

November, 2000
Volume 63, No. 11
Pages 1465-1624
CODEN: JFPRDR 63(11)1465-1624(2000)
ISSN:0362-028X

Journal of Food Protection



"The mission of the International Association for Food Protection is to provide food safety professionals worldwide with a forum to exchange information on protecting the food supply."



Published Monthly by

International Association for
Food Protection

6200 Aurora Avenue, Suite 200W
Des Moines, Iowa 50322-2863 USA
www.foodprotection.org

Journal of Food Protection

ISSN: 0362-028X
Official Publication



International Association for
Food Protection
Formerly IAMPES
Reg. U.S. Pat. Off.

Vol. 63

November 2000

No. 11

The Effect of Different Grain Diets on Fecal Shedding of <i>Escherichia coli</i> O157:H7 by Steers S. J. Buchko, R. A. Holley, W. O. Olson, V. P. J. Gannon, and D. M. Veira*	1467
Comparison of Aqueous Chemical Treatments To Eliminate <i>Salmonella</i> on Alfalfa Seeds W. R. Weissinger and L. R. Beuchat*	1475
Enrichment Procedures and Plating Media for Isolation of <i>Yersinia enterocolitica</i> G. C. Jiang, Dong-Hyun Kang,* and Daniel Y. C. Fung	1483
Genotypes and Enterotoxicity of <i>Staphylococcus aureus</i> Isolated from the Hands and Nasal Cavities of Flight-Catering Employees M. Hatacka,* K. J. Björkroth, K. Asplund, N. Mäki-Petäys, and H. J. Korkeala	1487
Stimulation of Starter Culture for Further Reduction of Foodborne Pathogens during Salami Fermentation Dong-Hyun Kang* and Daniel Y. C. Fung	1492
<i>Bacillus cereus</i> Group Strains, Their Hemolysin BL Activity, and Their Detection in Foods Using a 16S RNA and Hemolysin BL Gene-Targeted Multiplex Polymerase Chain Reaction System Hau-Yang Tsen,* Ming-Lun Chen, You-Miin Hsieh, Sen-Je Sheu, and Yan-Lian Chen	1496
Bacterial Spore Inhibition and Inactivation in Foods by Pressure, Chemical Preservatives, and Mild Heat Adrienne E. H. Shearer, C. Patrick Dunne, Anthony Sikes, and Dallas G. Hoover*	1503
Nonproteolytic <i>Clostridium botulinum</i> Toxigenesis in Cooked Turkey Stored under Modified Atmospheres Kathleen A. Lawlor,* Merle D. Pierson, Cameron R. Hackney, James R. Claus, and Joseph E. Marcy	1511
Detection of Gualacol Produced by <i>Allicyclobacillus acidoterrestris</i> in Apple Juice by Sensory and Chromatographic Analyses, and Comparison with Spore and Vegetative Cell Populations Rachel V. Orr, Robert L. Shewfelt, C. J. Huang, Sebat Tefera, and Larry R. Beuchat*	1517
Aerobiology of a High-Line Speed Cattle Abattoir K. W. F. Jericho,* J. Ho, and G. C. Kozub	1523
The Synergistic Effect of Excimer and Low-Pressure Mercury Lamps on the Disinfection of Flowing Water Ian A. Ramsay, Jean-Christophe Niedziela, and Iain D. Ogden*	1529
Ultraviolet Spectrophotometric Characterization and Bactericidal Properties of Electrolyzed Oxidizing Water as Influenced by Amperage and pH Soo-Voon Len, Yen-Con Hung,* Marilyn Erickson, and Chyer Kim	1534
Prevalence of High-Risk Food Consumption and Food-Handling Practices among Adults: A Multistate Survey, 1996 to 1997 Beletshachew Shiferaw,* Samantha Yang, Paul Cieslak, Duc Vugia, Ruthanne Marcus, Jane Koehler, Valerie Deneen, Frederick Angulo, and the FoodNet Working Group	1538
Influence of Hygienic Quality of Raw Materials on Biogenic Amine Production during Ripening and Storage of Dry Fermented Sausages Sara Bover-Cid, Maria Izquierdo-Pulido, and M. Carmen Vidal-Carou*	1544
Formation of Biogenic Amines in Raw Milk Hispánico Cheese Manufactured with Proteinases and Different Levels of Starter Culture E. Fernández-García,* J. Tomillo, and M. Nuñez	1551
Mixed Starter Cultures To Control Biogenic Amine Production in Dry Fermented Sausages Sara Bover-Cid, Maria Izquierdo-Pulido, and M. Carmen Vidal-Carou*	1556
Detection of Radiation-Induced Hydrocarbons and 2-Alkylcyclobutanones in Irradiated Perilla Seeds Hae-Jung Lee, Myung-Woo Byun, and Kyong-Su Kim*	1563
A Differential Medium for the Enumeration of the Spoilage Yeast <i>Zygosaccharomyces bailii</i> in Wine D. Schuller, M. Côte-Real,* and C. Leão	1570
Norwalk-like Virus Sequences Detected by Reverse Transcription-Polymerase Chain Reaction in Mineral Waters Imported into or Bottled in Switzerland Christian Beuret,* Dorothe Kohler, and Thomas Lüthi	1576
Research Notes	
Prevalence and Characteristics of Shiga Toxin-Producing <i>Escherichia coli</i> in Beef Cattle Slaughtered on Prince Edward Island R. Douglas Schurman, Harry Hariharan,* Susan B. Heaney, and Kris Rahn	1583
Analysis and Modeling of the Variability Associated with UV Inactivation of <i>Escherichia coli</i> in Apple Cider Siobain Duffy, John Churey, Randy W. Worobo, and Donald W. Schaffner*	1587
<i>Yersinia enterocolitica</i> Biogroup 1A, Serotype O:5 in Chicken Carcasses Mirtha E. Floccari, María M. Carranza, and Jose L. Parada*	1591
Reduction of <i>Campylobacter jejuni</i> in a Simulated Chicken Digestive Tract by Lactobacilli Cultures M. H. Chang and T. C. Chen*	1594
Thermal Stability of Moniliformin at Varying Temperature, pH, and Time in an Aqueous Environment Graciela Pineda-Valdes and Lloyd B. Bullerman*	1598
Review	
Detection and Analysis of Animal Materials in Food and Feed Momcilovic Dragan and Avraham Rasooly*	1602

* Asterisk indicates author for correspondence.

The publishers do not warrant, either expressly or by implication, the factual accuracy of the articles or descriptions herein, nor do they so warrant any views or opinions offered by the authors of said articles and descriptions.

Scientific Editors

DR. LARRY R. BEUCHAT, Center for Food Safety and Quality Enhancement, University of Georgia, Griffin, Georgia 30223-1797, USA

DR. JOHN N. SOFOS, Department of Animal Sciences, Colorado State University, Fort Collins, Colorado 80523-1171, USA

Journal Editorial Staff

DAVID W. THARP, CAE, *Executive Director*

LISA K. HOVEY, *Managing Editor*

BEV CORRON, *Administrative Editor*

DIDI LOYNACHAN, *Administrative Assistant*

Journal Management Committee Chairperson

DR. DONALD E. CONNER, Auburn University, Department of Poultry Science, 236 Ann Upchurch Hall, Auburn, Alabama 36849-5416, USA

Journal Editorial Office

International Association for Food Protection, 6200 Aurora Avenue, Suite 200W, Des Moines, Iowa 50322-2863, USA; Phone 515.276.3344; Fax 515.276.8655; E-mail: bcorron@foodprotection.org

Executive Board

President, JENNY SCOTT, National Food Processors Association, 1350 I Street N.W., Suite 300, Washington, D.C. 20005-3305, USA; Phone 202.639.5985; Fax 202.639.5991; E-mail: jscott@nfpa-food.org

President-Elect, JAMES S. DICKSON, Iowa State University, Department of Microbiology, 207 Science I, Ames, Iowa 50011-0001, USA; Phone 515.294.4733; Fax 515.294.6019; E-mail: jdickson@iastate.edu

Vice President, ANNA M. LAMMERDING, Health Canada, Health Protection Branch, 110 Stone Road W., Guelph, Ontario, Canada N1G 3W4; Phone 519.822.3300; Fax 519.822.2280; E-mail: anna.lammerding@hc-sc.gc.ca

Secretary, PAUL A. HALL, Kraft Foods, Inc., 801 Waukegan Road, Glenview, Illinois 60025-4312, USA; Phone 847.646.3678; Fax 847.646.4820; E-mail: phall@kraft.com

Past-President, JACK GUZEWICH, Food and Drug Administration, Division of Enforcement and Programs, HFS-605, 200 C Street S.W., Washington, D.C. 20204-0001, USA; Phone 202.260.3847; Fax 202.260.0133; E-mail: john.guzewich@cfns.fda.gov

Affiliate Council Chairperson, FRED WEBER, Weber Scientific, 2732 Kuser Road, Hamilton, New Jersey 08691-9430, USA; Phone 609.584.7677; Fax 609.584.8388; E-mail: fredweber@earthlink.net

Executive Director, DAVID W. THARP, CAE, International Association for Food Protection, 6200 Aurora Avenue, Suite 200W, Des Moines, Iowa 50322-2863, USA; Phone 800.369.6337; Fax 515.276.8655; E-mail: dtharp@foodprotection.org

Editorial Board

G. R. ACUFF, TX (00)	B. A. GLATZ, IA (01)	P. E. KOEHLER, GA (00)	E. J. RHODEHAMEL, SC (01)
W. H. ANDREWS, DC (01)	D. A. GOLDEN, TN (02)	R. G. LABBE, MA (01)	S. C. RICKE, TX (01)
J. S. BAILEY, GA (02)	L. G. M. GORRIS, NETH (02)	R. V. LACHICA, MA (02)	E. T. RYSER, MI (01)
T. J. BARRETT, GA (01)	M. W. GRIFFITHS, CAN (02)	R. E. LEVIN, MA (00)	D. W. SCHAFFNER, NJ (01)
S. E. BEATTIE, CA (01)	D. D. HANCOCK, WA (01)	D. L. MARSHALL, MS (01)	B. W. SHELTON, NC (00)
R. E. BRACKETT, DC (02)	M. A. HARRISON, GA (00)	R. T. MARSHALL, MO (00)	L. A. SHELER, MI (00)
R. L. BUCHANAN, DC (02)	R. P. HERWIG, WA (00)	S. E. MARTIN, IL (01)	M. F. SLAVIK, AR (01)
A. A. BUSHWAY, ME (02)	W. E. HILL, WA (00)	S. A. MCCARTHY, AL (02)	D. M. SMITH, MI (02)
A. CASTILLO, MEX (02)	B. H. HIMELBLOOM, AK (01)	R. F. McFEETERS, NC (02)	J. L. SMITH, PA (02)
J. G. CERVENY, WI (00)	A. D. HITCHINS, DC (02)	T. A. McMEEKIN, AUSTRAL (01)	S. S. SUMNER, VA (01)
F. S. CHU, WI (02)	A. D. HOCKING, AUSTRAL (01)	A. M. McNAMARA, VA (00)	M. L. TAMPLIN, FL (01)
D. O. CLIVER, CA (02)	D. G. HOOVER, DE (00)	J. MENG, MD (00)	D. W. THAYER, PA (00)
M. A. COUSIN, IN (00)	J. G. HOTCHKISS, NY (00)	C. MICHELS, BELG (02)	E. C. D. TODD, CAN (00)
J.-Y. D'AOUST, CAN (00)	R. W. HUTKINS, NE (00)	L. J. MOBERG, OH (99)	S. B. TURNIPSEED, CO (02)
P. M. DAVIDSON, TN (01)	S. C. INGHAM, WI (00)	P. V. NIELSEN, DEN (00)	K. S. VENKITANARAYANAN, CT (02)
A. DEMIRCI, PA (02)	L. A. JAYKUS, NC (02)	G.-J. E. NYCHAS, GRE (02)	A. VON HOLY, SAFR (01)
A. DePAOLA, AL (02)	E. A. JOHNSON, WI (00)	S. A. PALUMBO, PA (01)	M. R. WACHTEL, CA (01)
J. S. DICKSON, IA (02)	M. G. JOHNSON, AR (00)	M. W. PECK, UK (02)	I. T. WALLS, DC (02)
E. F. ESCARTIN, MEX (01)	R. JORDANO, SPA (01)	J. M. PEINADO, SPA (00)	M. M. WEKELL, NY (00)
J. M. FARBER, CAN (00)	V. J. JUNEJA, PA (02)	J. J. PESTKA, MI (01)	R. C. WHITING, DC (01)
P. FENG, DC (02)	C. W. KASPAR, WI (02)	M. E. POTTER, DC (00)	C. E. WOLF-HALL, ND (01)
J. F. FRANK, GA (02)	S. KATHARIOU, HI (00)	D. A. POWELL, CAN (02)	R. W. WOROBO, NY (02)
P. M. FRATAMICO, PA (02)	S. E. KATZ, NJ (01)	K. J. RAJKOWSKI, PA (01)	A. E. YOUSEF, OH (00)
C. O. GILL, CAN (00)	S. J. KNABEL, PA (00)	B. RAY, WY (01)	

Journal of Food Protection (ISSN-0362-028X) is published monthly beginning with the January issue by the International Association for Food Protection. Executive offices are located at 6200 Aurora Avenue, Suite 200W, Des Moines, Iowa 50322-2863, USA. Each volume consists of 12 issues. Periodical postage paid at Des Moines, Iowa 50318, and additional entry offices.

Postmaster: Send address changes to *Journal of Food Protection*, IAFP, 6200 Aurora Avenue, Suite 200W, Des Moines, Iowa 50322-2863, USA.

Microfilm of *Journal of Food Protection* is available from Xerox University Microfilms, 300 N. Zeeb Road, Ann Arbor, Michigan 48106-1346, USA. Permission to reprint any portion of *Journal of Food Protection* must be obtained from International Association for Food Protection. Claims for missing issues must be submitted to the Association within 30 days (US, Canada, and Mexico). International claims must be submitted within 60 days. Address changes and membership dues should be directed to the editorial office. Instructions for Authors are available at www.foodprotection.org or from the *Journal of Food Protection* Administrative Assistant.

Membership in the association is available to individuals. Dues including the *Journal of Food Protection* and *Dairy, Food and Environmental Sanitation* are \$150.00 US, \$175.00 Canada/Mexico, and \$220.00 International. Student rates are \$45.00 US, \$60.00 Canada/Mexico, and \$90.00 International for *Journal of Food Protection*; \$45.00 US, \$55.00 Canada/Mexico, and \$70.00 International for *Dairy, Food and Environmental Sanitation*; and \$75.00 US, \$100.00 Canada/Mexico, and \$145.00 for International for *Journal of Food Protection* and *Dairy, Food and Environmental Sanitation*. All membership dues include shipping and handling. *Journal of Food Protection* is available by subscription for \$284.00 US, \$299.00 Canada/Mexico, and \$329.00 International. Single copies are available for \$36.00 US and \$45.00 other countries. All rates include shipping and handling. No cancellations accepted.

The Effect of Different Grain Diets on Fecal Shedding of *Escherichia coli* O157:H7 by Steers

S. J. BUCHKO,^{1,2} R. A. HOLLEY,¹ W. O. OLSON,³ V. P. J. GANNON,⁴ AND D. M. VEIRA^{5*}

¹Department of Food Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2; ²Animal Diseases Research Institute, Lethbridge, Alberta, Canada T1J 3Z4; ³Canadian Food Inspection Agency, Animal Diseases Research Institute, Lethbridge, Alberta, Canada; ⁴Health Canada, Animal Diseases Research Institute, Lethbridge, Alberta, Canada; and ⁵Agriculture and Agri-Food Canada, Range Research Unit, Kamloops, British Columbia, Canada V2B 8A9

MS 00-58: Received 24 February 2000/Accepted 22 May 2000

ABSTRACT

Three groups of six yearling steers (three rumen fistulated plus three nonfistulated) fed one of three different grain diets (85% cracked corn, 15% whole cottonseed and 70% barley, or 85% barley) were inoculated with 10¹⁰ CFU of *Escherichia coli* O157:H7 strain 3081, and the presence of the inoculated strain was followed in the rumen fluid and feces for a 10-week period. *E. coli* O157:H7 was rapidly eliminated from the rumen of the animals on all three diets but persisted in the feces of some animals up to 67 days after inoculation, suggesting that the bovine hindgut is the site of *E. coli* O157:H7 persistence. A significant difference existed in the levels of *E. coli* O157:H7 shed by the animals among diets on days 5, 7, 49, and 63 after inoculation ($P < 0.05$). No significant difference was found between the levels shed among diets on days 9 through 42 and on day 67 ($P > 0.05$). The number of animals that were culture positive for *E. coli* O157:H7 strain 3081 during the 10-week period was significantly higher for the barley fed group (72 of 114 samplings) as opposed to the corn fed group (44 of 114 samplings) ($P < 0.005$) and the cottonseed and barley fed group (57 of 114 samplings) ($P < 0.05$). The fecal pH of the animals fed the corn diet was significantly lower ($P < 0.05$) than the fecal pH of the animals fed the cottonseed and barley and barley diets, likely resulting in a less suitable environment for *E. coli* O157:H7 in the hindgut of the corn fed animals. *E. coli* O157:H7 strain 3081 was present in 3 of 30 (corn, 1 of 10; cottonseed, 1 of 10; barley, 1 of 10) animal drinking water samples, 3 of 30 (corn, 1 of 10; cottonseed, 0 of 10; barley, 2 of 10) water trough biofilm swabs, 5 of 30 (corn, 0 of 10; cottonseed, 2 of 10; barley, 3 of 10) feed samples, and 30 of 30 manure samples taken from the pens during the entire experimental period. Mouth swabs of the steers were also culture positive for *E. coli* O157:H7 strain 3081 in 30 of 180 samples (corn, 7 of 60; cottonseed, 4 of 60; barley, 19 of 60) taken during the 10-week period. Minimizing environmental dissemination of *E. coli* O157:H7 in conjunction with diet modification may reduce numbers of *E. coli* O157:H7-positive cattle.

Since 1982, when it was first identified as a human pathogen, enterohemorrhagic *Escherichia coli* O157:H7 has been implicated in numerous outbreaks of hemorrhagic colitis and life-threatening hemolytic uremic syndrome (31, 32). Epidemiological investigations demonstrate that cattle, both beef and dairy, are a principal reservoir of *E. coli* O157:H7 (17, 38). This association is further supported by numerous field surveys and trace-back studies that link *E. coli* O157:H7 directly and indirectly with bovine sources (12, 17, 38). Although contaminated and improperly cooked ground beef has been implicated as the primary vehicle of transmission (15, 17), foods such as radish sprouts (23), apple cider (4), unpasteurized milk (6, 26), mayonnaise (30), yogurt (27), venison jerky (24), and water (1) have also been linked to *E. coli* O157:H7 outbreaks.

Fecal shedding of *E. coli* O157:H7 in cattle herds is widespread and intermittent in nature (17, 38). It is well known that the season and age of the animal have a significant effect on the level and duration of fecal shedding of *E. coli* O157:H7 by cattle and other ruminants. Several studies have reported peak fecal shedding of *E. coli* O157:

H7 during the spring and summer months (18, 26, 38). It has also been reported that weaned dairy calves and yearling beef cattle at slaughter are more likely to shed the organism in their feces than adult cattle (14, 19, 36). In addition to these factors, a number of recent studies suggest that diet also influences the fecal shedding of *E. coli* O157:H7 by cattle (8, 14, 17, 20).

Grain feeding is among the dietary factors that are considered to be important in respect to *E. coli* O157:H7 fecal shedding. Beef cattle are commonly fed energy-rich grain diets during the finishing periods of beef production before slaughter, and fecal shedding of *E. coli* O157:H7 at slaughter is considered to be a significant source of contamination for beef (36). Rapid fermentation of grains lowers the ruminal and intestinal pH of cattle, favoring acid-resistant *E. coli* such as *E. coli* O157:H7. Although it has been reported that grain feeding as opposed to hay feeding favors acid-resistant *E. coli*, a more recent study has indicated that the acid sensitivity of *E. coli* O157:H7 is not affected by the diet of cattle (9, 21). Much debate still exists concerning these findings, and it is not clear if the cattle feeding industry will advocate management changes until the issue resolves.

Information provided by numerous farm surveys sug-

* Author for correspondence: Tel: 250-554-5205; Fax: 250-554-5229; E-mail: veirad@em.agr.ca.

The Synergistic Effect of Excimer and Low-Pressure Mercury Lamps on the Disinfection of Flowing Water

IAN A. RAMSAY,¹ JEAN-CHRISTOPHE NIEDZIELA,² AND IAIN D. OGDEN^{2*}

¹Laser Installations Limited, Arbirlot, by Arbroath, DD11 2PY, UK; and ²Applied Food Microbiology Group, Department of Medical Microbiology, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK

MS 00-60: Received 24 February 2000/Accepted 22 May 2000

ABSTRACT

Microorganisms in flowing water were disinfected by UV radiation from two excimer (excited dimer) lamps (emitting at 172 and 222 nm) in combination with two low-pressure mercury lamps (emitting at 254 nm). Synergies were investigated among the three types of radiation in the treatment of water spiked in turn with *Escherichia coli*, *Listeria innocua*, *Shewanella putrefaciens*, and spores of *Bacillus subtilis* and *Bacillus cereus*. Synergy was demonstrated between radiations at 222 and 254 nm in the treatment of *E. coli*, *L. innocua*, and *S. putrefaciens*, but little or no synergy was observed in the treatment of *B. subtilis* and *B. cereus*. At maximum flow rates (60 liters/min), 5-log reductions in *E. coli* were achieved at 254 nm, although at 222 nm, less than 1-log reductions were observed. No bacterial kill was observed with 172-nm radiation alone, despite increasing exposure time by reducing flow rates to less than 3 liters/min.

The availability of safe drinking water is essential for human health and well-being. Water contamination and related food poisoning incidents cause considerable economic costs throughout the world (2). The social disruption and financial consequences are great, and the illness suffered can be debilitating and sometimes life threatening, e.g., *Escherichia coli* O157, which has been linked to waterborne incidents in the UK (12). The requirement for microbiologically pure water is further necessitated since some ready-to-eat foods, such as vegetables, are disinfected by aqueous treatments. The motivation for evolving new processes or new combinations of processes to disinfect and clean foods comes also from changing legislation. There are moves under way to eliminate the use of chlorine in the microbiological treatment of some foods, and processes that involve UV (4), ozone (6, 8), gamma irradiation (1, 5), and pulsed-electric fields (7, 13) are receiving increasing attention.

Surface water is often contaminated with waste from animal and human activity. Underground water supplies can also be contaminated, particularly so in the case of porous soils in periods of high rainfall. The microbiological safety limit for drinking water in the UK is less than 1 coliform per 100 ml. The use of mercury UV light at 254 nm is now widespread both in the production of potable water and, to a lesser extent, in the treatment of waste water. The maximum absorption of DNA occurs at 257 nm. Higher and lower wavelengths, e.g., 222 nm, are absorbed to a lesser extent. The UV absorption causes photocleavage (severing), resulting in bacterial death (5) at relatively small doses (typically <10 mJ/cm²). Higher doses are required to elimi-

nate some viruses and still higher doses (330 mJ/cm²) for *Cryptosporidium parvum* (10).

Oppenländer et al. (11) suggested that bactericidal action at 254-nm radiation could be improved by supplementary radiation from excimer lamps. An excimer is an excited dimer that is a combination of two adjacent atoms or molecules that behave as a unit. Excimer lamps, developed by Kogelschatz (9), make use of the excited dimer fluorescence from pairs of atoms that are excited by an electrical discharge. There are many excimer combinations, some with pairs of like atoms and others with pairs of different atoms. At the appropriate gas pressure for a chosen combination, the lamp gives an output of fluorescence centered on a specific wavelength in a narrow waveband, usually ± 5 nm.

Two lamps that exhibit a high, essentially monochromatic output are the Xe₂ at 172 nm and the KrCl at 222 nm. At the lower wavelength of 172 nm, high quantum energy is produced that is sufficient to hydrolyze water into hydrogen atoms and hydroxyl radicals (11). The hydroxyl radical is very short lived however (<10 μ s), and the penetration depth of 172-nm radiation in water is very short (approximately 30 μ m). For radiation at this wavelength, a vacuum is required between lamp and flow tube, and the envelope materials must be Spectrosil (or an equivalent) and not Vitreosil (fused silica). Photons at the higher wavelength, 222 nm, do not require a vacuum or the Spectrosil material, and they have a much deeper penetration into clear water, approximately 3 cm. In the theory of Oppenländer et al. (11), radiation at 222 nm catalyses the reaction between hydroxyl radicals (which are created by the 172-nm radiation) and water itself to produce hydrogen peroxide (H₂O₂) and ozone. These products are weaker oxidizing agents than the hydroxyl radical but exist for longer periods

* Author for correspondence. Tel: +44 1224 551132; Fax: +44 1224 685604; E-mail: i.ogden@abdn.ac.uk.

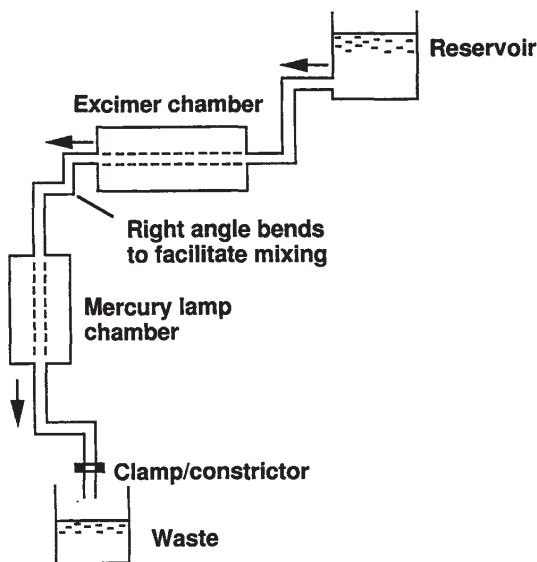


FIGURE 1. Schematic diagram of flow through UV disinfection system.

in water under normal conditions and have some bactericidal action.

For 172-nm radiation alone, only a marginal outer layer of water would receive treatment, and the hydroxyl radical would quickly decay as soon as the water flowed out of the region of excitation. For this reason, the logic of the theory requires that the 172- and 222-nm lamps should irradiate the Spectrosil flow tube simultaneously, although the diagram illustrating the theory (11) shows successive rather than simultaneous excitation by excimer lamps.

By causing mixing of the water with a series of right-angled bends as the water flows between the excimer chamber (where simultaneous excitation is assumed to have occurred) and the mercury lamp chamber, a dilute, uniform distribution of H_2O_2 is achieved before radiation by 254 nm. Then, according to Oppenländer et al. (11), this radiation dissociates the H_2O_2 into pairs of hydroxyl radicals that are now distributed over the entire cross section. So, in addition to the killing of bacteria by photocleavage by the radiations at 222 and 254 nm, there is the additional antimicrobial action of the hydroxyl radicals. This is the major source of synergy that is predicted (11) between the excimer lamps on one hand and the mercury lamps on the other. This theory also predicts a minor synergy between the two types of excimer lamp through killing by the less potent H_2O_2 .

The objective of this study was to validate the synergy theory and to develop an improved UV method for the disinfection of flowing liquids. The effect of excimer and mercury radiation on gram-positive and gram-negative microorganisms and bacillus spores was investigated.

MATERIALS AND METHODS

The flow-through system. A flow-through system was built to connect two UV chambers for dynamic experiments (Fig. 1).

An elevated reservoir (4 liters) was placed 1 m above the chambers sited on the laboratory bench. The reservoir was linked to a 30-mm-diameter Spectrosil tube in the excimer chamber with silicone rubber tubing. The connections to the 30-mm-diameter Vitreosil tube in the mercury chamber via three right-angled glass (Pyrex) bends were also made using silicone rubber tubing. A final length of silicone rubber tubing conducted the water under gravity from the mercury chamber into a waste receptacle on the floor. This tubing was clamped and released by hand to initiate flow.

Excimer lamps and the excimer chamber. The two lamp sets (KrCl Excimer and Xe^2 Excivac laboratory systems) were purchased from Heraeus Noblelight Ltd, Cambridge, UK. The steel excimer chamber held a Spectrosil tube (32 mm diameter by 60 cm length) that was submitted to UV light emitted by the two lamps. Two mirrors of polished stainless steel, curved to focus the irradiation onto the sample tube, were included. A vacuum pump created a vacuum to 0.3 mbar in the chamber. Before testing, it was important to exclude air bubbles manually from the sample tube that would otherwise prevent penetration by the 172-nm radiation.

Mercury lamp chamber. Two mercury-indium lamps (NNI, 120/80 from Heraeus, each having a discharge length of 80 cm) were housed parallel to a central Vitreosil sample tube within a double elliptical reflector (aluminum) to focus radiation from both sides onto the sample. Such a system prevents shadowing of target bacteria.

Bacterial strains under test. Isolates from the Applied Food Group's laboratory collection included six strains of *E. coli* (50, 60, 65, 67, 888, and D), three strains of *Listeria innocua* (GS1, MMB, and SC1), five strains of *Shewanella putrefaciens* (Ga2, Ga3, H1, Nn9, and Pp1), and one strain each of *Bacillus subtilis* and *Bacillus cereus*. *E. coli* and *L. innocua* were grown in 10-ml volumes of tryptone soya broth (Oxoid CM129) at 37°C for 18 h before use, and *S. putrefaciens* was grown in 10-ml volumes nutrient broth (Oxoid CM1) at 20°C for 24 h. *B. subtilis* and *B. cereus* spores were prepared by inoculation of vegetative cells onto nutrient agar plates incubated for 3 days at 20°C. The resulting growths were aseptically transferred to 100-ml sterile glass containers, heated in a water bath to 82°C for 10 min to kill vegetative cells, and the spore crops were quickly cooled and stored at 4°C before use. Different volumes (5 to 15 ml) of microorganisms under test were inoculated separately into the system reservoir and aliquots (10 ml) removed to estimate bacterial numbers by a modified method of Miles and Misra (3).

Disinfection experiments. The mercury lamps were warmed up for 10 min to reach full power before use. The excimer lamps warmed up instantaneously. The system was filled with 4 liters of bacterial suspension, and the flow rate on releasing the clamp at the output end was measured at 60 liters/min. Aliquots (10 ml) for bacterial analysis were aseptically collected after 2 liters had flowed to waste, which was the volume held in the system downstream of the second chamber and ensured that the test sample had been subjected to irradiation.

Synergy experiments. Preliminary tests showed that bacterial kill from the mercury lamps was significantly greater than with excimer radiation. To observe synergy among all lamps, it was therefore necessary to reduce the output power from the mercury lamps, set the 172- and 222-nm lamps at the highest power setting, and increase the contact time between sample and radiation source. The latter was achieved by the application of a constrictor on the output tube end of the system. Flow rates of 8

TABLE 1. Effect of different UV lamp combinations on *E. coli* 65 in water^a

Lamp combinations	Number of survivors
Control (no lamps)	$4 \times 10^5/\text{ml}$
172 nm	$4 \times 10^5/\text{ml}$
222 nm	$1 \times 10^5/\text{ml}$
254 nm	$<1 \times 10^1/\text{ml}$
172 + 222 nm	$1 \times 10^5/\text{ml}$
222 + 254 nm	$<1 \times 10^1/\text{ml}$
172 + 222 + 254 nm	$<1 \times 10^1/\text{ml}$

^a Flow rate was 60 liters/min.

liters/min were used for *E. coli*, *L. innocua*, and *S. putrefaciens* and 2.9 liters/min for bacillus spores.

Cleaning the system. Between replicate tests on the same microorganism, the system was rinsed thoroughly with tap water to ensure no residual contamination (confirmed by analysis of the water after such rinsing). At the end of each working day, the system was soaked overnight with proprietary hyperchlorite at the recommended strength.

Testing for hydrogen peroxide. The presence of H_2O_2 in water (after passing through both chambers) was determined using a test kit (HYP-1, HACH Company, Loveland, Colo.). Ammonium molybdate (1 ml) was added to a 10-ml aqueous sample followed by a measured quantity of sulfite reagent. The presence of H_2O_2 was indicated by a blue color. After 5 min, a specific number of drops of sodium thiosulfate titrant was added until the sample became faintly yellow. One drop of thiosulfate was equivalent to 0.2 mg/liter of H_2O_2 in the test sample.

Role of hydrogen peroxide in the disinfection. To test whether the production of H_2O_2 contributed to synergy between excimer and mercury radiation, tap water was allowed to flow through the system at a flow rate of 0.29 liter/min, exactly 10 times slower than the rate of flow used for 222- and 254-nm tests. Samples for bacterial analysis were taken after 5 s and 10.5 min under various power settings on the lamps (to maximize H_2O_2 production). To test whether the production of H_2O_2 by 222-nm radiation was responsible for the observed synergy, the 222-nm lamp was switched off and H_2O_2 (Sigma, Poole, Dorset, UK) added separately at 0.22- and 2.2-mg/liter concentrations to a suspension of *E. coli* located within the system.

Effects of temperature and pH. Water under treatment (before spiking) was left to equilibrate at 6, 18, and 42°C. After spiking with *E. coli*, volumes were in turn subjected to 222-, 254-, and 222- + 254-nm radiations at flow rates of 2.9 liter/min. At ambient temperature (20°C), the pH of water under treatment was

adjusted to 4.1 (with HCl) and 10.0 (with NaOH) and compared with neutral water (pH 7.4) when irradiated with the mercury-indium lamp alone.

RESULTS AND DISCUSSION

Preliminary disinfection experiments. Table 1 shows individual and combined effects of excimer and mercury radiation (all at maximum power) on *E. coli* 65. At the low pressure of 0.3 mbar in the excimer chamber, the radiation at 172 nm by itself had no measurable effect. A flow rate of 60 liters/min gave a total kill ($>10^5$) of *E. coli* with the mercury lamps. Excimer radiation of 222 nm alone produced a 0.3-log reduction, and subsequent repeat tests gave similar small reductions. Clearly, the mercury lamp system had greater bactericidal power than the excimer lamp systems in killing this microorganism. Replicate runs with the same strain (*E. coli* 65), different strains of *E. coli*, and suspensions of *L. innocua* and *S. putrefaciens* showed similar results (data not presented). Bacillus spores, as one might expect, were more resistant, and lamp combinations with 254-nm radiation showed on average a 4-log reduction. The combination of 172- and 254-nm radiation was not tested.

Synergy experiments. With the flow rates and power of the mercury lamps decreased, separate experiments were performed on the five microorganisms being tested. Table 2 shows that synergy between 222- and 254-nm radiations was indeed observed in the disinfection of *E. coli* and to a slightly lesser extent in the treatment of *L. innocua* and *S. putrefaciens*. Little or no synergy occurred in the treatment of *B. subtilis* and *B. cereus* spores. Contrary to the theory (11), however, the radiation at 172 nm was not a contributor to this synergy. In all tests at reduced flows in the synergy investigations, the 222-nm excimer lamp gave slightly greater bacterial kills than the mercury indium lamp with all species tested, but it must be noted that the 254-nm radiation was 5% of maximum power. The data presented in Table 2 are representative of a number of replicate experiments.

The role of hydrogen peroxide. The results cast doubt on the role of H_2O_2 in the observed synergy because the 172-nm lamp (supposedly the creator of the hydroxyl radical that leads to the formation of H_2O_2) had no microbial effect as shown in Table 1. When tap water was allowed to flow through the system at 0.29 liter/min, exactly 10 times slower than the rate of flow when synergy had been

TABLE 2. Effect of different lamp combinations on a range of microorganisms in flowing water

Lamp combinations	Number of survivors				
	<i>E. coli</i> 65 ^a	<i>L. innocua</i> GS1 ^a	<i>S. putrefaciens</i> Nn9 ^a	<i>B. cereus</i> ^b	<i>B. subtilis</i> ^b
Control (no lamps)	$5 \times 10^6/\text{ml}$	$8 \times 10^6/\text{ml}$	$7 \times 10^6/\text{ml}$	$5 \times 10^4/\text{ml}$	$3 \times 10^5/\text{ml}$
222 nm	$1 \times 10^4/\text{ml}$	$3 \times 10^3/\text{ml}$	$1 \times 10^3/\text{ml}$	$1 \times 10^2/\text{ml}$	$3 \times 10^3/\text{ml}$
254 nm	$3 \times 10^4/\text{ml}$	$1 \times 10^4/\text{ml}$	$2 \times 10^3/\text{ml}$	$7 \times 10^2/\text{ml}$	$2 \times 10^4/\text{ml}$
222 + 254 nm	$<1 \times 10^1/\text{ml}$	$<1 \times 10^1/\text{ml}$	$<1 \times 10^1/\text{ml}$	$4 \times 10^1/\text{ml}$	$2 \times 10^2/\text{ml}$

^a Flow rate was 8 liters/min.^b Flow rate was 2.9 liters/min.

TABLE 3. Effect of temperature on UV treatment of *E. coli* 65

Lamp combinations	Number of survivors		
	6°C	18°C	42°C
Control (no lamps)	$2 \times 10^6/\text{ml}$	$2 \times 10^6/\text{ml}$	$2 \times 10^6/\text{ml}$
222 nm	$6 \times 10^4/\text{ml}$	$5 \times 10^3/\text{ml}$	$6 \times 10^2/\text{ml}$
254 nm	$5 \times 10^5/\text{ml}$	$9 \times 10^4/\text{ml}$	$9 \times 10^4/\text{ml}$
222 + 254 nm	$1 \times 10^3/\text{ml}$	$2 \times 10^1/\text{ml}$	$<1 \times 10^1/\text{ml}$

observed between 222- and 254-nm radiations, and samples were taken after 5 s, it was found that 0.1 mg/liter of H_2O_2 had been produced. This applied to any lamp or combination of lamps and can be regarded as the baseline level. After 10.5 min, both the 172- and 254-nm radiations separately produced that baseline level (0.1 mg/liter). By contrast, after 10.5 min of irradiation at 222 nm, the sample contained approximately 2.2 mg/liter of H_2O_2 . This result was irrespective of vacuum application. The quantity produced was unchanged when the mercury lamp was also switched on. The other lamps neither produced nor destroyed H_2O_2 . In our tests and contrary to the theory, H_2O_2 was produced by 222-nm radiation alone.

When the 222-nm lamps were switched off and 0.22 and then 2.2 mg/liter of laboratory H_2O_2 added to a preparation of *E. coli*, it was found that after irradiating at 254 nm (at the same flow rate as had been used in the synergy demonstrations) bacterial kill was the same as with the mercury lamp alone. Hydrogen peroxide had made no measurable contribution to the kill and was unrelated to the observed synergy. There is a possibility that surviving bacteria, i.e., those not killed by the 222-nm radiation, were injured to the degree that subsequent radiation at 254 nm would result in death. We propose this as the explanation of the observed synergy rather than the complicated description in the literature (11) involving 172-nm radiation and H_2O_2 .

Effects of temperature and pH. The effect of temperature on the UV treatment of bacteria is shown in Table 3. For the range of 6 to 42°C, the bacterial kill of *E. coli* increased with temperature at 222-, 254-, and 222- + 254-nm radiations, but from an industrial perspective, it is unlikely that the cost of raising water temperature could be justified. The reason for the effect of temperature was unclear.

The effect of pH was assessed because its value in water varies widely across the United Kingdom. In areas of high peat content, the value can be less than 4, and in areas containing limestone, it can be more than 8. The number of survivors of *E. coli* in tests at pH values of 4.1, 7.4, and 10.0 showed no significant difference under radiation at 254 nm.

This work showed that H_2O_2 was formed by the 222-nm lamp acting alone. Any additional H_2O_2 formed when supplemented with 172-nm radiation was not detectable, presumably because of the low penetration ($<30 \mu\text{m}$) of 172-nm radiation into water. No H_2O_2 was formed by the mercury lamps at any intensity tested. The amount of H_2O_2

formed by the 222-nm lamp is very small and, with the flow rate reduced to 0.29 liter/min, 2.2 mg/liter of H_2O_2 was produced as measured by a chemical detection method. Separate addition of this amount of H_2O_2 to *E. coli* produced no reduction in viable numbers.

This study showed that several points from the literature (11) were either incorrect or irrelevant to studies of microorganisms in liquids. First, no contribution was verified from the 172-nm lamp in experiments with water spiked with bacteria. This was most probably because the penetration of the 172-nm radiation into the water was so much shorter than the penetration of the other two radiations (222 and 254 nm). Second, hydrogen peroxide was formed in water by radiation from the 222-nm lamp alone. The presence or absence of a vacuum made no difference, but the concentration was small compared with the concentrations found by others to have a bactericidal effect (14). Third, the small concentration of H_2O_2 did not contribute to the observed synergy between exposures at 222 and 254 nm.

We assume that synergy occurred, because, under suitable conditions of flow rate and bacterial concentration, some of the bacteria that survive 222-nm irradiation were damaged and were further killed by subsequent 254-nm radiation. Synergy between the sterilizing effects of irradiation at 222 and 254 nm in aqueous solutions has been established in the case of *E. coli*, *L. innocua*, and *S. putrefaciens* but at insignificant levels for the bacterial spores of *Bacillus* spp.

In conclusion, this study confirmed the feasibility of 254-nm radiation from low-pressure mercury lamps as a means of disinfecting clear water. Large bacterial reductions were achieved at high flow rates (60 liters/min) using maximum intensity optimized by focusing lateral radiation with elliptical reflectors of polished aluminum. When supplemented with excimer radiation at 222 nm, no additional bacterial kill was observed at flow rates high enough to be of value to industry. Excimer radiation at 172 nm had no measurable bactericidal action.

The design of elliptical reflectors within the mercury lamp housing is subject to patent application UK 9902773.2.

ACKNOWLEDGMENTS

This work was supported by Food Research Programme Projects FS1044 and FS1054 from the Ministry of Agriculture, Fisheries and Food, UK.

REFERENCES

1. Anonymous. 1988. Food irradiation: a technique for preserving and improving the quality of food. World Health Organization, Geneva.
2. Baird-Parker, A. C. 1994. Foods and microbiological risks. Microbiology 140:687-695.
3. Bousfield, I. J., J. L. Smith, and R. W. Trueman. 1973. The use of semi-automatic pipettes in the viable counting of bacteria. J. Appl. Bacteriol. 36:297-299.
4. Chang, J. C. H., S. F. Ossof, D. C. Lobe, M. H. Dorfman, C. M. Dumais, R. G. Qualls, and J. B. Johnson. 1985. UV inactivation of pathogenic and indicator microorganisms. Appl. Environ. Microbiol. 49:1361-1365.
5. Clavero, M. R. S., J. D. Monk, L. R. Beuchat, M. P. Doyle, and R.

- E. Brackett. 1994. Inactivation of *Escherichia coli* O157:H7, salmonellae and *Campylobacter jejuni* in raw ground beef by gamma irradiation. *Appl. Environ. Microbiol.* 60:2069–2075.
6. Franz, J., and A. Gagnaux. 1971. Sterilisation with ozone under extremely high sterilisation requirements. *Wasser Luft Betrieb.* 15:393–396.
7. Jeyamkondan, S., D. S. Jayas, and R. A. Holley. 1999. Pulsed electric field processing of foods. *J. Food Prot.* 62:1088–1096.
8. Jin-Gab, K., A. E. Yousef, and D. Sandhya. 1999. Application of ozone for enhancing the microbiological safety and quality of foods. *J. Food Prot.* 62:1071–1087.
9. Kogelschatz, U. 1993. UV production in dielectric barrier discharges for pollution control, p. 339–354. In B. M. Penetrante and S. E. Schultheis (ed.), *Non thermal plasma techniques for pollution control*, vol. G 34, part B. NATO ASI Series. Springer-Verlag, Berlin.
10. Lorenzo-Lorenzo, M. J., M. E. Ares-Mazas, I. Villacorta-Martinez de Maturana, and D. Duran-Oreiro. 1993. Effect of ultraviolet disinfection of drinking water on the viability of *Cryptosporidium parvum* oocysts. *J. Parasitol.* 79:67–70.
11. Oppenländer, T., G. Baum, W. Egle, and T. Hennig. 1994. Novel vacuum-UV-(VUV) and UV-excimer flow through photoreactors for waste water treatment and for wavelength-selective photochemistry. In *Proceedings of the Third International Conference on Solar Energy Storage and Applied Photochemistry*, Cairo, Egypt.
12. Pebody, R. G., C. Furtado, A. Rojas, N. McCarthy, G. Nylen, P. Ruutu, T. Leino, R. Chalmers, B. de Jong, M. Donnelly, I. Fisher, I. C. Gilham, L. Graverson, T. Cheasty, G. Willshaw, M. Navarro, R. Salmon, P. Leinikki, P. Wall, and C. Bartlett. 1999. An international outbreak of Vero cytotoxin-producing *Escherichia coli* O157 infection amongst tourists: a challenge for the European infectious disease surveillance network. *Epidemiol. Infect.* 123:217–223.
13. Vego-Mercado, H., U. P. Pothakamury, F. J. Chang, G. V. Barbosa-Canovas, and B. G. Swanson. 1996. Inactivation of *Escherichia coli* by combining pH, ionic strength, and pulsed electric fields hurdles. *Food Res. Int.* 29:119–129.
14. Waites, W. M., S. E. Harding, D. R. Fowler, S. H. Jones, D. Shaw, and M. Martin. 1988. The destruction of spores of *Bacillus subtilis* by the combined effects of hydrogen peroxide and ultra-violet light. *Lett. Appl. Microbiol.* 7:139–140.