CONSTANT SYSTEMS
CELL DISRUPTION MADE EASY

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Application Note

Early activation of bioenergetic metabolism powers bacterial spore germination

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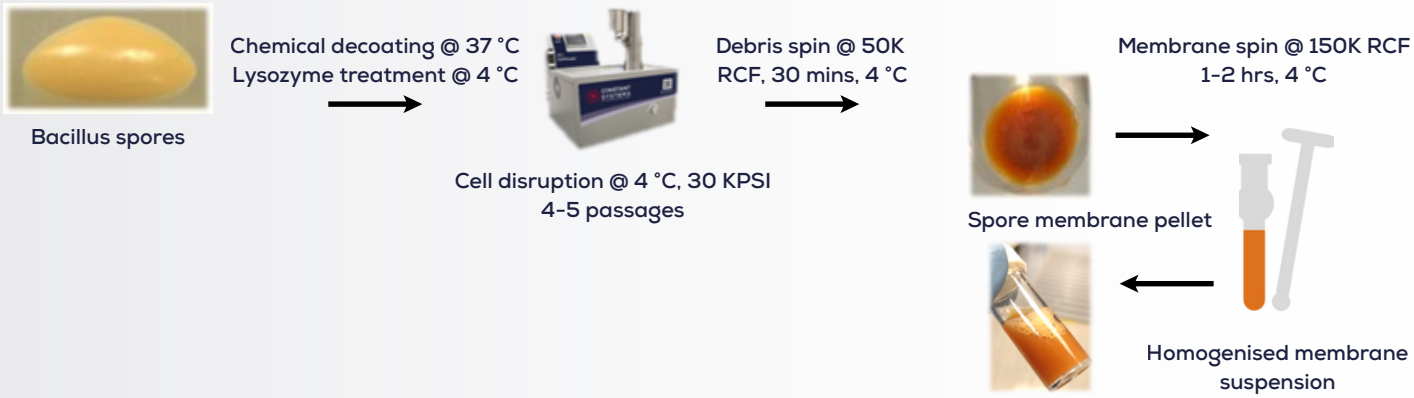
Introduction

Bacterial spores are nature's ultimate survivors, enduring extremes and remaining dormant for centuries—but how they power rapid awakening was long unclear. We discovered a spore-specific respiratory system that energizes germination, reshaping basic biology and enabling biotech and biopharma applications, from maximizing industrial fermentation and spore-based delivery systems to improved probiotics, vaccines, and pathogen control.
Dormant spores are energetically depleted (low ATP/reduced power) and dehydrated, leaving the source of germination energy uncertain.
In a recent **PNAS** paper Gupta et al. (2025) showed that two spore-specific enzymes, YumB and YthAB, tune the inner-membrane respiratory apparatus to drive early germination. Energized by cytoplasmic metabolism, this respiratory chain activates before the spore core is fully hydrated, challenging the view that energy metabolism resumes only later. This study is among the few examining the bioenergetics of life emerging from dormancy—a process seen across all domains of life.



The Constant Systems CF instrument proved essential for key aspects of this work. To characterise the respiratory apparatus present in dormant spores, we needed to optimise spore disruption and membrane isolation protocols so that we could then analyse these membrane preparations by blue-native gel electrophoresis and mass spectrometry. In addition to the composition of the respiratory chain, we wanted to compare the rates of respiratory activity between membranes isolated from spores and late exponential-phase bacterial cells. We needed **reproducibility** across preparations to be able to compare such complex samples. Spore disruption is non-trivial and involves removal of outer proteinaceous and peptidoglycan layers first with chemical and enzymatic treatments. After this, we used the CF at high pressures to effectively fragment the decoated spores and release the membranes, which were later pelleted by ultracentrifugation. Using CF enabled us to get **high membrane yields**, and **consistent mechanical disruption** across biological replicates to keep the orientation of the resulting membrane vesicles comparable – this was crucial for reliable measurements of respiratory activity. The ability to keep samples cold during disruption also contributed to the overall stability of respiratory complexes and other delicate proteins in the membrane preparations.

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About The Continuous Flow Cell Disruptor

The CF Cell Disruptor offers Continuous Flow Processing with two models, the CF1 and CF2. Both models offer the same process and differ only by process speed.
The CF1 offers up to 6L per hour and the CF2 offers up to 24L per hour processing rates.

Both models benefit from a HMI control, a maximum process pressure of 40kpsi (2700 bar), integrated sample cooling jacket, 200mL inlet reservoir (hopper), auto shut down feature for when the process is complete and both models take advantage of Constant Systems precise and consistent hydraulic control which is fully scaleable through the whole product range from 0.5mL single preparation processing to large volume processing at 150L per hour. This precise control ensures that the entire sample is processed at the operators set pressure to ensure accurate and consistent results and in most cases a single process or pass is adequate meaning multiple passes are not required. Both models are capable of processing fluid or re-suspended sample types and are utilised for many sample types such as bacteria, yeast algae and mammalian / insect cells.

CF1 Model
At approximately 700mm^2 the CF1 is small enough to bench mount if required but is best suited when used on its tailor made trolley. The CF1 trolley ensures that the equipment is situated at its optimum working height and is mounted on full swivel and lockable casters that enables safe and free movement of the equipment when needed. The CF1 is recommended for process volumes in the range of 15mL through to 10L. For those processing larger volumes in this range, the CF1 can be offered with an integrated peristaltic pump fully controlled through the HMI which will ensure that the inlet reservoir is continually fed whilst recirculating the sample to help avoid any settling until the entire sample is processed.

CF2 Model
With a footprint of approx. 700mm^2 the CF2 is a floor standing model that is recommended for process volumes from 15mL through to 100L. The CF2 is offered with an integrated peristaltic pump as standard. The CF2 is mounted on full swivel and lockable castors that enables safe and free movement of the equipment when needed.

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