Mat Res Innovat (2002) 6:291–295
DOI 10.1007/s10019-002-0214-2

ORIGINAL ARTICLE

**Kelli Hoover · Mahesh Bhardwaj · Nancy Ostiguy
Owen Thompson**

Destruction of bacterial spores by phenomenally high efficiency non-contact ultrasonic transducers

Received: 2 October 2002 / Revised: 28 October 2002 / Accepted: 31 October 2002 / Published online: 23 November 2002

Abstract Conventional wisdom stipulates that high power ultrasound without direct or indirect transducer contact with the medium to be treated is not possible. This seemingly correct notion is based upon two major hurdles: inefficient transmission of ultrasound from the piezoelectric material into air/gases and exorbitant attenuation of ultrasound by gases. The latter is a natural phenomenon about which nothing can be done, and the former requires an un-conventional approach to transducer design. After many years of R& D in this area, we have finally succeeded in producing transducers that generate immense acoustic pressure in air in the frequency range of ~50 kHz→10 MHz. By using these transducers without any contact with the material, we demonstrate destruction of 99.9% of dried bacterial spore samples of a close relative of anthrax, *Bacillus thuringiensis*. Following further refinement of the transducers and the mechanism of their excitation, we anticipate that non-contact ultrasound will have numerous applications including inactivation of agents of bioterrorism and sterilization of medical and surgical equipment, food materials, and airduct systems of buildings, airplanes, space stations, and others.

Keywords Ultrasound · Non-contact · Air-coupled · Power · Germicide · Bacteria · Bioterrorism · *Bacillus thuringiensis*

Introduction and significance of non-contact ultrasound

Since its first practical use for detection of underwater objects in the U.K. [1], non-destructive and non-invasive applications of ultrasound have advanced significantly. Low power ultrasound is widely used for non-destructive evaluation of industrial materials for defect, microstructure, and property characterization, as well as in medical diagnostics for fetus development and tissue analysis. High power ultrasound is used for cell disruption, particle size reduction, welding, vaporization, etc. It is being further developed for chemical reaction acceleration, invasive and non-invasive therapeutics and surgical procedures, and levitation.

A common denominator of all conventional applications of ultrasound is that the ultrasound source – the transducer – is physically coupled, either directly or indirectly to the medium to be tested or treated. Generally, the coupling agents are liquids such as water, oils, gels, or grease. Physical coupling is necessary in order to efficiently transmit ultrasound in the materials. Despite the obvious value of ultrasound, its ***modus operandi*** is severely stifled by the necessity of physical contact of the transducer to the medium. Elimination of contact will facilitate:

a. The analysis of green, un-polymerized, liquid-sensitive, porous, and other materials, or when contact is simply a nuisance. Non-invasive medical diagnostics when contact with a patient is harmful or painful.
b. Destruction of unwanted germs in the environment or containers and surface and subsurface treatment of wounds, scars, malignant tissue, etc.
c. Sterilization of medical, food, and pharmaceutical components and equipment.
d. Disinfection and decontamination of foods.

While Non-Contact Ultrasound (NCU) is highly desirable, in practical terms it is extremely difficult to accomplish. The natural barrier to NCU is the gross attenuation of ultrasound (particularly, in frequencies >100 kHz) by deformed and atomically weak media such as gases. Since it is a natural phenomenon, there is nothing one can do about it. Another impediment to NCU is the exorbitant acoustic impedance mismatch between the piezo-

K. Hoover · N. Ostiguy · O. Thompson
Department of Entomology, 501 ASI, University Park,
PA 16802 USA,

M. Bhardwaj (✉)
Ultran Laboratories, Inc., Boalsburg, PA 16827 USA,
Phone: +1-814-4666200
e-mail: mcb@ultranlabs.com

electric materials and air, which can run as high as six orders of magnitude. Consequently, nearly all of the ultrasonic energy is reflected back into the piezoelectric material and air interface, thereby generating a non-usable ultrasound field in air. Due to the extremely high frequency-dependence of ultrasound attenuation by air, this problem is further exacerbated in the MHz frequencies [2]. In this context, NCU has been generally considered an impossible dream. Nevertheless, the desire to generate high power ultrasound in air/gases has not abated.

There are two ways by which high power ultrasound can be generated in air/gases: by applying extremely high voltage to the transducer, or by designing a transducer that is inherently characterized by very high transmission in gases. The former, for obvious reasons, is dangerous and detrimental to the transducer. The latter requires development and applications of suitable acoustic impedance (Z) matching layers on a high coupling and high electro-mechanical efficiency piezoelectric material. After nearly 20 years of intense struggle, we have finally succeeded in developing such a transducer, which is at the center stage of bacterial spore destruction by NCU mode.

High power non-contact ultrasonic transducers

The efficiency of an ultrasonic transducer is dependent on the coupling co-efficients and other electro-mechanical properties of the piezoelectric material. It also depends upon the mechanism by which ultrasound is transferred from the piezoelectric material to the medium in which ultrasound needs to be propagated. In the non-contact mode, this medium is air. Since the acoustic impedances of piezoelectric materials (PZTs) are several orders of magnitude higher than that of air, it is usually necessary to implant transitional (acoustic impedance matching) layers of various materials in front of it (the piezoelectric material). Ultimately it is the characteristics of the final layer that determines the transduction efficiency of a transducer device. Since the properties of a given piezoelectric material can be considered constant for a given device, the transfer of ultrasonic energy in air is entirely controlled by the acoustic and other characteristics of the final matching layer on the piezoelectric material. Since the early 1980's a few transducer researchers, including one of us (MB), have used a number of non-porous, porous, and filled polymers of low Z as the final matching layer on PZT in order to enhance transduction efficiency in air [2]. These transducer designs increase the ultrasound transmission efficiency in air, but they are far from being capable of generating high power in air.

Based upon compressed fiber as the final matching layer, in 1997 we succeeded in producing transducers that are characterized by 100% transmission of ultrasound into air from the matching layer [3]. Known as NCU transducers, these devices generate immense acoustic pressures in ambient air. For example, pressures from 20 Pa/V→150 Pa/V have been observed between 100 kHz–4 MHz frequencies. Despite the high attenuation of ultrasound by air, these acoustic pressures are substantial, though smaller than for similar transducers in water (1 KPa/V→5 KPa/V). Figure 1 shows acoustic pressures for some NCU transducers as a function of transducer to reflecting target distance in ambient air. This acoustic pressure represents the integrated response over the entire area of the transducer. It does not specifically define localized pressure/temperature that is generated in the axial compressional and transversal nodal zones in the field of ultrasound.

Since their invention, the devices based upon this design have been successfully made and applied for a variety of gas flow metering, industrial non-destructive and bio-medical non-invasive applications [4, 5, 6, 7, 8]. In this paper, for the first time, we are pleased to introduce a non-contact ultrasound power application aimed at the destruction of deadly bacterial spores. It should be evident that this breakthrough has opened not only another source of power, but also one that potentially has numerous uses.

Methods for destruction of microbial pathogens

Disease-causing microorganisms can be highly resistant to killing and can exhibit high toxicity in low numbers, making it difficult to control human exposure through air delivered mechanisms. For example, bioterrorism in the form of aerosolized anthrax spores (*Bacillus anthracis)* passing through the mail presents a serious challenge for the postal services; as a consequence of this threat to human health, mail to governmental offices must be decontaminated before it can be delivered, often resulting in long delivery delays. A search of the literature using the databases PubMed, CAT, and Proquest revealed that the technologies currently available to accomplish decontamination of microbes have significant limitations, in part because organisms like *Bacillus* spores, protozoan cysts, and some viruses are resistant to drying, heat, ultraviolet light, gamma radiation, and many disinfectants [9]. For example, radiation from sources such as Cobalt 60, electron beams, and X-rays can be used to destroy bacterial spores [10], but a large stationary concrete reinforced facility is needed to protect workers and exposure times to hazardous radiation can be lengthy. UV can inactivate microbes on surfaces, but has very limited penetration ability [11, 12]. Sonication (high power low frequency ultrasound with water as the wave carrier medium) has been demonstrated to destroy bacterial spores [13, 14], but for obvious reasons, it would not be useful for applications in which contact with the material to be treated is impractical. Thus, the ability to deal with the threat posed by dangerous airborne microbes has been hampered by limitations of the current technologies. To this effect we investigated the ability of new high efficiency NCU transducers to destroy bacterial spores.

In order to destroy biological compounds, an extremely high intensity ultrasound field is needed in the carrier medium before it reaches the material to be treated. High intensity ultrasound fields can be created by ei-

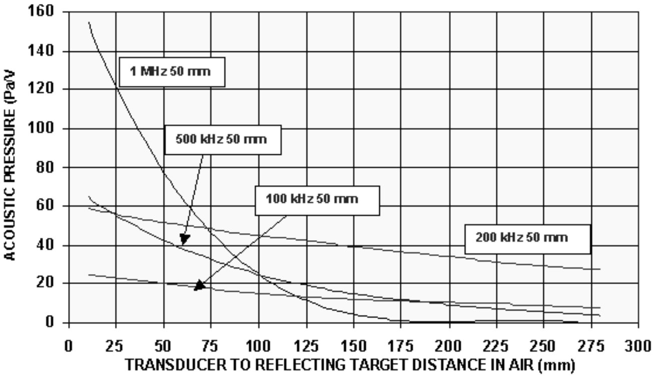

Fig. 1 Acoustic pressures for some NCU transducers as a function of transducer to reflecting target distance in ambient air. This acoustic pressure represents the integrated response over the entire area of the transducer. It does not specifically define localized pressure/temperature that is generated in the axial compressional and transversal nodal zones in the field of ultrasound

ther placing the transducer with liquid contact on the material to be treated, or by using water as the carrier of ultrasound. However, achievement of similar effects by using air as the carrier medium is difficult, as described above. In order for NCU to be practical for diagnostic or for power applications the transducers must be characterized by very high transmission efficiency in air, which is the main characteristic of NCU transducers [2, 3]. We hypothesized that sufficient power could be generated with NCU transducers to transmit ultrasound through air to destroy highly resistant bacterial spores.

Experimental procedure

To determine the feasibility of NCU to kill bacterial spores, a series of experiments were conducted in which lyophilized *Bacllius thuringiensis kurstaki* (Bt) spores were irradiated with NCU transducers in the frequency range of 70 kHz–200 kHz, varying the exposure time from 10–180 seconds. We used lyophilized (freeze-dried) spores of Bt as a model for evaluating destruction of anthrax spores because it is very closely related to *B. anthracis*, the causal agent of anthrax, and it is safe to work with outside of a biocontainment facility. *B. anthracis* and Bt can be distinguished by only a few plasmid genes that code for proteins toxic to humans and insects, respectively [15].

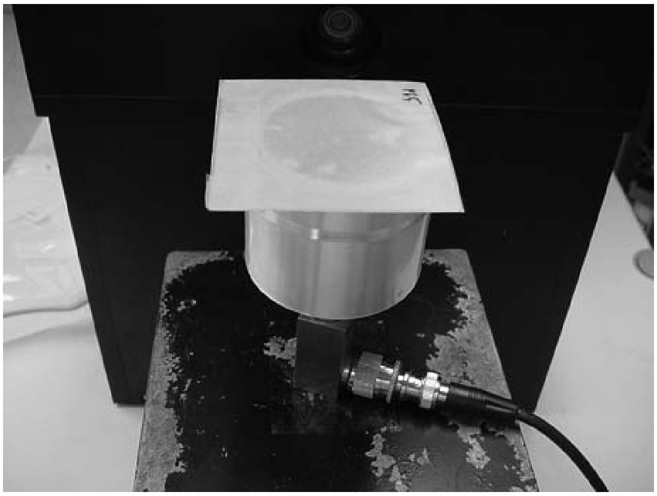

Fig. 2 Non-Contact Ultrasound transducer with an envelope containing spores of *Bacillus thuringiensis* in treatment position. The space between the envelope and the transducer is approximately 3 mm. Note that there is no medium present to provide any contact between the envelope and the transducer

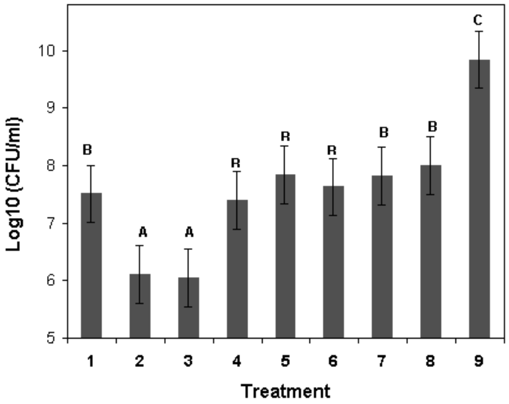

Fig. 3 The number of viable *Bacillus thuringiensis kurstaki* (Bt) spores following treatment with pulsed non-contact ultrasound varying in frequency is presented as $\log_{10}$ colony forming units per ml ($\log_{10}$ CFU/ml). Treatments 1–4: were treated for 10, 30, 60 or 180 seconds, respectively at a frequency of 93 kHz; Treatments 5–8 were treated for 10, 30, 60 or 180 seconds, respectively, at a frequency of 161 kHz. Treatment 9: Control. Error bars represent the average percentage standard error of the means (3%); treatments were replicated three times. Means were compared by one-way ANOVA ($F = 42$, $df = 8, 18$, $p < 0.0001$) and means separation was performed by the Newman-Keuls test. Bars labeled with a different capital letter are significantly different at the 0.05 level. Spores of Bt were grown at 25 °C on a shaking incubator in LB broth [19], washed in sterile MilliQ water and lyophilized before treatment. After treatment, spores were suspended in sterile MilliQ water on a weight to volume basis and plated in serial dilutions on LB agar followed by incubation for 2 days at 25 °C

Spores of Bt were grown at 25 °C on a shaking incubator in LB broth [16], washed in sterile MilliQ water and lyophilized before treatment. After treatment, spores were suspended in sterile MilliQ water on a weight to volume basis and plated in serial dilutions on LB agar followed by incubation for 2 days at 25 °C. Spore samples were sealed in weigh paper envelopes and irradiated with ultrasound at a distance of 3 mm from the transducer surface in ambient air (Fig. 2). For all treatments, the NCU transducers (50 mm active area diameter with planar geometrical configuration) were excited in pulsed mode with a 50 dB power amplifier, generating approximately 10 MPa of acoustic pressure in ambient air. The acoustic pressure estimation is based upon transducer excitation by 2000 bursts of 0.25 V sine wave augmented with another 50 dB amplification. In all experiments the pulse repetition rate was kept at 50 ms.

Observations and inferences

Based upon the procedure described in the previous section, inactivation of spores was determined as a function of non-irradiated control samples.The percentage inactivation of spores ranged from 98.12% to 99.99% at 161 and 93 kHz, respectively (Fig. 3). Exposure at 93 kHz inactivated 99.99% of the spores (Fig. 3), which represents a reduction in spore loads of 4900-fold and 6500-fold following 30 and 60 seconds of exposure, respectively. This exceeds the requirement of 90% inactivation (D_{90}) for evaluating methods to kill pathogens in food and on medical devices [10, 17]. Treatment for longer than 60 seconds did not improve the efficacy.

Scanning electron micrographs of treated and untreated spore samples were prepared to examine the impact of NCU on the structure of the spores. Lyophilized samples of the ultrasound treated and untreated spores were directly transferred to conductive carbon tabs adhered to aluminum stubs (EMS, Fort Washington, PA), sputter-coated with 20 nm of Au/Pd in a BAL-TEC SCD 050 sputter-coater (Techno Trade, Manchester, NH), and imaged at 20 kV accelerating voltage using a JSM 5400 SEM, (JEOL USA, Peabody, MA).

Potential mechanism of spore destruction in NCU mode

Interpretation of the SEM images suggested to us that treatment with NCU fragmented the spores and fused them together (Fig. 4), as if the physical and/or physiological nature of the spores had been altered. This observation would not be inconsistent with the lethal effect of high pressure used to inactivate microbes during food preservation [18]. It is generally thought that high pressure induces a number of changes to microbes' morphology, biochemical reactions, genetic mechanisms, and cell wall morphology. High intensity acoustic energy has been shown to agglomerate fine particles suspended in a fluid (presumably, also including gases) into larger particles [19]. During this process, it is possible that sufficient mechanical energy is released to alter the original nature of the spores.

In a separate experiment we have observed regions of intense ultrasonic fields in air not only in the direction of propagation from the face of the transducer, but also perpendicular to it (Fig. 5). This experiment was performed by placing 120 μm SiC particles on the surface of a high power 500 kHz ultrasonic transducer. The regions where particles have coalesced in the nodal regions close to and on the surface of the transducer are shown in Fig. 6. We strongly suspect that the spores are caught between the axial compressional and transversal nodal zones where intense acoustic pressures and high temperatures due to friction between the particles exist – eventually damaging the original nature of the spores. While this is just a hypothesis, the ultimate reason for the lethality of ultrasound, including the frequency-dependent effects on microbes in non-contact mode is still a subject of inquiry.

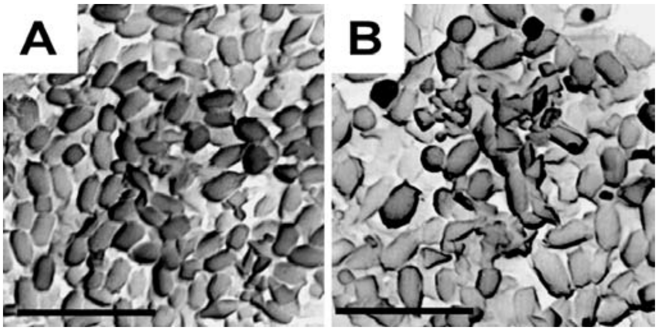

Fig. 4 Scanning electron micrographs of spores at a magnification of 5,000x treated with NCU at 93 kHz for 30 seconds. 1 bar = 5 μm. Irradiated spores are on the left (A), non-irradiated spores on the right (B). Observe the fusion of spores in the irradiated image. These images were recorded on Polaroid 55 film and archived using PGT's IMIX software (IMIX-PC v.10, Princeton, NJ)

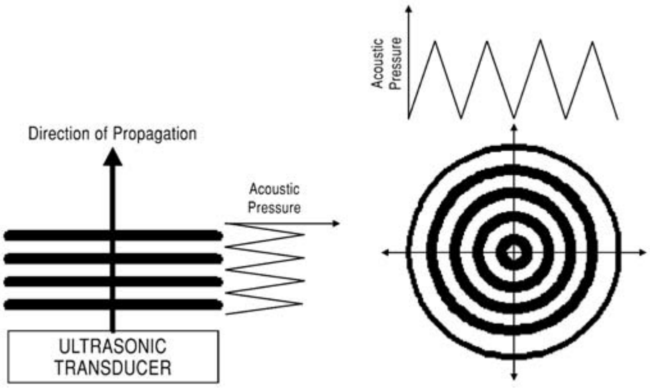

Fig. 5 Particle motion from an ultrasonic transducer. LEFT: Particle motion in the direction of ultrasound propagation creating the regions of compression (dark bands) and of rarefaction (clear bands). The former and latter are characterized by high and low acoustic pressure zones, respectively. The distance between two adjacent peaks or troughs corresponds to one wavelength in the medium of ultrasound propagation. For 100 kHz in air it is 3.4 mm. RIGHT: Particle motion in the direction perpendicular to the direction of propagation, i.e., on the transducer face or close to it. This creates nodes (dark circles) and anti-nodes (clear circles) in the medium of ultrasound propagation. The former and latter are characterized by high and low acoustic pressure zones, respectively. The distance between two adjacent rings corresponds to one wavelength in the medium. For 100 kHz in air it is 3.4 mm. When the transducer operates at high power, the zones of compression (axial direction) and nodes (transverse direction) where particles coalesce (see Fig. 6)

Conclusion

Traditional contact ultrasound is commonly used to test materials for defects and properties, and to examine the human body for diagnostic evaluations, e.g., to image a fetus, malignant tissue, or kidney stones. Contact ultrasound procedures, however, have a significant limitation in that they require a coupling medium, such as water or gel, between the transducer and test specimen. Physical coupling is necessary to increase ultrasound transmission

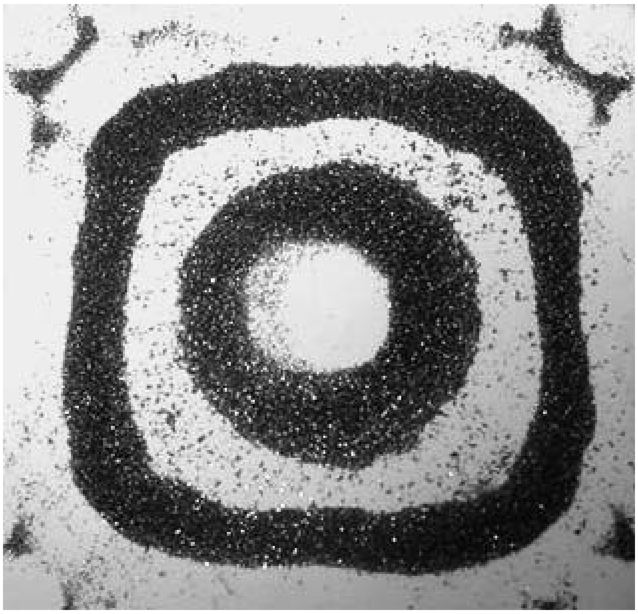

Fig. 6 Nodes and anti-nodes demonstrated by the particle vibration on the surface of a quasi-circular high power transducer. Note the pattern created when the particles coalesced in the nodal regions close to and on the surface of the transducer are shown. Not shown here is the similar particle motion that also occurs in air. This digital photographic image was created by placing 120 µm SiC particles on the surface of a high power 500 kHz ultrasonic transducer

efficiency and to circumvent the attenuation caused by air between the transducer and the test medium [2]. In order for NCU to be practical for diagnostic or for power applications the transducers must be characterized by very high transmission efficiency in air. Such transducers have been successfully produced. They generate 20 Pa/V→150 Pa/V of acoustic pressure in ambient air in frequencies from <50 kHz→10 MHz. Despite extremely high attenuation of ultrasound by air, these acoustic pressures are substantial, though smaller than for similar transducers in water (1 KPa/V→5 KPa/V). Although these pressures are considerable, we suspect that minimal to no shielding will be required for operators because the beam spread is very small. For example, for a 100 kHz and 50 mm active area diameter transducer, it <5° and occurs at a distance of >180 mm away from the transducer face in ambient air. Furthermore, before any deleterious effects could occur, practically all of the ultrasonic energy is absorbed by the material to be irradiated.

The task of reducing transmission losses in air, to the extent that the efficiency of the transducer device is ultimately high enough for diagnostic and power applications, has captivated the imagination. Following further refinement of the NCU parameters, including the mechanism of transducer excitation, we anticipate that this technology has the potential to be used as a general germicide for the sterilization of medical and surgical equipment and food materials, in addition to destruction of anthrax spores and other highly toxic microbes that may be used as an agent of bioterrorism. The method described in this paper also has the potential to be used in large-scale situations, such as in decontamination of airduct systems of buildings, airplanes, and space stations.

Acknowledgements We thank Mike Grove for technical support and Rosemary Walsh, Former Director of Research Projects, EM Facility, Life Sciences Consortium at Penn State, for the EM photos. We also gratefully acknowledge the assistance of Bernard Hosten and Christine Biateau of Laboratoire de Mecanique Physique, Universite Bordeaux, France for measuring the absolute acoustic pressure of reference NCU transducers. Correspondence and requests for materials should be addressed to Kelli Hoover at kxh25@psu.edu or Mahesh Bhardwaj at mcb@ultranlabs.com.

References

1. Richardson RL (1913) British Patent 11
2 Bhardwaj MC (2002) Non-destructive evaluation: Introduction of non-contact ultrasound. In: Schwartz, M (ed) Encyclopedia of Smart Materials. John Wiley and Sons, NY, pp 690–614
3. Bhardwaj MC (2001) U.S. Patent 6 311 573
4. Brassier P, Hosten B, Vulovic F (2001) Flow Measurements & Instrumentation 12: 201–211
5. Carneim T, Green DJ, and Bhardwaj MC (1999) Cer Bull 1: 88–94
6. Jones JP, Lee D, Bhardwaj M, Venderkam V, and Achauer B (1997) Acoust Imaging 23: 89
7. Tittmann BR, Bhardwaj M, Neeson I, and Vandervalk L (1999) Non-contact ultrasonic NDE of carbon/carbon composite. In: Ceramic Engineering and Science Proceedings, 23rd Annual Conference on Composites, Advanced Ceramics, Materials, and Structures. The American Ceramic Society, Westerville, OH
8. Vun RY, Wu C, Bhardwaj M, Stead G (2000) Through-thickness ultrasonic transmission properties of oriented strandboard. In: Proceedings of the 12th International Symposium on non-destructive testing of wood, University of Western Hungary, Sopron
9. Watson A, Keir D (1994) Epidemiol Infect 113: 479
10. Grecz N, Brannon RB, Killgore G (1987) Am J Infect Control 15: 101
11. Warriner K, Rysstad G, Murden A, Rumsby P, Thomas D, Waites WM (2000) J Food Protect 63: 753
12. Warriner K, Rysstad G, Murden A, Rumsby P, Thomas D, Waites WM (2000) J Appl Microbiol 88: 678
13. Chandler DP, Brown J, Bruckner-Lea CJ, Olson L, Posakony GJ, Stults JR, Valentine NB, Bond LJ (2001) Anal Chem 73: 3784
14. Taylor MT, Belgrader P, Furman BJ, Pourahmadi F, Kovacs GTA, Northrup MA (2001) Anal Chem 73: 492
15. Helgason E, Okstad OA, Caugant DA, Johansen HA, Fouet A, Mock M, Hegna I, Kolsto A (2000) Appl Environ Microbiol 66(6): 2627
16. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular Cloning: a laboratory manual, 2nd edn. vol. 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor
17. Prestamo G, Arabas J, Fonberg-Broczek M, Arroyo G (2001) J Agric Food Chem 49: 2830
18. Chefte JC (1995) Food Sci Technol Int 1: 79
19. Gallego-Juarez JA (1994) New technologies in high power ultrasound industrial applications. In: Proceedings IEEE Ultrasonics Symposium, Cannes