Hydrodynamic Focusing Bioreactor – Space, KC-135 Bubble Removal Studies

Current GRC/JSC Collaboration

JSC Project Manager

____Steve R. Gonda, Ph.D.

Biotechnology Cell Science Program

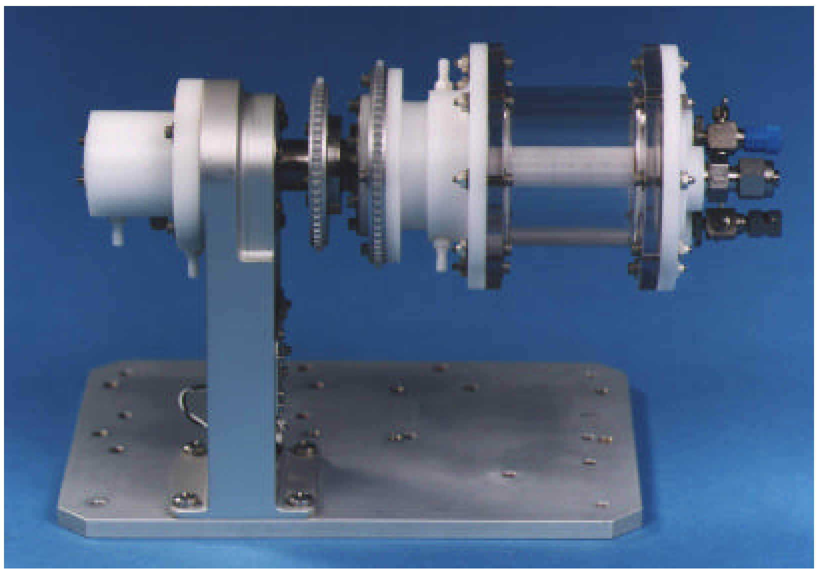
GRC Personnel

Marsha Nall	Howard Ross
Charles Niederhaus	Chanthy Iek
John Kizito	Emily Nelson
Henry Nahra	Pat Parsons

JSC Continuing Project: Inception 1999

Rotating Wall Perfused Vessel (RWPV)

Flown on STS-70, -79, -85, -89, & -107, and operational on NASA/Mir Increment 3 & 7



NASA 1991 Invention of the Year

Astronaut David Wolf, Ray Schwartz, and Tinh Trinh

Size

- Diameter 5 cm
- Filter diameter 1.25 cm
- Disc diameter 3.86 cm
- Length 6.77 cm
- Volume 125 mL.

Rotation

- Inner disc/axis filter and outer wall rotate independently.
- Ground-based studies use solid body rotation at 10-40 rpm.
- Microgravity studies rotate inner disc/filter at ~10 rpm, outer wall at ~1 rpm.

Sample withdrawal

- 3 sample ports on end opposite rotating disc.

Long-Term Mir Operation

Problem:

Bubbles unexpected arose in Mir studies, worst case 80% volume gas.

Cause:

Long duration operation required external replenishment of fresh media which contained gas.

Reason:

Fresh media was hydrated on orbit and gas formed in bag.

Astronaut tried to centrifuge bag to prevent gas ingestion with only partial success.

Result:

Small bubbles entered bioreactor and coalesced into larger bubbles.

Free-floating bubbles as well as bubbles along the filter formed.

Bubble Observations

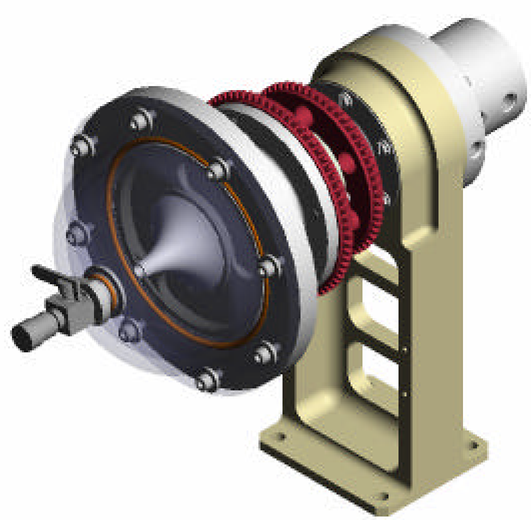
From multiple bubble events

Flushing: When the bioreactor had a large volume of gas, flushing the system by simultaneously adding and removing large volumes (4x) of fluid had partial success. Small bubbles remained on the filter surface as well as free-floating bubbles.

Filter: When the bubbles attached to the filter grew to a large size from coalescence, it was easier to remove the gas by flushing.

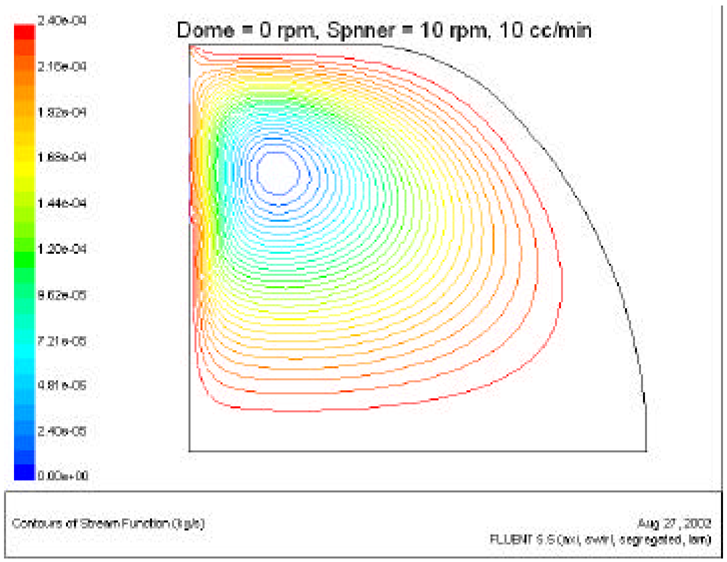
Size: Bubbles would initially increase in size from coalescence and then decrease as the gas was absorbed into the fluid and removed by the exchange membrane.

Hydrodynamic Focusing Bioreactor (HFB)

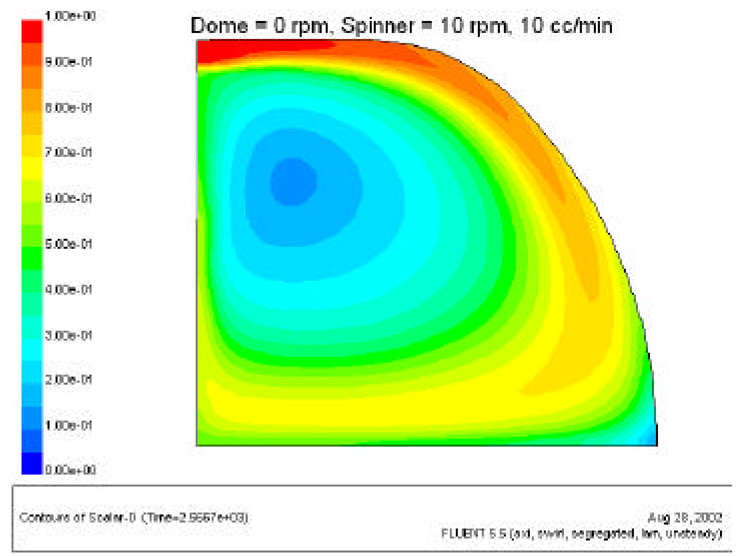


- HFB was an alternative developed prior to Mir flights.
- Was not designed specifically to solve bubble problem.
- Central axis port believed to allow bubble removal using centrifugal forces.
- Auxiliary hardware identical to RWPV.

HFB Computations, 4th Generation



Flow Streamlines



Oxygen Concentration

Stanley Kleis, University of Houston

Glenn Contributions to HFB Program

- Measure fluid properties
  - Density
  - Viscosity
  - Surface tension
- Cooperate in KC-135 campaign
  - Observe bubble migration
  - Evaluate bubble removal strategies
- Validate computer model
  - Flow visualization
  - Velocity measurement
- Develop Science Requirements Document for DTO

KC-135 Bubble Migration Studies

Objectives

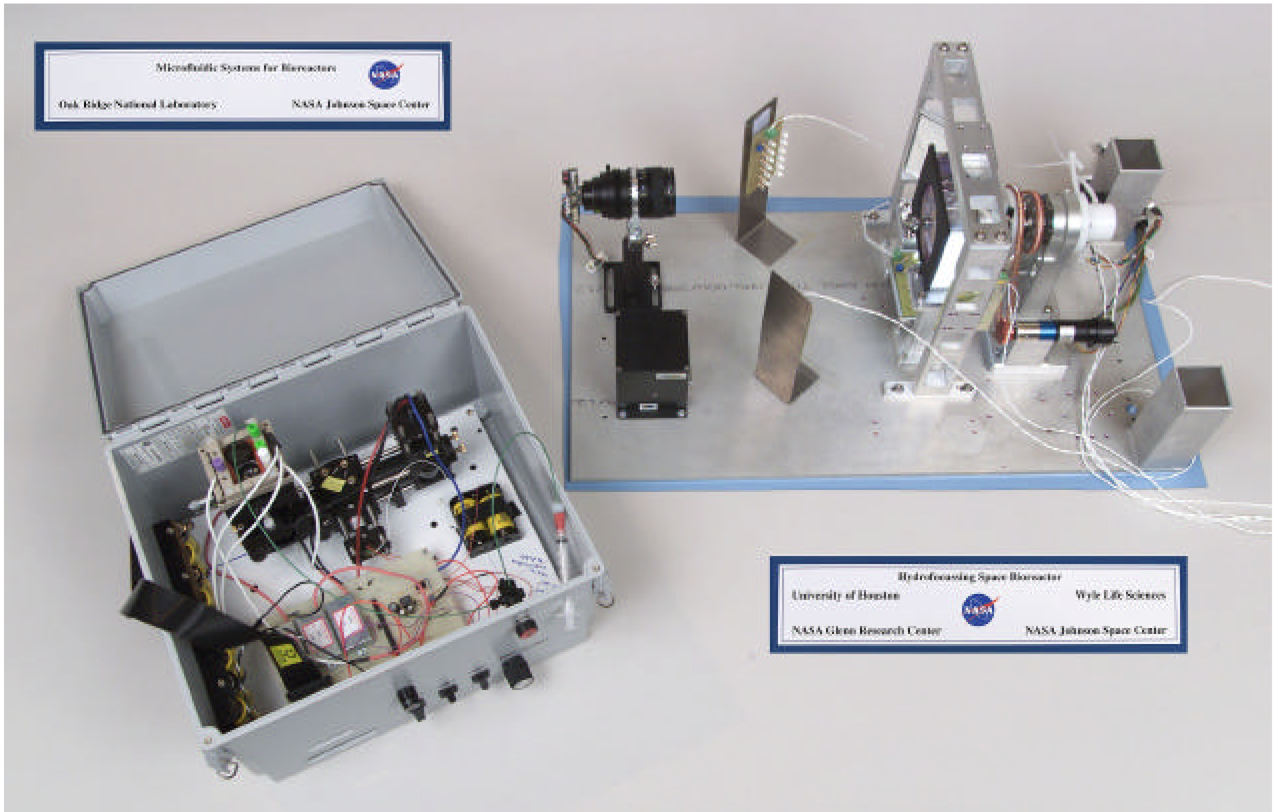
- Verify code predictions of bubble/particle movement.
- Forces include buoyancy, inertia, drag, lift, virtual mass, and Basset history integral.
- Front, side, and top views allow tracking of bubbles and different density particles in the bioreactor.
- 6 component acceleration measurements will be combined with initial conditions in an attempt to validate code particle trajectory predictions.

KC-135 Bubble Migration Studies

Operating Parameters

- Growth media at room temperature as bulk fluid.
- Heavy particles representative of cell aggregates.
- Light particles (s.g. < 0.1) without surface tension effects.
- Various size bubbles.
- Various dome and spinner rotation rates.

KC-135 Apparatus, Flight Week 1

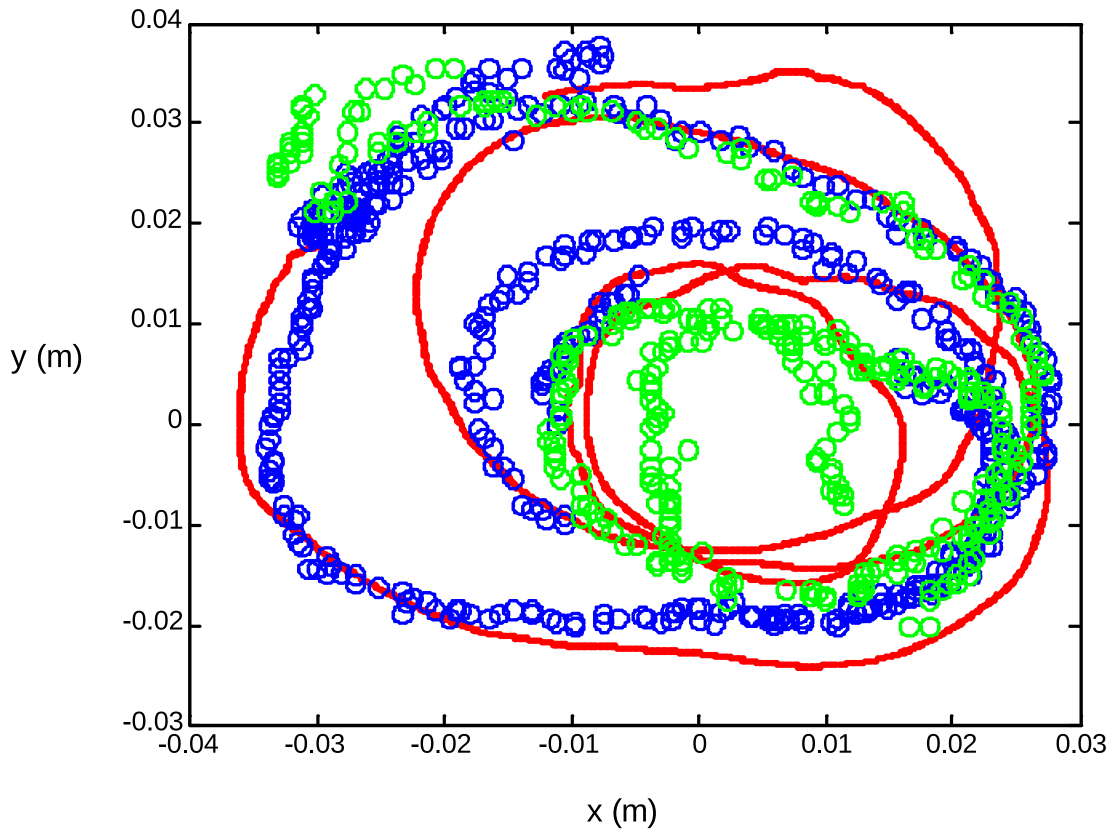


Microgravity Science Division

Space Directorate

KC-135 Bubble Trajectory Comparison

Open points measurements, solid line computations



Sandra Geffert, University of Houston

KC-135 Bubble Removal Studies

Objectives

- Determine if bubble on central axis can be easily removed without excessive fluid removal.
- Attempt to determine if bubbles have a tendency to attach to bioreactor surfaces.
- Attempt to remove bubbles from bioreactor surfaces with sufficiently low shear forces that do not damage cells.
- Determine if excessively large bubbles have other effects.

KC-135 Bubble Removal Studies

Test Conditions

Day 1: Bubble trajectories with media and various size bubbles. Initial attempts at bubble removal.

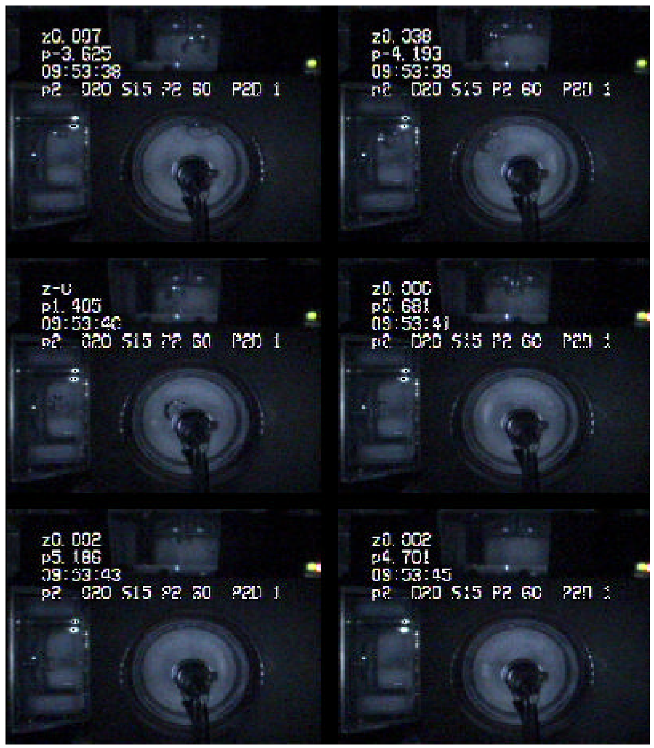
Day 2: Bubble removal with media using flush outlet port.

Day 3: Bubble removal with media using 16 gauge Teflon-coated needle 1 cm into vessel.

Day 4: Bubble removal with sterile water using 16 gauge Teflon-coated needle 1 cm into vessel.

KC-135 Apparatus, Flight 2

KC-135 Bubble Removal



Conclusions

- First set of flights are providing useful comparisons to CFD predictions of bubble motion.
- Central cone prevented large bubbles from being removed in microgravity and inhibited bubble migration to the central port.
- Central cone also provided no advantage during normal operation, leading to its removal in current design.
- Second set of flights demonstrated the latest internal configuration of the HFB–S with a flat disk and peripheral inlet.
- Bubbles were successfully removed in the imperfect KC-135 environment, and valuable information was gained for implementing a DTO.