U. S. Food and Drug Administration
Center for Food Safety and Applied Nutrition
June 2, 2000

Kinetics of Microbial Inactivation for Alternative Food Processing Technologies

Ultraviolet Light

(Table of Contents)

Scope of Deliverables

This section covers information, where available, on microbial kinetics and relevant safety considerations when ultraviolet (UV) radiation is used for the purposes of food preservation. The section pays particular attention to the growing interest in using UV light to treat fruit juices, especially apple juice and cider. It does not include pulsed UV, which is being actively investigated by Dr. Lagunas-Solar and co-workers at the University of Calif., Davis, nor does it address combinations of other alternative processing technologies with UV processing.

1. Definition, Description and Applications

Ultraviolet processing involves the use of radiation from the ultraviolet region of the electromagnetic spectrum for purposes of disinfection. Typically, the wavelength for UV processing ranges from 100 to 400 nm. This range may be further subdivided (Bolton 1999) into UVA (315 to 400 nm) normally responsible for changes in human skin that lead to tanning; UVB (280 to 315 nm) that can cause skin burning and eventually lead to skin cancer; UVC, (200 to 280 nm) called the germicidal range since it effectively inactivates bacteria and viruses, and the vacuum UV range (100 to 200 nm) that can be absorbed by almost all substances and thus can be transmitted only in a vacuum. The germicidal properties of UV irradiation are mainly due to DNA mutations induced through absorption of UV light by DNA molecules. This mechanism of inactivation results in a sigmoidal curve of microbial population reduction.

To achieve microbial inactivation, the UV radiant exposure must be at least 400 J/m^2 in all parts of the product. Critical factors include the transmissivity of the product, the geometric configuration of the reactor, the power, wavelength and physical arrangement of the UV source(s), the product flow profile, and the radiation path length. UV may be used in combination with other alternative processing technologies, including various powerful oxidizing agents such as ozone and hydrogen peroxide, among others.

Applications include disinfection of water supplies and food contact surfaces. Recently, interest has increased in using UV to reduce microbial counts in juices.

2. Pathogens of Public Health Concern

2.1. Pathogens of Concern in Apple Juice and Cider

Escherichia coli O157:H7 is a nonsporeforming bacterium that has been implicated in numerous foodborne illness outbreaks of contaminated, raw apple cider. *Cryptosporidium parvum* is a protozoan parasite that has the capability of forming oocysts and has also been implicated as the causative agent in a foodborne illness outbreak of apple cider in New York State.

2.2. Shape of Inactivation Curve

The shape of the curve for microbial inactivation by UV light is sigmoidal. The initial plateau is due to an injury phase of the microorganism in response to UV exposure. After the initial plateau, the maximum amount of injury has been surpassed; thus, minimal additional UV exposure would be lethal for microorganisms and survivor numbers rapidly decline. The end of the curve has a tailing phase due to UV resistance of the microorganisms and to experimental components, such as suspended solids that may block the UV irradiation.

Much of the prior literature has focused on UV disinfection of water supplies. The literature is insufficient to develop comprehensive microbial inactivation reaction kinetics data or models. There are, however, studies relative to the UV radiant exposure required to obtain a 4-log reduction of various microorganisms, using 254 nm UV light (Table 1). These data suggest that the log reduction is related to the UV radiant exposure (J/m^2). The curve has a linear section with a shoulder and tailing effects (Hoyer 1998). All tested organisms were reduced by 4-log cycles with UV radiant exposure less than 400 J/m^2 (Hoyer 1998). With some bacterial cells, photoreactivation, a repair mechanism that is enhanced by visible light in the blue spectral range, may occur. Photoreactivated cells show greater resistance to UV radiation than non-reactivated cells (Table 1).

Table 1. UV 254 nm radiant exposure (J/m^2) for 4-log reduction (from Hoyer 1998)

Microorganism	Exposure required without photoreactivation (J/m^2)	Exposure required with photoreactivation (J/m^2)
Escherichia coli ATCC 11229	100	280
E. coli ATCC 23958	50	200
E. coli NCTC 5934	90	215
E. coli NCIB 9481	100	180

E. coli wild isolate	110	270
Enterobacter cloacae	100	330
Klebsiella pneumoniae	110	310
Citrobacter freundii	80	250
Yersinia enterocolitica	100	320
Salmonella Typhi	140	190
Salmonella Typhimurium	130	250
Serratia marcescens	130	300
Enterocolitica faecium	170	200
Vibrio cholerae wild isolate	50	210
Pseudomonas aeruginosa	110	190
Mycobacterium smegmatis	200	270
Polio virus (Mahoney)	290	
Rotavirus SA 11	350	
Staphylococcus aureus phage A994	380	

3. Mechanisms of Microbial Inactivation

3.1. Pathogen Culture Maintenance and Growth

The *E. coli* strains were kept in 30% glycerol at "80 0C and, when needed, were inoculated onto Tryptic Soy Agar, grown at 37 0C for 18 h with 250 rpm shaking agitation that provided the necessary aeration for good growth. The inoculation procedure was repeated 3 times before use (Worobo 1998).

3.2. Microbial Enumeration Conditions and Methods

Apple cider was inoculated with *E. coli* O157:H7 to achieve an initial level of approximately 7- to 8-logs cfu/ml. High inoculum levels are required to ascertain a 5-log or greater reduction in the target pathogen. The initial levels of *E. coli* O157:H7 were determined and then cider was processed through the CiderSure machine. Enumeration of

aseptically drawn samples was done with Tryptic Soy Agar and incubation at 37 0C for 24 to 48 h (Worobo 2000). The primary reference used in identifying these standard enumeration methods is: Compendium of Methods for the Microbiological Examination of Foods, Third Edition. 1992. Edited by: Carl Vanderzant, Ph.D. and Don F. Splittstoesser, Ph.D. compiled by the American Public Health Association (APHA) Technical committee on Microbiological Methods for Foods

3.3. Inactivation Mechanism(s)

The germicidal properties of ultraviolet irradiation are due to the DNA absorption of the UV light, causing crosslinking between neighboring pyrimidine nucleoside bases (thymine and cytosine) in the same DNA strand (Miller and others 1999). Due to the mutated base, formation of the hydrogen bonds to the purine bases on the opposite strand is impaired. DNA transcription and replication is thereby blocked, compromising cellular functions and eventually leading to cell death. The amount of crosslinking is proportional to the amount of UV exposure. The level of mutations that can be reversed depends on the UV repair system present in the target microorganism. Once the threshold of crosslinking has been exceeded, the number of crosslinks is beyond repair, and cell death occurs (Miller and others 1999). This phenomenon is reflected in the shape of the inactivation curve described before. The crosslinking threshold corresponds to the point of rapid decline after the initial plateau phase on the sigmoidal survival plot of UV exposure.

3.4. Methods to Measure, Quantify or Mathematically Model Pathogen Inactivation

Ultraviolet light from germicidal lamps was exposed to inoculated apple cider by passing it through the processing tube at a constant flow rate. The UV exposure was monitored using a sensor placed on the outermost exterior wall of the inner surface of the flow chamber. The D-value of *E. coli* O157:H7 due to ultraviolet exposure was calculated by increasing the exposures in the same apple cider. *Escherichia coli* O157:H7 survival was determined for each successive level of UV exposure and D-value was extrapolated from this multiple point data (Worobo, 2000).

4. Validation/Critical Process Factors

4.1. Identification and Description of Critical Process Factors

Little data is available on critical process factors affecting microbial inactivation. Pressure, temperature, and pH of the medium appear to have little effect on the absorption properties. Product composition, solids content, color, starches, and the overall chemistry of the food have a major effect. The effects of these individual factors are not available.

For disinfection of water, it is essential that all parts (each volume element) of the product receive a UV radiant exposure of at least 400 J/m^2 (at 254 nm) to reduce human pathogens and virus by at least 4-log cycles (Bernhardt 1994). Thus, the homogeneity of

the flow pattern and the radiation field may have critical effects on disinfection. An additional critical factor is the transmissivity of the material being disinfected. If the material is highly transparent to UV light, disinfection may be more effective; however, turbid materials would attenuate and scatter UV radiation, resulting in less microbial inactivation. The thickness of the radiation path through the materials is also of importance, since attenuation increases with the length of passage; thus, the geometric configuration of process systems is critical. Another critical factor to consider is the UV wavelength used since this will affect microbial inactivation.

It is important to note some of the key terms used in the UV literature. The common expressions of irradiance, fluence rate, and fluence, and their distinction (Bolton 1999), are defined in the Glossary. It is important to point out that fluence rate refers to power passing through a sphere, while irradiance refers to power passing through a surface. For a parallel and perpendicularly incident beam, not scattered or reflected, irradiance and fluence rate become identical. For any UV source within a 3-dimensional volume, the integration of UV irradiance over the interior surface of the volume yields the UV power of the lamp. This is not true for UV fluence rate. The appropriate term for UV disinfection is "UV fluence rate" because a microorganism can receive UV power from any direction, especially when multiple lamps are used. The light dose or fluence is the total radiant energy of all wavelengths passing from all directions through an infinitesimally small sphere. It is given by the average fluence rate times the exposure time in s. The term "UV dose" is often used in UV disinfection literature and represents UV exposure of a given organism in the germicidal range.

It may be noted that although Bolton (1999) prefers the use of UV fluence rate, the term "irradiance" is commonly used in the water disinfection literature. Indeed, it appears that in some of the German standards the word irradiance was used (Hoyer 1998). Nevertheless, the use of the more technically accurate concept of UV fluence rate is preferred.

4.2. Describe Methods to Measure/Monitor Critical Process Factors

In one system, sensors placed at a precise depth in the fluid stream measure the actual power through the material. The unit is programmed to deliver the same energy level to the material and, therefore, adjusts the exposure time to achieve the proper energy levels. In addition, if the material□s absorption is very high, the unit does not operate.

UV irradiance may be monitored by radiometers, either thermal or photonic, UV sensors, or actinometer; however, the most reliable method is still biodosimetry, where the sample is inoculated with a surrogate microorganism and log reductions are counted at the outlet. The protocols for monitoring many of the other critical factors are not yet in place, and little or no information exists about them.

Detailed considerations monitoring UV radiant exposure have been described by Hoyer (1998) and are described below. Specific reference standards are in place in Germany for water disinfection.

To verify that UV radiant exposure exceeds 400 J/m^2 during operation requires a link that assures the conditions approved as sufficient by the biodosimetric test are met. This includes flow control, achieved with calibrated UV selective monitoring sensors mounted in a port at a defined position of the UV system. In a specified range of flow, the UV irradiance (W/m^2) at that port must be kept above the minimum level of the identical prototype during the biodosimetric test. It must allow for independent control with a reference sensor. This monitoring ensures that changes which may reduce the disinfection effect are detected and measures to handle insufficient disinfection are taken.

UV sensors must be standardized. For independent control, a single type of reference sensor should monitor the irradiance for every UV system. This includes a standardized size for a measuring port enclosed with a quartz glass panel of greater than 90 percent UV transparency. The manufacturer decides whether to use the standard port for system monitoring or an independent system sensor in the equivalent position, provided the signal is the same compared with a reference sensor. Prerequisites for a reproducible measurement of UV irradiance are a defined opening angle, spectral selectivity, and a defined physical size of UV sensors. These are detailed within German standards (DVGW 1997).

Because the mercury arc also emits radiation of higher wavelength, UV sensors must have a spectral selectivity radiation of 240-290 nm. Sensors sensitive to longer wavelengths would not detect the decay of microbiocidic radiation with sufficient liability because spectral absorption of FeO(OH), $MnO(OH)_2$, and humic acids decreases with increasing wavelength and because aging of UV lamps predominantly occurs in the low wavelength range, leaving the long-range radiation intensity almost unchanged. Therefore, radiation above 290 nm on stock contributes to more than 10 percent of the sensors signal. Calibration of sensors for irradiance in W/m^2 is standardized at 253.7 nm, the resonant band of mercury. This standardization is done by actinometry with uridine, via a transfer standard calibrated by a national standardization body. The advantage of uridine is that its spectral absorption is nearly identical with the DNA. Radiant exposure is simply determined using a common laboratory spectrophotometer.

The sensor port position must also allow for the detection of water transmission changes to nearly the same degree as the decrease of irradiation from aging or deposits. The position depends on individual construction of UV units and is tested with changing UV lamps and changing the UV transmission properties of the water by adding a UV dye. Along with this procedure the sensitivity and spectral selectivity of the sensor is verified by comparison with the reference system.

Because one sensor can only monitor 1 or 2 lamps, in systems with several lamps all UV lamps need to be monitored through electrical parameters. Additionally, all UV lamps in a system unit must be of the same age and quality and the quartz of the glass panel must be guaranteed by the manufacturer and documented through identification marks.

4.3. Description of Microbial or Chemical Surrogates/Indicators

Escherichia coli ATCC 25922 shows similar ultraviolet irradiation sensitivity to *E. coli* O157:H7 and therefore would seem like an appropriate surrogate microorganism. For the validation work, apple cider would be inoculated with the surrogate microorganism at high levels to determine the inactivation effect of UV on the surrogate microorganism. The validations were repeated in triplicate and the microbiological plating performed in duplicate (Worobo, 2000).

Hoyer (1998) describes the following set of recommendations for testing drinking water systems. According to Hoyer (1998) the minimum required irradiance (W/m^2) should be tested by biodosimetry measured by the system sensor (and in parallel with the reference sensor) at the minimum and maximum throughput as declared by the manufacturer. The biodosimetric test with minimum flow is necessary due to the incomplete mixing expected under laminar flow. The most important features of the biodosimetry test are the mixing devices before and after the UV unit, proper mixing to better than 99% of added germs, and representative sampling.

The disinfection performance at the minimum required irradiance at the sensor port of the UV system is tested in 2 ways (Hoyer 1998):

1. At the lowest 254 nm spectral absorption coefficient of water (less than or equal to 1 1/m), reducing the UV output of the lamps to the minimum required irradiance.

2. At full-lamp power and higher UV absorption of the water by adding a UV dye until the minimum required irradiance is read from the sensor.

To test both sets of conditions (that is, effects of varying lamp power or increasing UV absorption in the medium), a 4-log reduction with proper hydraulics and a 400 J/m^2 UV radiant exposure should be achieved. An ideal surrogate microorganism should have a 5-log reduction at 400 J/m^2. At present, such a germ is not available. Therefore a split test with 2 different bacteria is performed. Germs of *E. coli* ATCC 11229 being too sensitive to test for 400 J/m^2 but suitable to test for hydraulics, and spores of *Bacillus subtilis* ATCC 6633 (2.5-log reduction at 400 J/m^2) being too insensitive to test for hydraulics but suitable to test for 400 J/m^2 are used.

5. Process Deviations

Process deviations in this technology are equipment-specific. Since no standard equipment design currently exists, the character of the deviation and the corrective action cannot be characterized in a simple manner within the scope of this document; however, the procedures described by Hoyer (1998) would appear to provide some hints.

To provide the required dosage, adjustment of the exposure time is done by computers linked to sensors. The scan rate is about 20 ms. This information is used for varying lamp power and/or changes in the absorption. UV irradiance measurement should be done at various points in the system, but protocols are not available at this time.

When the UV radiant exposure drops below a particular value, as described by the above descriptions, the product would have to be diverted and reprocessed or the system shut down.

6. Research Needs

Research that needs to be addressed includes:

- Effects of individual parameters, such as suspended and dissolved solids concentration.
- Identification of the pathogens most resistant to UV light
- Identification of surrogate microorganisms for pathogens.
- Development of validation methods to ensure microbiological effectiveness.
- Development and evaluation of kinetic models.
- Studies to optimize critical process factors.

GLOSSARY

Fluence rate, Eo. The radiant power of all wavelengths passing from all directions through an infinitesimally small sphere of cross-sectional area dA, divided by dA (W/m^2).

Irradiance, E. The total radiant power of wavelengths incident on an infinitesimal element of surface area dS containing the point under consideration divided by dS. Note that for the receipt of light, "irradiance" is the counterpart to "emittance" for the emission of light (W/m^2).

Light dose, fluence, or UV radiant exposure, H. The total radiant energy of all wavelengths passing from all directions through an infinitesimally small sphere of cross-sectional area dA divided by dA. It is given by the average fluence rate times the exposure time in s. The term UV dose is often used in UV disinfection literature. It represents UV exposure of a given organism in the germicidal range (J/m^2).

REFERENCES

Anonymous. 1999. UV light provides alternative to heat pasteurization of juices. Food Technol. 53(9):144

Bernhardt, H. 1994. Desinfektion aufbereiteter oberflachenwasser mit UV-Strahlen. Wasser-Abwasser. 135(12):677-689

Bolton, J. R. 1999. Ultraviolet Applications Handbook. Bolton Photosciences, Inc. Ayr, ON, CA

N0B 1E0.

DVGW Arbeitsblatt W 294, 1997. Desinfektionsanlagen fur die Trinkwasserversorgung -- Anforderungen und Prufung. Bonn.

Hoyer, O. 1998. Testing performance and monitoring of UV systems for drinking water disinfection. Water Supply. 16(1/2):419-442

IUVA. 2000. IUVA Website [International UltraViolet Association]. 2000. http://www.iuva.org

Miller, R., Jeffrey, W., Mitchell, D. and Elasri, M. 1999. Bacterial responses to ultraviolet light. Am Soc Microbiol. 65(8):535-541

Worobo, R. W., Churey, J. J. and Padilla-Zakour, O. 1998. Apple cider: treatment options to comply with new regulations. J Assoc Food Drug Officials. December:19-25

Worobo, R. W. 2000. Efficacy of the CiderSure 3500 ultraviolet light unit in apple cider. Ithaca, NY. Cornell University, Department of Food Science and Technology. 1-6.

Contacts

Randy Worobo, Ph.D.
Assistant Professor
Cornell University
New York State Agricultural Experiment StationW. North Street
Geneva, NY 14456

Phil Hartman
FPE Inc.
2453 Canandaigua Road
Macedon, NY 14502

Table of Contents

Home | HACCP

Hypertext updated by ear 2001-JAN-05