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We report on the development of a novel, continuous-flow, radially focused ultrasonic disruptor capable of lysing *Bacillus* spores in the absence of added chemical denaturants, enzymes, or microparticles. Greater than 99% disruption was achieved for *Bacillus globigii* spores and *Escherichia coli* and *Bacillus subtilis* vegetative cells with sample residence times of 62, 12, and 12 s, respectively. Microscopic and SEM images indicated that at equivalent power levels, the incidence of cell death or loss of viability typically exceeded the efficiency of (visible) cell lysis. However, semiquantitative PCR showed up to a 1000-fold increase in intracellular DNA availability from ultrasonically disrupted spores, and liberated DNA was intact and available for subsequent detection.

The likelihood that foreign or domestic terrorists will use biological weapons with devastating consequences is both real and alarming; thus, significant research and development has been devoted to the development of aerosol sampling devices and whole-cell, protein, antibody, or nucleic acid-based detectors for biological pathogens, culminating in micro-PCR devices with real-time fluorescence detection of amplification products,[1] nucleic acid microarrays,[2−5] and mass spectrometry instruments for both protein and nucleic acid characterization.[6−8]

A fully integrated pathogen detection system, however, requires sample preparation functions, including spore/cell lysis, analyte isolation, concentration, and purification. There are now a multitude of nucleic acid isolation and purification techniques available for processing samples recovered from any source, some of which have been adapted for manual implementation in the field.[9] Many of these techniques, however, still require significant manual intervention (e.g., bead-mill homogenization, centrifugation, pipeting, vortexing, precipitation, filtration) and consumables, practical limitations that are especially relevant for the unattended, timely detection of biological warfare agents (or other microorganisms) in complex genetic and chemical backgrounds. With technologies under continued development for microbial and nucleic acid separation and detection in soils, wastewater, sediments, food, sludge, and other environmental matrixes, the primary technology gaps remaining for integrated biodetection devices that can interface with large-volume (solid, liquid, or gaseous) environmental samples are automated sample acquisition, cell concentration, and cell lysis from solid and aqueous samples.

To make nucleic acids available for subsequent manipulation, an integrated lysis device must be effective against the most refractory microorganisms, including protozoan cysts, fungal hyphae, Gram positive bacteria, and spores. Routine laboratory methods for cell lysis of these organisms include freeze/thaw, proteinase K, lysozyme, and guanidium salt treatments followed by ethanol or 2-propanol precipitation of liberated DNA; ballistic disintegration; or sonication in a static chamber at kilohertz (kHz) frequencies after cell (spore) pretreatment with detergent or other chemicals.[9−11] Ultrasonic horns operating at kilohertz frequencies are the primary ultrasonic treatment for many microbiological applications, and such devices have recently been developed for incorporation into portable nucleic acid analysis devices.[10,12] These previous reports describe rapid spore disruption in batch mode with spores either contained within a static chamber[10] or trapped behind a filter membrane.[12] However, these devices require a 90-min lysozyme pretreatment or the addition of glass beads in the ultrasonic chamber to effect spore disruption.

A continuous-flow cell disruption system would be advantageous for analyzing multiple samples in series or for the development of unattended biomonitoring systems. In the former application, continuous-flow cell disruption would allow the immediate

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delivery of a sample, without additives (enzymes, chemicals, particles) that complicate the sample processing (or detection) procedure, to a downstream detector. In the latter case, human intervention and disposable cartridges would be eliminated from the analytical process. The objective of this work, then, was to move upstream in the sample processing chain and develop a rapid, continuous-flow device capable of disrupting *Bacillus* spores and vegetative cells in the *absence* of added chemical denaturants, enzymes, or particles, with liberated nucleic acids available for subsequent nucleic acid purification and detection. The resulting device was designed to focus megahertz ultrasonic energy along the entire length of a flow channel. To the best of our knowledge, the cylindrically focused sonicator design and operating frequency are both novel analytical principles for spore disruption.

MATERIALS AND METHODS

Spore and Cell Preparations. *Bacillus globigii* (BG) spore suspensions were a generous gift from Dr. Mark Kingsley (Pacific Northwest National Laboratory) and originated from stock preparations maintained at Dugway Proving Grounds. These spores are part of a panel of standards for evaluating the performance of new bioagent instrumentation. Spores were resuspended in sterile water and subjected to several rounds of vigorous mixing, settling and decanting to eliminate spore clumps. Plate counts and microscopy were used to confirm the consistency of the stock spore suspension and verify that a single spore gave rise to a single colony. A suspension of 10^8 spores mL^{-1} was used for all ultrasound experiments. *Escherichia coli* DH5α was grown in LB broth (Difco, Detroit, MI), and vegetative *Bacillus subtilis*, *Bacillus aetrophaeus* and *Bacillus thuringiensis kurstaki* cells were cultivated in trypticase soy broth (TSB; Difco) according to standard procedures. Prior to ultrasonic treatment, cells were washed several times in phosphate-buffered saline (PBS; 8.1 g NaCl, 1.18 g NaH_2PO_4, 0.22 g $Na_2HPO_4 \cdot H_2O$/liter; pH 7.3) and diluted to a final concentration of 10^8 cells mL^{-1} in PBS.

Plate Counts and Microscopy. Plate counts were used to quantify cell or spore viability, and microscopy (optical or SEM) was used to assess physical damage to cells or spores. Ten-fold serial dilutions of spore suspensions (treated or untreated) were prepared in sterile water within 2 h of all lysis experiments; *E. coli* and *B. subtilis* were diluted in LB or TSB, respectively. Three × 20 μL aliquots were spotted directly onto LB or trypticase soy agar plates and incubated at 30 °C for 36 h. All sonication and plating experiments were performed in triplicate, resulting in at least 9 data points (spore or cell counts) for each treatment. Sonicator performance and efficacy were measured by comparing viable spore/cell concentrations before and after treatment. Cell or spore viability was calculated as $100(C_0 - C_{sonic})/C_0$; where C_0 is the average viable spore/cell count before ultrasonic treatment, and C_{sonic} is the average viable spore count after ultrasonic treatment. Differences between treatment means were assessed by analysis of variance (ANOVA).

Cell lysis was observed by direct microscopic analysis. Vegetative cells of *E. coli* and *Bacillus* were heat-fixed to glass slides, stained with methylene blue and observed at 1000× magnification under the phase-contrast microscope. Vegetative cells (and cell lysis) were quantified in a Petroff Hausser counting chamber at 400× according to standard procedures. Due to the small size of *Bacillus* spores and the tendency for spores to clump together, spores and spore lysis were (nonquantitatively) visualized by scanning electron microscopy (SEM).

Spore Surface Decontamination. The original *B. globigii* spore preparation had extracellular DNA on the spore coat that could be detected by PCR in the absence of any spore treatment, a common situation arising from spore preparation procedures (e.g., Belgrader et al.[10]). We, therefore, employed a spore surface decontamination procedure to remove or minimize the impact of extracellular DNA on PCR assessments of spore disruption and the release of intracellular DNA. Aliquots (800 μL) of BG spores were collected by centrifugation and resuspended in 200 μL of 10% sodium hypochlorite for 1−10 min. After decontamination, spores were recovered by centrifugation, washed extensively in sterile water, and then subjected to on-line ultrasonic treatment and PCR amplification as described below. Control experiments showed that the hypochlorite treatment had no effect on the physical appearance of spores, spore viability, or carryover of PCR inhibitors (not shown).

Sonicator Systems. A standard humidifier bath (Holmes Ultrasonic humidifier, model HM-460B, ∼10 W cm^{-2} peak power) served as the initial test bed for spore disruption in a 1 MHz sonic zone. Preliminary experiments were conducted with the sample contained within a 1-mL polypropylene tube and placed in a water bath within the region of maximum sonic energy. The same humidifier bath was used for initial flow-through ultrasonic treatments in conjunction with a standard sequential injection system (FIAlab 3000, FIAlab Instruments, Bellevue, WA) that included a 1-mL syringe pump (Cavro, Sunnyvale, CA) and a 10-port selection valve (Valco, Cheminert, Houston, TX). The flow-through lysis tube was 1.5-mm i.d./3.2-mm o.d. Teflon or PEEK tubing; other tubing was 0.8 mm i.d. Teflon.

Successful spore disruption in the flow-through humidifier configuration led to the development and testing of the novel, cylindrically focused sonicator design (Figure 1B). The cylindrically focused, flow-through lysis system was tested on Gram negative cells (*E. coli*), Gram positive vegetative cells (*B. subtilis*), and spores (*B. globigii*). A Hewlett-Packard 33120A 15 MHz function/arbitrary waveform generator and ENI A-300 rf power amplifier were used for driving a 1.4 MHz cylindrical piezoelectric transducer. The piezoelectric element was a 160° segment of a 34-mm-diameter tube, with a 1.5-mm wall thickness. The transducer was cut to 3.5 cm in length and mounted in high-density foam, and a 1.5-mm i.d. flow-through tube was placed along the length of the transducer. Input frequency was monitored with a Tektronix TDS 460-A 4-channel, digitizing oscilloscope, and in-tube temperatures were recorded using a digital-output thermocouple. Power densities were determined using a calibrated hydrophone (Specialty Engineering Associates, Soquel, CA; model SPRH-S-1000) by centering the hydrophone 15 mm above the transducer (location of maximum power). The linear correlation between input voltage and power density was measured from 0.15 W cm^{-2} to 1.8 W cm^{-2} and extrapolated to higher energies, because the generation of bubbles interfered with direct power measurements at the higher energies.

Experimental variables tested in batch mode included residence time and microparticle amendments. Experimental variables tested in flow-through mode included tubing material, air bubble amendments, flow rate, power, and distance from the piezoelectric

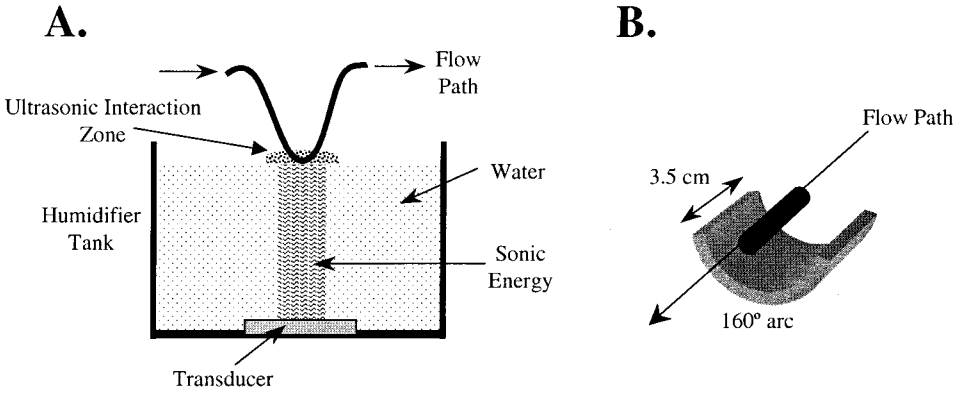

Figure 1. (A) Initial experiments with the flow-through lysis concept, utilizing a FIAlab flow injection system and MHz sonic zone. (B) The cylindrically focused, flow-through sonicator design.

transducer. Explanations of individual tests are provided in the Results section. The ultrasonic devices used in this study are capable of generating several watts cm^{-2} ultrasonic energy, which may result in cell/tissue damage and ultrasonic "burns"; therefore, several safety precautions were routinely employed for all of the experiments. Operator contact with the region of maximum ultrasonic energy was avoided, and safety glasses were always worn when the devices were in operation. The transducers were always operated in a water bath for both ultrasonic coupling to the reaction tube and heat dissipation. Care was taken not to contact the electrical output signal from the power amplifier, because 600 W and several hundred volts could be delivered to the operator, depending on the electrical load of the system.

PCR Detection. DNA availability after ultrasonic treatment was assessed by a dilution-to-extinction PCR method.[13] Genomic DNA (for positive controls) was isolated from *B. globigii* spores by bead-mill homogenization and quantified by UV spectrometry. PCR primer sequences Bg215f (5′-ACCAGACAATGCTCGACGTT) and Bg345r (5′-CCCTCTTGAAATTCCCGAAT), targeting *recF*, were kindly provided by Dr. Gary Long (Navy Medical Research Institute; NMRI) and synthesized by Keystone Labs (Camarillo, CA). PCR amplification was carried out in 25 μL, total volume, utilizing an MJ Research (Watertown, MA) Tetrad thermal cycler and 0.2-mL thin-walled reaction tubes. Spore preparations (sonicated or untreated) were serial diluted in a 10-fold series immediately prior to PCR, and purified *B. globigii* DNA was serially diluted in PCR-grade water at 100 pg to 1 fg μL^{-1} as a positive control template. Final reaction conditions were 5 μL of DNA or sonicated/untreated spore preparation, 10 mM Tris pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 200 μM each dNTP, 0.2 μM each primer, and 0.625 U LD-*Taq* polymerase (Perkin-Elmer, Foster City, CA), which had been pretreated with TaqStart antibody at the recommended concentration (Sigma, St. Louis, MO). Assembled reactions were amplified with 45 cycles at 94 °C for 15 s, 56 °C for 30 s, and 72 °C for 30 s with a 2-s extension per cycle. The entire contents of each PCR were analyzed on 1% NuSieve and 1% Seakem GTG agarose (FMC Bioproducts, Rockland, ME) gels run at 100 V for 2 h in 1× TAE running buffer, both containing ethidium bromide. Gel images were captured using a BioRad (Hercules, CA) Fluor-S imager and Molecular Analyst software.

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Table 1. Percent Loss of Viability of BG Spores after Treatment in a 1 MHz Sonic Field[a]

4 × 30 s with[b]	vertical	av temp ± SD, °C
no beads	99.4	104 ± 3
glass beads	99.6	104 ± 1
magnetic beads	99.1	103 ± 2

[a] Values are the geometric mean from three trials and nine plate counts. [b]60 mg of 50-μm glass microspheres (Potters Industry, Inc., Cleveland, OH) or 40 μg of paramagnetic particles (MagneSphere, Promega Corp., Madison, WI) were added to 200 μL of spore suspension to induce bubble formation for cavitation-like phenomena or to enhance collision rates.

RESULTS AND DISCUSSION

Proof-of-Concept. Batch experiments were conducted to determine the conditions (time, frequency, amendments) that were required to disrupt spores with 1 MHz ultrasound. Experiments were performed in standard polypropylene microfuge tubes and a 1 MHz sonic zone generated using a household ultrasonic humidifier bath that harbored a planar transducer. Glass or paramagnetic particles were included to address the inertial cavitation and bubble nucleation lysis mechanism proposed by others.[10] The tube was placed at the base of the "ultrasonic fountain" of atomized water droplets in the humidifier tank. Results from these experiments (Table 1) clearly indicate that spore disruption and death can be obtained in a megahertz sonic zone. The batch system also caused up to a 5-log reduction in cell viability for vegetative *E. coli*, *B. aetrophaeus*, and *B. thuringiensis kurstaki* cells with extensive cell lysis, as determined by phase-contrast microscopy (not shown). The presence or absence of microparticles had no appreciable effect on spore death or in-tube temperature, suggesting that the added particles were acting neither as an energy sink nor as a primary microbubble source for spore disruption during the course of the experiment. All subsequent experiments and device development were, therefore, performed without microparticles.

The physical geometry of a microfuge tube (i.e., thick bottom, thinner sides), atomization of the coupling fluid (water) in a discrete 3-dimensional zone, and in-tube temperature variations among multiple replicates suggested that the angle of incident energy, degree of immersion in the sonic zone, ultrasonic coupling efficiency, and resulting thermal effects may be important elements of ultrasonic disruption at the megahertz frequency. We,

Table 2. Percent Loss of Viability for BG Spores with a Planar Transducer and Flow-Through System (Figure 1A)[a]

	no air	plus air
	Teflon Tubing	
1 μL s^{-1}	82.4	99.3
5 μL s^{-1}	21.2	72.0
	PEEK Tubing	
1 μL s^{-1}	90.4	88.9

[a] *B. globigii* spores (200 μl at 10^8 spores mL^{-1}) were continuously delivered through a 5-mm interaction zone (9 μL volume) at the indicated flow rates. The sequential injection system was used to introduce air by first alternately stacking zones of 15 μL of air and 10 μL of spore suspension and then delivering the air/spore sample to the lysis device at a constant flow rate.

Table 3. Lysis Efficiency for Vegetative *E. coli* and *B. subtilis* Cells Relative to Untreated Controls

power density W cm^{-2}	organism	%[a] viability	%[b] lysis	temp range[c] °C
0.3	*E. coli*	96	20	26−37
	B. subtilis	79	ND	
0.5	*E. coli*	76	32	29−37
0.7	*E. coli*	<1	72	34−47
	B. subtilis	13	21	
0.9	*E. coli*	0	82	41−63
1.2	*B. subtilis*	<1	ND[d]	43−90
1.8	*B. subtilis*	0	4	60−115

[a] Cells were subjected to a 1.4 MHz sonic field at a flow rate of 5 μL s^{-1} (12-s sample residence time) in a cylindrically focused device (Figure 1B). At power densities >4.6 W cm^{-2}, all cells were visibly lysed, including other species of *Bacillus* (not shown). Percent viability was assessed by plate count and represents the average of three trials and three enumerations. [b] Percent lysis efficiency is the mean from three trials and was assessed by direct microscopic counts in a Petroff-Hausser counting chamber. [c] Temperature ranges are the in-tube temperatures during the lysis experiment as manually measured with a thermocouple probe. [d] ND = not determined. Large temperature spikes at power densities >1 W cm^{-2} were due to air bubbles passing by the thermocouple, which resulted in the large temperature ranges for these experiments.

therefore, varied the angle of the microfuge tube relative to the transducer and assessed spore death versus sonication time. We easily achieved 90% spore disruption and (usually) >99% reduction in spore viability regardless of the angle of incident energy. At 2 min of continuous lysis with the incident energy transmitted through thinner sections of polypropylene, we achieved 3−4 log reductions in spore viability. At 4 min of continuous power, all of the tubes melted, precluding further analysis of time-dependent effects. Nonetheless, these results suggested that efficient spore and cell disruption could be achieved through fairly thin plastic containers without equipment failure and prompted us to investigate a flow-through lysis configuration with the planar transducer.

Flow-Through Lysis. The first flow-through sonicator design is illustrated in Figure 1A. The spore/sonicator interaction zone encompassed ~5 mm of the flow path (9 μL). The maximum power density delivered by the humidifier was 10 W cm^{-2}, but peak power density occurred at a very small point in the humidifier tank (<1 μL volume). Calibrated hydrophone measurements indicated that the rest of the flow path (outside the 1 μL interaction zone) was exposed to <0.8 W cm^{-2}. At 1−5 μL s^{-1}, the spore or cell suspension was exposed to the zone of maximum energy for only 1.8−9 s, as opposed to the 1−2 min of exposure employed in batch experiments. We also introduced 15 μL air segments at every 10 μL of spore suspension to mimic the air/liquid interface present in batch mode. Results from these experiments are shown in Table 2 that demonstrate that a megahertz sonic zone can rapidly effect spore disruption in a flow-through format.

Air segmentation appeared to enhance spore disruption efficiency in Teflon tubing, but this interaction was not pursued because Teflon tubing likewise melted during the course of the trials (40−200 s continuous power). PEEK tubing, on the other hand, withstood the sonic energy and maintained in-tube temperatures near 100 °C, but air segmentation had no significant effect on spore viability. Hydrophone measurements did not show the characteristic energy spikes indicative of inertial cavitation. Control plating experiments (no ultrasonic treatment) showed that BG spores were not retained within the fluidics system, such that all plate counts associated with Table 2 reflect treatment effects on BG spores rather than spore loss or carryover during lysis trials. On the basis of these results, all further experiments were conducted in PEEK tubing in the absence of any air segmentation or microbubble amendments.

Novel Cylindrically Focused Sonicator. Results in Table 2 clearly show that residence time in the sonic zone is an important variable for effective cell and spore disruption. We, therefore, designed a cylindrically focused device (Figure 1B), wherein the sonic zone is radially focused at a distance *along* the linear flow path, throughout the entire length of the transducer (3.5 cm). Radially focusing the sonic energy along a flow path is a truly novel sonicator design that enables most of the sonic energy to be applied to and focused on the fluid channel. Sample residence time in the ultrasonic field is, therefore, a function of flow rate and transducer length, with no disruption of continuous-flow operation. The cylindrically focused design may be scaled (up or down) to accommodate flow channels of various size (from industrial scale processing to microfabricated chips).

In this device, a flow rate of 1 μL s^{-1} resulted in a spore/cell exposure time of 62 s (spore treatments), but a flow rate of 5 μL s^{-1} resulted in an exposure time of 12.4 s (vegetative cell experiments). Initial results with vegetative cells of *E. coli* and *B. subtilis* are shown in Table 3. Interestingly, 100% cell death (or loss of viability) was achieved at much lower energies than 100% cell lysis, an observation that may be related to in-tube temperatures and a characteristic heat shock response in bacteria (especially vegetative cells). However, significant cell lysis did occur at low ultrasound energies (0.3 to 0.5 W cm^{-2} average power density; Table 3) in which the in-tube temperature remained at or below 37 °C.

To further examine the effects of incident energy on spore disruption and death, we examined flow rate, input power levels, and flow-channel distance from the transducer. Table 4 summarizes results from these experiments, and again shows that near 100% reduction in spore viability is possible after continuous flow in a megahertz sonic zone and average power densities ranging from 4.5 to 8.3 W cm^{-2}. Analysis of variance showed a significant ($p = 0.0004$) interaction between distance from the transducer and loss in viability indicating that optimal spore disruption depends on the particular flow-cell design. Results in Table 4 also

Table 4. Effect of Distance from the Transducer on Spore Viability[a]

distance	power, W cm^{-2}	loss in viability	in-tube temp, °C
15 mm	4.5	99.7	78−101
	5.6	99.9	100−101
	6.9	99.8	100−101
	8.3	99.2	100−102
20 mm	4.5	99.6	95−103
	5.6	98.7	95−104
	6.9	97.8	101−106
	8.3	98.1	101−105
26 mm	4.5	88.3	45−72
	5.6	97.8	80−92
	6.9	99.7	95−101
	8.3	99.9	99−101

[a] *B. globigii* spores were treated using the flow-through cylindrically focused device at 1.4 MHz and a flowrate of 1 μL s^{-1} (62-s sample residence time). Distance is the distance between the flow path and the bottom of the transducer. In-tube temperatures were manually recorded using a thermocouple probe.

show that the in-tube temperature never exceeded 106 °C, and that 98−99% spore death was achieved in some flow cell configurations at temperatures well below 100 °C.

SEM images of spore suspensions before and after treatment confirmed spore disruption and lysis using the cylindrically focused, flow-through system at power densities between 4.5 and 8.3 W cm^{-2}. As shown in Figure 2, untreated spores were smooth and regular. In contrast, the treated spore preparation showed evidence of lysis, spore damage, "pustules", and debris on or near the spore coat. The number of physically damaged spores (those that appeared as in Figure 2) was qualitatively less than expected on the basis of measures of spore viability, but is in agreement with vegetative cell lysis/viability data that shows complete cell

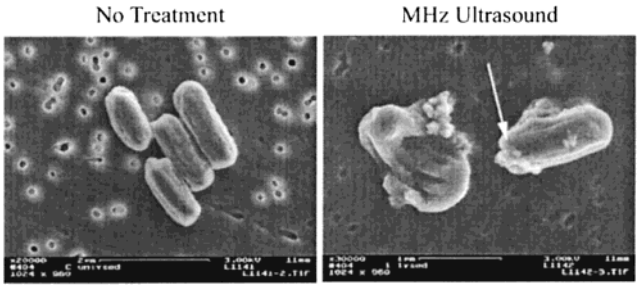

Figure 2. Scanning electron microscope images of *B. globigii* spores before (left panel) and after (right panel) megahertz ultrasonic treatment. Sonicated spore preparations showed evidence of cracked spores and "pustules" emanating from the spores themselves (arrow). 50−100 nm circles in both images are support membrane pores, not cellular debris. Spore death (plate counts) did not correlate with lysis efficiency as measured by SEM, in keeping with results for *E. coli* and vegetative *Bacillus* cells.

death occurs at lower ultrasonic energies than complete cell lysis (Table 3). It is, therefore, probable that internal spore damage results in spore death before the onset of external, visible physical damage. It is also possible that megahertz ultrasound causes imperceptible spore coat damage (e.g. "pinholes") that is not detectable by SEM, leading to more sensitive PCR detection of intracellular DNA (below). Further experimentation and electron microscopy will be required to fully understand the relationship between ultrasonic energy, spore death, and lysis efficiency in megahertz sonic zones, which will then lead to flow cell designs with enhanced performance.

DNA Analysis. Plate counts alone are not enough to confirm spore lysis or to verify that target analytes (nucleic acids, protein) are available for subsequent detection. In addition to SEM imaging, we therefore measured the release of intracellular DNA after cylindrically focused ultrasonic treatment at 4.5 W cm^{-2}. As

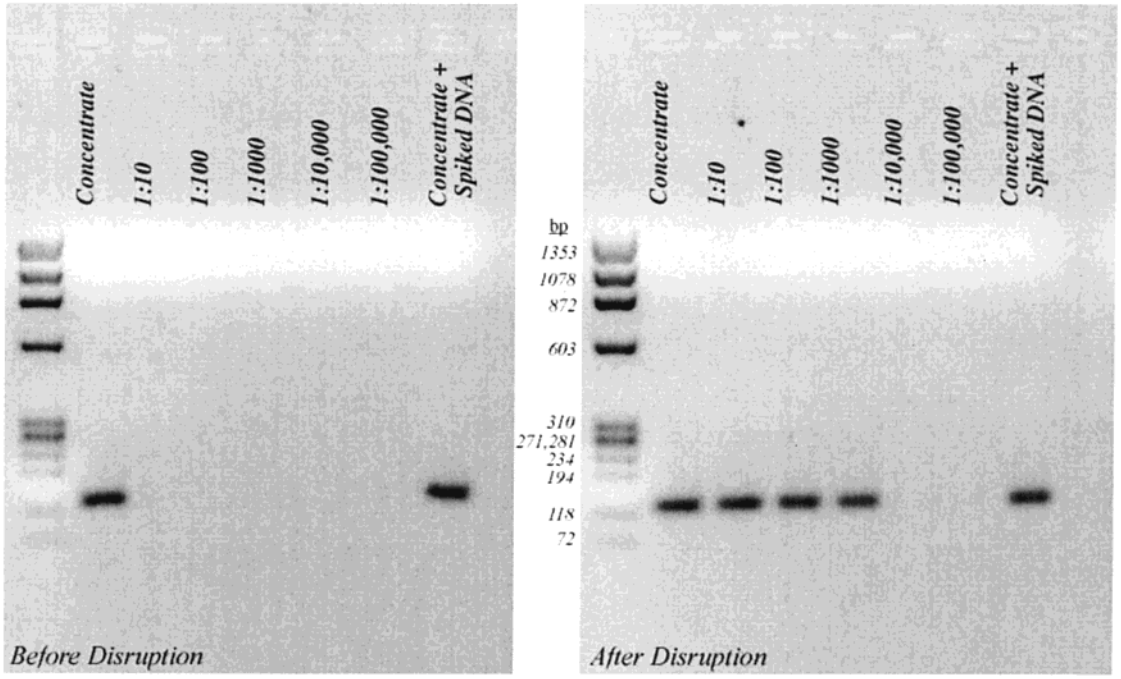

Figure 3. *B. globigii* spore DNA availability before and after treatment in a cylindrically focused sonic field. The molecular weight marker is ϕX174 cut with *Hae*III, with fragment sizes shown in the margin. The "concentrate" is 5 μL of spore preparation at 10^8 spores mL^{-1} in water. The "concentrate + spiked DNA" is 5 μL of spore preparation at 10^8 spores mL^{-1} in water amended with 200 fg of *B. globigii* DNA (to test for PCR inhibition). The detection limit for this PCR assay is 50 fg of genomic DNA (not shown).

shown in Figure 3, we observed up to a 3-log increase in available BG DNA after spore disruption. These results demonstrate that DNA is, indeed, released from intact spores and available for subsequent detection by PCR after megahertz treatment (results that are consistent with those of Belgrader et al.[10,12] operating at kilohertz frequencies with a 90-min spore:lysozyme pretreatment and microbead amendment.) We were unable to visualize total DNA from 10^8 spores on agarose gels after flow-through lysis, and therefore, we did not address the issue of genomic DNA fragmentation or degradation in megahertz ultrasound.[14] With the continuous-flow ultrasonic device reported here, we nonetheless routinely observed a 100-fold increase in available DNA after treatment, which coincides with the typical 100-fold decrease in cell viability (above). Further studies are required to optimize intracellular DNA availability as a function of flow-cell design and increase the availability of interior spore DNA for subsequent detection.

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CONCLUSIONS

In this study, we have demonstrated that megahertz ultrasound can be used to rapidly and continuously disrupt *Bacillus* spores and vegetative cells in the *absence* of any physical or chemical pretreatment. We have further described a unique sonicator geometry that radially focuses the sonic energy along an axial flow path or fluid channel, resulting in a continuous cell and spore lysis system that is compatible with unattended bio-monitoring devices and applications.

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