) on_num3 = 14; /*
else on_num3 = 16;
if ((dy3 < 10.0) && (dy3 > -5))
  {
    if (dty3 < -1.0)
      on_num3 += 16;
    else if (dty3 < -0.7)
      on_num3 += 12;
    else if (dty3 < -0.2)
      on_num3 += 8;
  }
else if (readings3 > 300)
{
  if (dy3 < 0) on_num3 = 0;
  else if (Get.data[2] > (0.995*readings3)) on_num3 = 2;
  else if (Get.data[2] > (0.99*readings3)) on_num3 = 3;
  else if (Get.data[2] > (0.96*readings3)) on_num3 = 3;
}
else if (Get.data[2] > (0.9*readings3)) on_num3 = 4;
else if (Get.data[2] > (0.85*readings3)) on_num3 = 4;
else if (Get.data[2] > (0.8*readings3)) on_num3 = 5;
else if (Get.data[2] > (0.7*readings3)) on_num3 = 6;
else if (Get.data[2] > (0.6*readings3)) on_num3 = 7;
else on_num3 = 8;
if((dy3 < 80.0) && (dy3 > -10))
{ if (dty3 < -2.0)
    on_num3 += 6;
    else if (dty3 < -0.7)
    on_num3 += 4;
    else if (dty3 < -0.2)
    on_num3 += 3;
    else if (dty3 < 0)
    on_num3 += 2;
}
else if (readings3 > 150)
{
    if (dy3 < 0)
        on_num3 = 0;
    else if (Get.data[2] > (0.99*readings3)) on_num3 = 0;
    else if (Get.data[2] > (0.96*readings3)) on_num3 = 2;
    else if (Get.data[2] > (0.9*readings3)) on_num3 = 3;
    else if (Get.data[2] > (0.85*readings3)) on_num3 = 4;
    else if (Get.data[2] > (0.8*readings3)) on_num3 = 5;
    else if (Get.data[2] > (0.7*readings3)) on_num3 = 6;
    else if (Get.data[2] > (0.6*readings3)) on_num3 = 7;
    else on_num3 = 8;
    if((dy3 > -1) && (dty3 < 0))
    on_num3 += 2;
}
else
{ if (dy3 < 0)
    on_num3 = 0;
    else if (Get.data[2] > (0.99*readings3)) on_num3 = 0;
    else if (Get.data[2] > (0.96*readings3)) on_num3 = 0;
    else if (Get.data[2] > (0.9*readings3)) on_num3 = 1;
    else if (Get.data[2] > (0.85*readings3)) on_num3 = 2;
    else if (Get.data[2] > (0.8*readings3)) on_num3 = 3;
    else if (Get.data[2] > (0.7*readings3)) on_num3 = 4;
    else if (Get.data[2] > (0.6*readings3)) on_num3 = 5;
    else on_num3 = 7;
    if((dy3 > -1) && (dty3 < 0))
    on_num3 += 2;
}
//on_num3 = 16;
go_num3++;  
go_num3 = go_num3 % 16;  
if ((go_num3 < on_num3) && (onandoff3))
  // if (onandoff3)
  {
    word3 = 0x04;
    SetCtrlVal (panelHandle, PANEL_LED3, ON);
  }
else
  {
    word3 = 0x00;
    SetCtrlVal (panelHandle, PANEL_LED3, OFF);
  }
// number 4
if (readings4 > 500)
{
  if (dy4 < 0 )  on_num4 = 0;
  else if (Get.data[3] > (0.999*readings4))  on_num4 = 1;
  else if (Get.data[3] > (0.998*readings4))  on_num4 = 3;
  // else if (Get.data[3] > (0.997*readings4))  on_num4 = 6;
  // else if (Get.data[3] > (0.996*readings4))  on_num4 = 8;
  // else if (Get.data[3] > (0.994*readings4))  on_num4 = 9;
  // else if (Get.data[3] > (0.992*readings4))  on_num4 = 10;
  else if (Get.data[3] > (0.99*readings4))  on_num4 = 11; /*
  else if (Get.data[3] > (0.95*readings4))  on_num4 = 13;
  else if (Get.data[3] > (0.85*readings4))  on_num4 = 14; */
else on_num4 = 16;
if((dy4 < 10.0) && (dy4 > -5))
  { if (dty4 < -1.0)
     on_num4 += 16;
    else if (dty4 < -0.7)
     on_num4 += 12;
    else if (dty4 < -0.2)
     on_num4 += 8;
  }
}
else if(readings4 > 300)
{
  if (dy4 < 0 )  on_num4 = 0;
  else if (Get.data[3] > (0.995*readings4))  on_num4 = 2;
  else if (Get.data[3] > (0.99*readings4))  on_num4 = 3;
  else if (Get.data[3] > (0.96*readings4))  on_num4 = 3;
  else if (Get.data[3] > (0.9*readings4))  on_num4 = 4;
  else if (Get.data[3] > (0.85*readings4))  on_num4 = 4;
  else if (Get.data[3] > (0.8*readings4))  on_num4 = 5;
else if (Get.data[3] > (0.7*readings4))    on_num4 = 6;
else if (Get.data[3] > (0.6*readings4))    on_num4 = 7;
else on_num4 = 8;
if((dy4 < 80.0) && (dy4 > -10))
{ if (dty4 < -2.0)
   on_num4 += 6;
   else if (dty4 < -0.7)
     on_num4 += 4;
   else if (dty4 < -0.2)
   on_num4 += 3;
   else if (dty4 < 0)
   on_num4 += 2;
}
else if ( readings4 > 150)
{ if (dy4 < 0 ) on_num4 = 0;
 else if (Get.data[3] > (0.99*readings4)) on_num4 = 0;
 else if (Get.data[3] > (0.96*readings4)) on_num4 = 2;
 else if (Get.data[3] > (0.9*readings4)) on_num4 = 3;
 else if (Get.data[3] > (0.85*readings4)) on_num4 = 4;
 else if (Get.data[3] > (0.8*readings4)) on_num4 = 5;
 else if (Get.data[3] > (0.7*readings4)) on_num4 = 6;
 else if (Get.data[3] > (0.6*readings4)) on_num4 = 7;
 else on_num4 = 8;
 if((dy4 > -1) && (dty4 < 0))
   on_num4 += 2;
}
else
{ if (dy4 < 0 ) on_num4 = 0;
 else if (Get.data[3] > (0.99*readings4)) on_num4 = 0;
 else if (Get.data[3] > (0.96*readings4)) on_num4 = 0;
 else if (Get.data[3] > (0.9*readings4)) on_num4 = 1;
 else if (Get.data[3] > (0.85*readings4)) on_num4 = 2;
 else if (Get.data[3] > (0.8*readings4)) on_num4 = 3;
 else if (Get.data[3] > (0.7*readings4)) on_num4 = 4;
 else if (Get.data[3] > (0.6*readings4)) on_num4 = 5;
 else on_num4 = 7;
 if((dy4 > -1) && (dty4 < 0))
   on_num4 += 2;
}
//on_num4 = 16;
go_num4++;
go_num4 = go_num4 % 16;
if ((go_num4 < on_num4) &&(onandoff4))
// if (onandoff4)
    
    {  
        word4=0x08;  
        SetCtrlVal (panelHandle, PANEL_LED4, ON);  
    }  
else
    
    {  
        word4=0x00;  
        SetCtrlVal (panelHandle, PANEL_LED4, OFF);  
    }

// number 5
if(readings5 > 500)

    {  
        if (dy5 < 0 ) on_num5 = 0;  
        else if (Get.data[4] > (0.999*readings5)) on_num5 = 1;  
        else if (Get.data[4] > (0.998*readings5)) on_num5 = 3;  
        /* else if (Get.data[4] > (0.997*readings5)) on_num5 = 5;  
        // else if (Get.data[4] > (0.996*readings5)) on_num5 = 7;  
        // else if (Get.data[4] > (0.994*readings5)) on_num5 = 10;  
        // else if (Get.data[4] > (0.992*readings5)) on_num5 = 13;  
        else if (Get.data[4] > (0.9*readings5))  on_num5 = 14; */  
        else if (Get.data[4] > (0.95*readings5)) on_num5 = 10;  
        else on_num5 = 16;  
    }  
else if(readings5 > 300)

    {  
        if (dy5 < 0 )   on_num5 = 0;  
        else if (Get.data[4] > (0.995*readings5)) on_num5 = 2;  
        else if (Get.data[4] > (0.99*readings5)) on_num5 = 3;  
        else if (Get.data[4] > (0.96*readings5)) on_num5 = 3;  
        else if (Get.data[4] > (0.9*readings5)) on_num5 = 4;  
        else if (Get.data[4] > (0.85*readings5)) on_num5 = 4;  
        else if (Get.data[4] > (0.8*readings5)) on_num5 = 5;  
        else if (Get.data[4] > (0.7*readings5)) on_num5 = 6;  
        else if (Get.data[4] > (0.6*readings5)) on_num5 = 7;  
        else on_num5 = 8;  
}
if((dy5 < 80.0) && (dy5 > -10))
{ if (dty5 < -2.0)
    on_num5 += 6;
else if (dty5 < -0.7)
    on_num5 += 4;
else if (dty5 < -0.2)
    on_num5 += 3;
else if (dty5 < 0)
    on_num5 += 2;
}
else if (readings5 > 150)
{
    if (dy5 < 0 )   on_num5 = 0;
else if (Get.data[4] > (0.99*readings5)) on_num5 = 0;
else if (Get.data[4] > (0.96*readings5)) on_num5 = 2;
else if (Get.data[4] > (0.9*readings5)) on_num5 = 3;
else if (Get.data[4] > (0.85*readings5)) on_num5 = 4;
else if (Get.data[4] > (0.8*readings5)) on_num5 = 5;
else if (Get.data[4] > (0.7*readings5)) on_num5 = 6;
else if (Get.data[4] > (0.6*readings5)) on_num5 = 7;
else on_num5 = 8;
    if((dy5 > -1) && (dty5 < 0))
        on_num5 += 2;
}
else
{
    if (dy5 < 0 )   on_num5 = 0;
else if (Get.data[4] > (0.99*readings5)) on_num5 = 0;
else if (Get.data[4] > (0.96*readings5)) on_num5 = 0;
else if (Get.data[4] > (0.9*readings5)) on_num5 = 1;
else if (Get.data[4] > (0.85*readings5)) on_num5 = 2;
else if (Get.data[4] > (0.8*readings5)) on_num5 = 3;
else if (Get.data[4] > (0.7*readings5)) on_num5 = 4;
else if (Get.data[4] > (0.6*readings5)) on_num5 = 5;
else on_num5 = 7;
    if((dy5 > -1) && (dty5 < 0))
        on_num5 += 2;
}
// on_num5 = 16;
go_num5++;
go_num5 = go_num5 % 16;
if ((go_num5 < on_num5) && (onandoff5))
    // if (onandoff5)
{ word5=0x10;
SetCtrlVal (panelHandle, PANEL_LED5, ON);
}
else
{
{ word5=0x00;
SetCtrlVal (panelHandle, PANEL_LED5, OFF);
}

// number 6
if(readings6 > 500)
{
if (dy6 < 0 ) on_num6 = 0;
else if (Get.data[5] > (0.999*readings6)) on_num6 = 1;
else if (Get.data[5] > (0.998*readings6)) on_num6 = 3;
else if (Get.data[5] > (0.997*readings6)) on_num6 = 5;
else if (Get.data[5] > (0.996*readings6)) on_num6 = 7;
else if (Get.data[5] > (0.994*readings6)) on_num6 = 9;
else if (Get.data[5] > (0.992*readings6)) on_num6 = 10;
else if (Get.data[5] > (0.99*readings6)) on_num6 = 11; /*
else if (Get.data[5] > (0.95*readings6)) on_num6 = 10; /*
else if (Get.data[5] > (0.9*readings6)) on_num6 = 13; /*
else if (Get.data[5] > (0.75*readings6)) on_num6 = 14; /*
else on_num6 = 16;
if((dy6 < 10.0) && (dy6 > -5))
{ if (dty6 < -1.0)
    on_num6 += 16;
    else if (dty6 < -0.7)
    on_num6 += 12;
    else if (dty6 < -0.2)
    on_num6 += 8;
}
}
else if(readings6 > 300)
{
if (dy6 < 0 ) on_num6 = 0;
else if (Get.data[5] > (0.995*readings6)) on_num6 = 2;
else if (Get.data[5] > (0.99*readings6)) on_num6 = 3;
else if (Get.data[5] > (0.96*readings6)) on_num6 = 3;
else if (Get.data[5] > (0.9*readings6)) on_num6 = 4;
else if (Get.data[5] > (0.85*readings6)) on_num6 = 4;
else if (Get.data[5] > (0.8*readings6)) on_num6 = 5;
else if (Get.data[5] > (0.7*readings6)) on_num6 = 6;
else if (Get.data[5] > (0.6*readings6)) on_num6 = 7;
else on_num6 = 8;
if((dy6 < 80.0) && (dy6 > -10))
{
    if (dty6 < -2.0)
        on_num6 += 6;
    else if (dty6 < -0.7)
        on_num6 += 4;
    else if (dty6 < -0.2)
        on_num6 += 3;
    else if (dty6 < 0)
        on_num6 += 2;
}
else if (readings6 > 150)
{
    if (dy6 < 0 )   on_num6 = 0;
    else if (Get.data[5] > (0.99*readings6))    on_num6 = 0;
    else if (Get.data[5] > (0.96*readings6))    on_num6 = 2;
    else if (Get.data[5] > (0.9*readings6))     on_num6 = 3;
    else if (Get.data[5] > (0.85*readings6))    on_num6 = 4;
    else if (Get.data[5] > (0.8*readings6))     on_num6 = 5;
    else if (Get.data[5] > (0.7*readings6))     on_num6 = 6;
    else if (Get.data[5] > (0.6*readings6))     on_num6 = 7;
    else on_num6 = 8;
    if((dy6 > -1)&&(dty6 < 0))
        on_num6 += 2;
}
else
{
    if (dy6 < 0 )   on_num6 = 0;
    else if (Get.data[5] > (0.99*readings6))    on_num6 = 0;
    else if (Get.data[5] > (0.96*readings6))    on_num6 = 0;
    else if (Get.data[5] > (0.9*readings6))     on_num6 = 1;
    else if (Get.data[5] > (0.85*readings6))    on_num6 = 2;
    else if (Get.data[5] > (0.8*readings6))     on_num6 = 3;
    else if (Get.data[5] > (0.7*readings6))     on_num6 = 4;
    else if (Get.data[5] > (0.6*readings6))     on_num6 = 5;
    else on_num6 = 7;
    if((dy6 > -1)&&(dty6 < 0))
        on_num6 += 2;
}
// on_num6 = 16;
go_num6++;
go_num6 = go_num6 % 16;
if ((go_num6 < on_num6) &&(onandoff6))
    // if (onandoff6)
    {
}
word6=0x20;
SetCtrlVal (panelHandle, PANEL_LED6, ON);
}
else
{
  word6=0x00;
  SetCtrlVal (panelHandle, PANEL_LED6, OFF);
}
GetCtrlVal (panelHandle, PANEL_FAN, &fan);
if (fan) word7=0x40;
else word7=0x00;
/* word5=word6>>1;
word4=word6>>2;
word3=word6>>3;
word2=word6>>4;
word1=word6>>5; */
//sent_word = 0x42;
if (Get.data[9]>80)
{
  sent_word = 0x40;
  SetCtrlVal (panelHandle, PANEL_ALARM, ON);
  SetCtrlVal (panelHandle, PANEL_LED1, OFF);
  SetCtrlVal (panelHandle, PANEL_LED2, OFF);
  SetCtrlVal (panelHandle, PANEL_LED3, OFF);
  SetCtrlVal (panelHandle, PANEL_LED4, OFF);
  SetCtrlVal (panelHandle, PANEL_LED5, OFF);
  SetCtrlVal (panelHandle, PANEL_LED6, OFF);
}
else
{
  sent_word = word1 | word2 | word3 | word4 | word5 | word6 | word7;
  SetCtrlVal (panelHandle, PANEL_ALARM, OFF);
}
Fmt(sent_word1,"%s<%i %i %i %i %i %i %i",sent_word, on_num1, on_num2, on_num3, on_num4, on_num5, on_num6, go_num6);
SetCtrlVal (panelHandle, PANEL_TEST, sent_word1);
outp(LPT1,sent_word);
}
int CVICALLBACK Change_display (int panel, int control, int event,
void *callbackData, int eventData1, int eventData2)
{
  switch (event) {
    case EVENT_COMMIT:
      break;
  }
  return 0;
}
int CVICALLBACK set_wave (int panel, int control, int event,
void *callbackData, int eventData1, int eventData2)
{
    double wave;
    char TEMPSTR[7];
    switch (event) {
    case EVENT_COMMIT:
        GetCtrlVal(setHandle, SET_RING1, &wave);
        SetCtrlVal(setHandle, SET_SETPOINT, decalib(wave));
        break;
    }
    return 0;
}

int CVICALLBACK set_wave1 (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2)
{
    double wave;
    switch (event) {
    case EVENT_COMMIT:
        GetCtrlVal(setHandle, SET_RING2, &wave);
        SetCtrlVal(setHandle, SET_SETPOINT_2, decalib(wave));
        break;
    }
    return 0;
}

int CVICALLBACK set_wave2 (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2)
{
    double wave;
    switch (event) {
    case EVENT_COMMIT:
        GetCtrlVal(setHandle, SET_RING3, &wave);
        SetCtrlVal(setHandle, SET_SETPOINT_3, decalib(wave));
        break;
    }
    return 0;
}

int CVICALLBACK set_wave3 (int panel, int control, int event,
    void *callbackData, int eventData1, int eventData2)
{
    double wave;
    switch (event) {
    case EVENT_COMMIT:
        GetCtrlVal(setHandle, SET_RING4, &wave);
        SetCtrlVal(setHandle, SET_SETPOINT_4, decalib(wave));
        break;
    }
    return 0;
}

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int CVICALLBACK set_wave4 (int panel, int control, int event, 
    void *callbackData, int eventData1, int eventData2) 
{
    double wave;
    switch (event) {
    case EVENT_COMMIT:
        GetCtrlVal (setHandle, SET_RING5, &wave);
        SetCtrlVal(setHandle, SET_SETPOINT_5, decalib(wave));
        break;
    }
    return 0;
}

int CVICALLBACK set_wave5 (int panel, int control, int event, 
    void *callbackData, int eventData1, int eventData2) 
{
    double wave;
    switch (event) {
    case EVENT_COMMIT:
        GetCtrlVal (setHandle, SET_RING6, &wave);
        SetCtrlVal(setHandle, SET_SETPOINT_6, decalib(wave));
        break;
    }
    return 0;
}

int CVICALLBACK timer3_control (int panel, int control, int event, 
    void *callbackData, int eventData1, int eventData2) 
{
    char save_fmt[30];
    char sent_word2[200];
    switch (event) {
    case EVENT_TIMER_TICK:
        // SetSystemAttribute
        (ATTR_ALLOW_UNSAFE_TIMER_EVENTS, 1);
        if (goflag2 ==1)
        {
        // Fmt(sent_word2,"%s<%f[p50]",Rbuf);
            SetCtrlVal (panelHandle, PANEL_TEST_2, Rbuf);
            if (choice1)
            {
%f[p2]  %f[p2]" , datanum, Get.data[0], 
            Get.data[1],Get.data[2],Get.data[3],Get.data[4],Get.data[5],Get.data[6]);

    221
} else
{
}
InsertListItem (panelHandle, PANEL_TEMP_LIST, -1, buf, 0);
GetNumListItems (panelHandle, PANEL_TEMP_LIST, &index_num);
SetCtrlIndex (panelHandle, PANEL_TEMP_LIST, index_num-1);
GetCtrlVal (graphHandle, GRAPH_BINARYSWITCH, &read);
graphdata7[0] = Get.data[0];
graphdata7[1] = Get.data[2];
graphdata7[2] = Get.data[3];
graphdata7[3] = Get.data[9];
graphdata7[4] = Get.data[8];
if (read == 0)
{
    graphdata1[0] = calib(Get.data[0]);
    graphdata2[0] = calib(Get.data[1]);
    graphdata3[0] = calib(Get.data[2]);
    graphdata4[0] = calib(Get.data[3]);
    graphdata5[0] = calib(Get.data[4]);
    graphdata6[0] = calib(Get.data[5]);
    graphdata1[1] = calib(readings1);
    graphdata2[1] = calib(readings2);
    graphdata3[1] = calib(readings3);
    graphdata4[1] = calib(readings4);
    graphdata5[1] = calib(readings5);
    graphdata6[1] = calib(readings6);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH1, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH2, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH3, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH4, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH5, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH6, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH1_1, ATTR_VISIBLE, 1);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH2_1, ATTR_VISIBLE, 1);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH3_1, ATTR_VISIBLE, 1);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH4_1, ATTR_VISIBLE, 1);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH5_1, ATTR_VISIBLE, 1);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH6_1, ATTR_VISIBLE, 1);
if (choice1)
{
    SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH5_1, ATTR_VISIBLE, 0);
    SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH6_1, ATTR_VISIBLE, 0);
if(dim1) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH1_1, graphdata1, 2, 0, 0, VAL_DOUBLE);
if(dim2) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH2_1, graphdata2, 2, 0, 0, VAL_DOUBLE);
if(dim3) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH3_1, graphdata3, 2, 0, 0, VAL_DOUBLE);
if(dim4) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH4_1, graphdata4, 2, 0, 0, VAL_DOUBLE);
if(dim5) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH5_1, graphdata5, 2, 0, 0, VAL_DOUBLE);
if(dim6) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH6_1, graphdata6, 2, 0, 0, VAL_DOUBLE);
PlotStripChart (panel Handle, PANEL_TEMP_GRAPH7, graphdata7, 2, 0, 0, VAL_DOUBLE); /*Kadir added if(dim7) - */
}
else
{
  graphdata1[0] = Get.data[0];
  graphdata2[0] = Get.data[1];
  graphdata3[0] = Get.data[2];
  graphdata4[0] = Get.data[3];
  graphdata5[0] = Get.data[4];
  graphdata6[0] = Get.data[5];
  graphdata1[1] = readings1;
  graphdata2[1] = readings2;
  graphdata3[1] = readings3;
  graphdata4[1] = readings4;
  graphdata5[1] = readings5;
  graphdata6[1] = readings6;
  SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH1, ATTR_VISIBLE, 1);
  SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH2, ATTR_VISIBLE, 1);
  SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH3, ATTR_VISIBLE, 1);
  SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH4, ATTR_VISIBLE, 1);
  SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH5, ATTR_VISIBLE, 1);
  SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH6, ATTR_VISIBLE, 1);
  if (choice1)
  {
    SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH5, ATTR_VISIBLE, 0);
  }
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH6, ATTR_VISIBLE, 0);
}
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH1_1, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH2_1, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH3_1, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH4_1, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH5_1, ATTR_VISIBLE, 0);
SetCtrlAttribute(graphHandle, GRAPH_TEMP_GRAPH6_1, ATTR_VISIBLE, 0);
if(dim1) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH1, graphdata1,
            2, 0, 0, VAL_DOUBLE);
if(dim2) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH2, graphdata2,
            2, 0, 0, VAL_DOUBLE);
if(dim3) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH3, graphdata3,
            2, 0, 0, VAL_DOUBLE);
if(dim4) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH4, graphdata4,
            2, 0, 0, VAL_DOUBLE);
if(dim5) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH5, graphdata5,
            2, 0, 0, VAL_DOUBLE);
if(dim6) PlotStripChart (graphHandle, GRAPH_TEMP_GRAPH6, graphdata6,
            2, 0, 0, VAL_DOUBLE);
PlotStripChart (panelHandle, PANEL_TEMP_GRAPH7, graphdata7,
            5, 0, 0, VAL_DOUBLE);
}
datapoints[0][datanum] = Get.data[0];
datapoints[1][datanum] = Get.data[1];
datapoints[2][datanum] = Get.data[2];
datapoints[3][datanum] = Get.data[3];
datapoints[4][datanum] = Get.data[4];
datapoints[5][datanum] = Get.data[5];
datapoints[6][datanum] = Get.data[6];
datapoints[7][datanum] = Get.data[7];
datapoints[8][datanum] = Get.data[8];
datapoints[9][datanum] = Get.data[9];
datapoints[10][datanum] = Get.data[10];
datapoints[12][datanum] = Get.data[12];
GetCtrlVal (panelHandle, PANEL_HEATING, &sign);
GetCtrlVal (panelHandle, PANEL_COMPARISON, &save1);
if (sign == 1)
{
  sum=Get.data[4]-datapoints[4][0];
  save=save + sum;
  Fmt(save_fmt, "%s<%f", save);
  SetCtrlVal (panelHandle, PANEL_TEST2, save_fmt);
}
if ((save1 !=0)&(&(save1 - save)<1))
```c
{ 
goflag=0;
goflag1=0;
goflag2=0;
SetCtrlVal (panelHandle, PANEL_HEATING,0);
//SetCtrlAttribute(panelHandle,PANEL_STOPBUTTON,ATTR_VISIBLE,1);
//SetCtrlAttribute(panelHandle,PANEL_GOBUTTON,ATTR_VISIBLE,0);
}
datnum++;
SetCtrlVal (panelHandle, PANEL_LED, OFF);
}
break;
}
return 0;
}
int CVICALLBACK fan_control (int panel, int control, int event,
   void *callbackData, int eventData1, int eventData2)
{
   switch (event) {
   case EVENT_COMMIT:
      if (end)
      { 
         GetCtrlVal (panelHandle, PANEL_FAN, &fan);
         if (fan) word=0x40;
         else word= 0x00;
         outp(LPT1,word);
      } else
      break;
   }
   return 0;
}
int CVICALLBACK close_graph (int panel, int control, int event,
   void *callbackData, int eventData1, int eventData2)
{
   switch (event) {
   case EVENT_COMMIT:
      HidePanel (graphHandle);
   break;
   }
   return 0;
}
int CVICALLBACK display_graph (int panel, int control, int event,
   void *callbackData, int eventData1, int eventData2)
{
   switch (event) {
   case EVENT_COMMIT:
      DisplayPanel (graphHandle);
   break;
   }
   return 0;
}
```
Vita
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EDUCATION

- Pennsylvania State University, University Park, PA (2002-2005).
  - Doctor of Philosophy in Agricultural & Biological Engineering (Expected: Summer 2006) - GPA 3.73/4.00.
  - Master of Science in Agricultural & Biological Engineering - GPA 3.55/4.00.
- Tamil Nadu Agricultural University, Tamil Nadu, India (1995-1999).
  - Bachelor of Engineering in Agricultural Engineering - GPA 8.84/10.00.

SELECTED AWARDS AND HONORS

- Annual graduate exhibition, Pennsylvania State University
  - Third place, 2005; First place, 2003
- Annual College of Agricultural Sciences, Gamma Sigma Delta Undergraduate and Graduate Research Expo, Pennsylvania State University
  - Third place, 2005; First place, 2004; Gerald T. Gentry award, 2002
- Outstanding paper presentation award, Evans family lecture for graduate research, College of Agricultural Sciences, Pennsylvania State University, April 14, 2004.

PEER REVIEWED PUBLICATIONS

- Krishnamurthy, K. and A. Demirci. 2006. Disinfection of water through flow-through ultraviolet light disinfection system. Ultrapure Water (In review)