



Abstract

Claremont BioSolutions (CBS), a spin-off company out of the Bioengineering and Microfluidics Laboratories at the Keck Graduate Institute (KGI), has been developing a variety of compact components that are amenable for integration into pathogen detection systems. One such component, known as the Micro Bead-Beater™ (μBB™), is a compact device that is capable of ultra-rapid lysis (>90% lysis in 15 seconds) of micro volumes (<80ul) of Bacillus spores in a continuous-flow format (batch mode) or in a disposable single-tube format. The μBB™ is also capable of processing much larger volumes (milliliters) of spores or vegetative cells using a continuous-flow mode. DNA quantification results using dsDNA binding fluorescence dyes and real-time PCR is presented, comparing the lysis of Bacillus subtilis (B. s) spores using the μBB™ versus other well-known spore lysis techniques. Nanoscale imaging results performed at KGI, using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and atomic force microscopy (AFM), on B. subtilis spores lysed using the μBB™ is also presented.

The micro-bead beater (μBB)

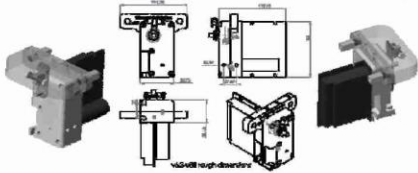


Figure 1: Representations of the micro bead beater and its connections for incorporation into a fluidic system. The device is approx. 4 inches high.

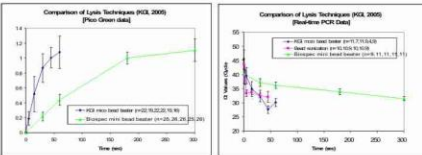


Figure 2: PicoGreen (left) and real-time PCR (right) assays comparing the lysis performance of μBB to the Biospec™ and bead sonication on Bacillus subtilis spores.

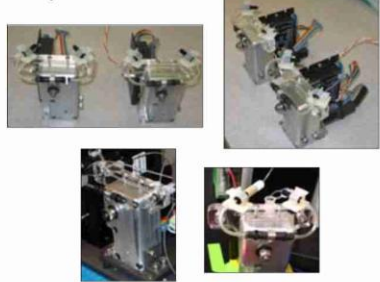


Figure 3: Images of the micro bead beater and examples of it being incorporated into a fluidic system.

Flow-thru capability

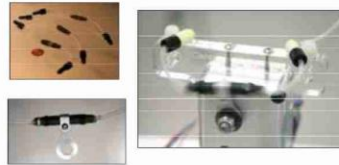


Figure 4: Easily removable and refillable PEEK cartridges use standard fluidic 1/4-inch connectors.

Figure 5: PicoGreen data showing absolute lysis efficiency of Bacillus subtilis spores using the μBB in flow-through batch mode (80ul samples).

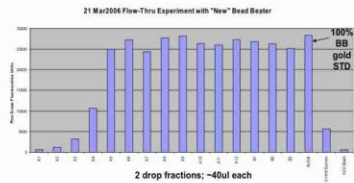
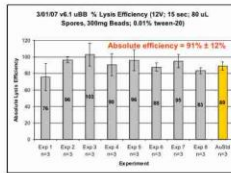


Figure 6: PicoGreen data showing an increase in fluorescence as absolute steady-state lysis efficiency of Bacillus subtilis spores is achieved μBB in flow-through batch mode (100ul/min; 40ul fractions).

Flow-through lysis unaffected by bead volume; lower residence time is offset by more efficient lysis

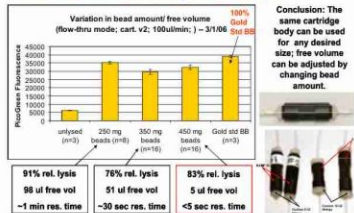


Figure 7: Bead volume of cartridges is adjustable through a variety of variables.

>31 Day Endurance Testing:

The mechanical mechanism, motors, tubing, cartridges, bead integrity, consistent lysis efficiency, and non-clogging have all been shown to endure at least 31 days of continuous use [On for 15 seconds every 4 minutes; approx. 22,000 lysis cycles]. (Data available)

Figure 8: Agilent Bioanalyzer analysis of gDNA shearing. 20 ng/uL CalT Thymus gDNA in TE. Lanes 1-6, bead beating for 15 seconds. Lanes 7-9 probe-sonicated for 20 seconds. Lanes 10-11 untreated 20 ng/uL. 1500bp marker included.

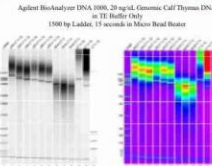
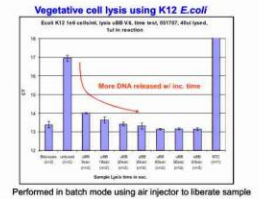


Figure 9: Real-time PCR data showing lysis of K12 E. coli efficiency using the μBB performed in flow-through batch mode (40ul samples).



Post-lysis imaging

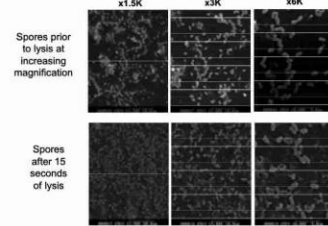


Figure 10: Lysis time-course of micro bead beaten Bacillus subtilis spores visualized using a Hitachi S520 SEM.

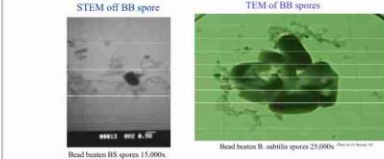


Figure 11: Examples of bead beaten B.s. spores as seen in a Hitachi H600

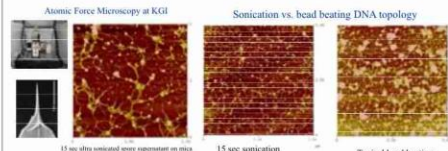


Figure 12: High resolution AFM images of the supernatant of spore lysate on mica.

Microfluidic bead blenders

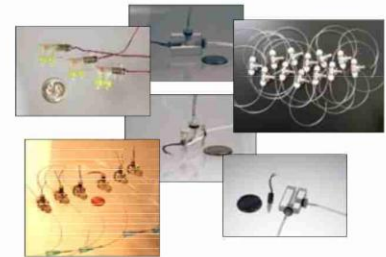


Figure 13: Micro Bead Blenders. Chambers are made of milled acrylic. Inlets and outlets are composed of custom fittings that trap zirconium beads. A disposable 4mm diameter motor drives a custom laser-carved impeller.



Figure 14: Image of bead blending at 250μsec exposure.

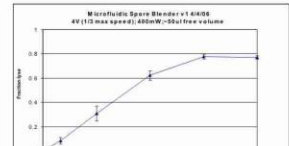


Figure 15: Bead Blender Flow-through mode results show 50% lysis in under 30 sec and 75% in 45 sec. Fraction lysed as percentage yield of Biospec™ Bead Beating for 3 minutes. (Determined using PicoGreen assay).

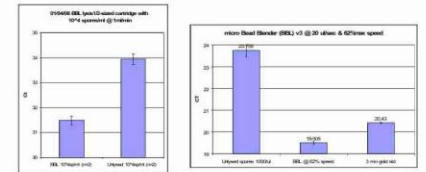


Figure 16: Real-time PCR data of BBL in continuous flow-through mode.

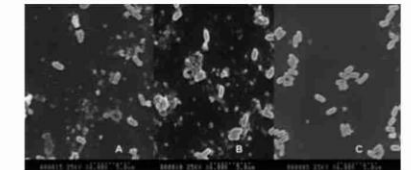


Figure 17: Electron micrographs of B. subtilis spores. Panel A: 1ml sample treated by Bead Blender in flow through mode at high velocity, for 90 sec (11ul/sec). Panel B: 80 ul aliquot treated for 40 second. Panel C: Untreated spores.

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