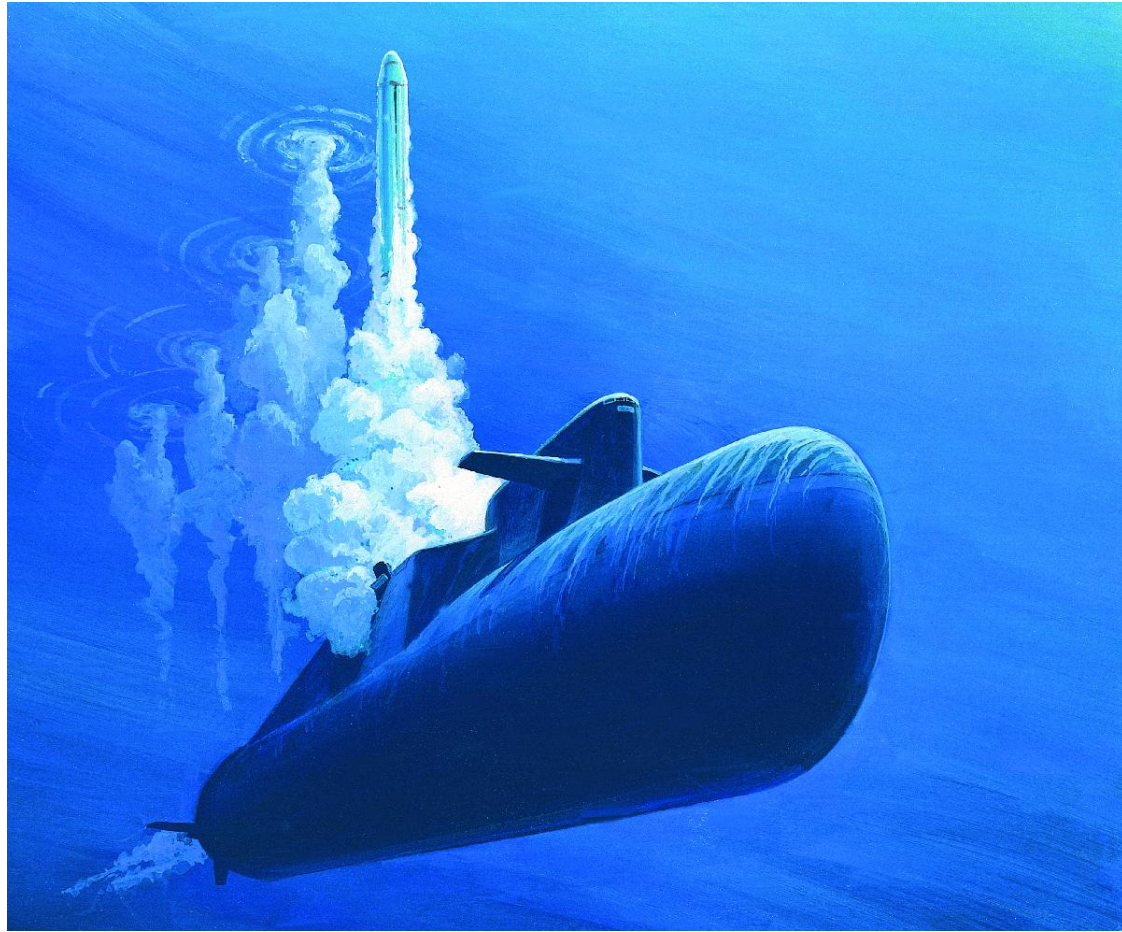


Biosafety Concerns with Single Use Bioprocess Technology

Brian Petuch RBP CBSP

Merck Global Safety & Environment

SUBS



SUBS



SUBs (Single Use Bioprocess)



Areas Benefitting from SUBs

- rDNA protein production
- mAb production
- Therapeutic protein production
- Viral vector production
 - Lentivirus
 - AAV
 - Adenovirus



Bioprocessing Steps



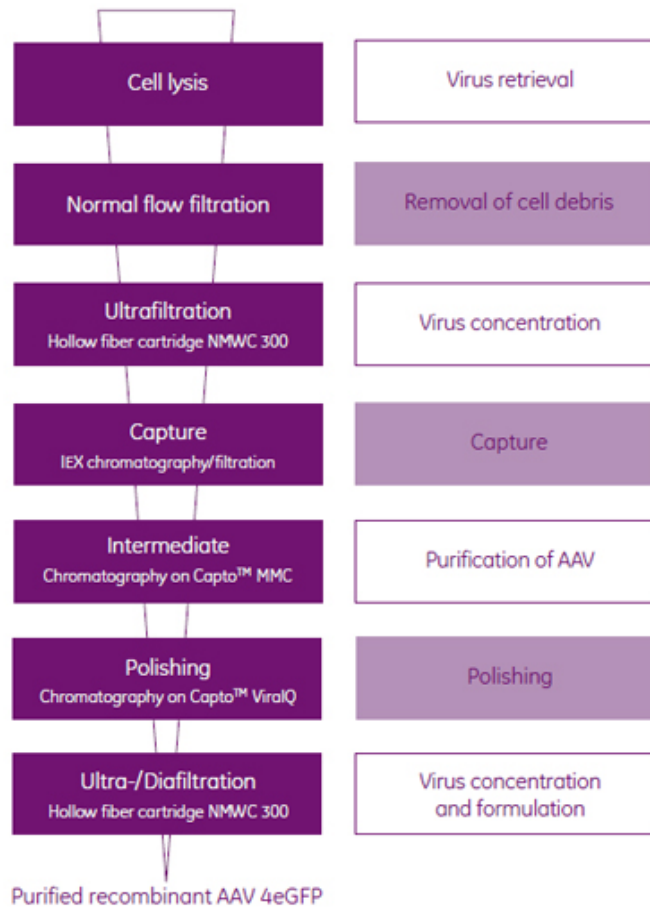
Process for Bacterial & Yeast Products

| Unit Process | Procedure |
|---|--|
| Cell Separation | Centrifugation |
| Cell lysis | Homogeniser |
| Inclusion body isolation | Centrifugation |
| Inclusion body solubilisation; protein refolding | Resolubilise in chaotropic agent in presence of reducing agent; air oxidise |
| Product concentration | Ultrafiltration; precipitation |
| Buffer exchange/adjustment | Diafiltration |
| Purification | Chromatography Two-phase extraction |
| Product formulation, | Buffer formulation Lyophilisation Packaging |

Process for Mammalian & *Pichia* Products

| Unit Process | Procedure |
|------------------------------|---|
| Cell Separation → | Filtration/Centrifugation |
| Clarification → | Centrifugation Microfiltration |
| Product concentration → | Ultrafiltration Precipitation |
| Buffer exchange/adjustment → | Diafiltration |
| Purification → | Chromatography Two-phase extraction |
| Product formulation, → | Buffer formulation Lyophilisation Packaging |

Process for Viral Vector Production



Large Scale Biosafety Guidelines



Large Scale Biosafety Guidelines

Appendix K - Table 1. Comparison of Good Large Scale Practice (GLSP) and Biosafety Level (BL) - Large Scale (LS) Practice (See Appendix K-VI-A, Footnotes Of Appendix K)

| CRITERION [See Appendix K-VI-B, Footnotes of Appendix K] | | GLSP | BL1-LS | BL2-LS | BL3-LS |
|---|--|---------------------------------|--|---|---------------------------------------|
| 1. | Formulate and implement institutional codes of practice for safety of personnel and adequate control of hygiene and safety measures. | K-II-A | G-I | | |
| 2. | Provide adequate written instructions and training of personnel to keep work place clean and tidy and to keep exposure to biological, chemical or physical agents at a level that does not adversely affect health and safety of employees. | K-II-B | G-I | | |
| 3. | Provide changing and hand washing facilities as well as protective clothing, appropriate to the risk, to be worn during work. | K-II-C | G-II-A-1-h | G-II-B-2-f | G-II-C-2-i |
| 4. | Prohibit eating, drinking, smoking, mouth pipetting, and applying cosmetics in the work place. | K-II-C | G-II-A-1-d G-II-A-1-e | G-II-B-1-d G-II-B-1-e | G-II-C-1-c G-II-C-1-d |
| 5. | Internal accident reporting. | K-II-G | K-III-A | K-IV-A | K-V-A |
| 6. | Medical surveillance. | NR | NR | | |
| 7. | Viable organisms should be handled in a system that physically separates the process from the external environment (closed system or other primary containment). | NR | K-III-B | K-IV-B | K-V-B |
| 8. | Culture fluids not removed from a system until organisms are inactivated. | NR | K-III-C | K-IV-C | K-V-C |
| 9. | Inactivation of waste solutions and materials with respect to their biohazard potential. | K-II-E | K-III-C | K-IV-C | K-V-C |
| 10. | Control of aerosols by engineering or procedural controls to prevent or minimize release of organisms during sampling from a system, addition of materials to a system, transfer of cultivated cells, and removal of material, products, and effluent from a system. | Minimize Procedure K-II-F | Minimize Engineer K-III-B K-III-D | Prevent Engineer K-IV-B K-IV-D | Prevent Engineer K-V-B K-V-D |
| 11. | Treatment of exhaust gases from a closed system to minimize or prevent release of viable organisms. | NR | Minimize K-III-E | Prevent K-IV-E | Prevent K-V-E |
| 12. | Closed system that has contained viable organisms not to be opened until sterilized by a validated procedure. | NR | K-III-F | K-IV-F | K-V-F |
| 13. | Closed system to be maintained at as a low pressure as possible to maintain integrity of containment features. | NR | NR | NR | K-V-G |
| 14. | Rotating seals and other penetrations into closed system designed to prevent or minimize leakage. | NR | NR | Prevent K-IV-G | Prevent K-V-H |
| 15. | Closed system shall incorporate monitoring or sensing devices to monitor the integrity of containment. | NR | NR | K-IV-H | K-V-I |
| 16. | Validated integrity testing of closed containment system. | NR | NR | K-IV-I | K-V-J |
| 17. | Closed system to be permanently identified for record keeping purposes. | NR | NR | K-IV-J | K-V-K |
| 18. | Universal biosafety sign to be posted on each closed system. | NR | NR | K-IV-K | K-V-L |
| 19. | Emergency plans required for handling large losses of cultures. | K-II-G | K-III-G | K-IV-L | K-V-M |
| 20. | Access to the work place. | NR | G-II-A-1-a | G-II-B-1-a | K-V-N |
| 21. | Requirements for controlled access area. | NR | NR | NR | K-V-N&O |

NR = not required



Classic Bioprocess Technology



Classical Bioreactors



14 L Fermentor photo above is a
copyright of New Brunswick Scientific

Classical Filtration



Classical Chromatography



be manufactured according to specifications.



your local

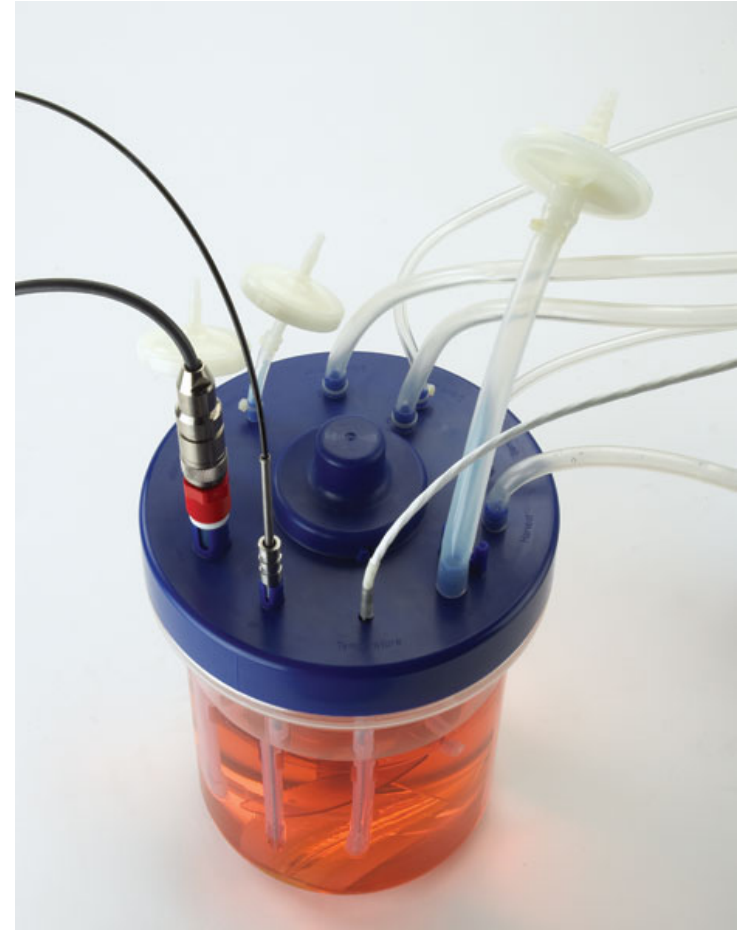




SUB Technology



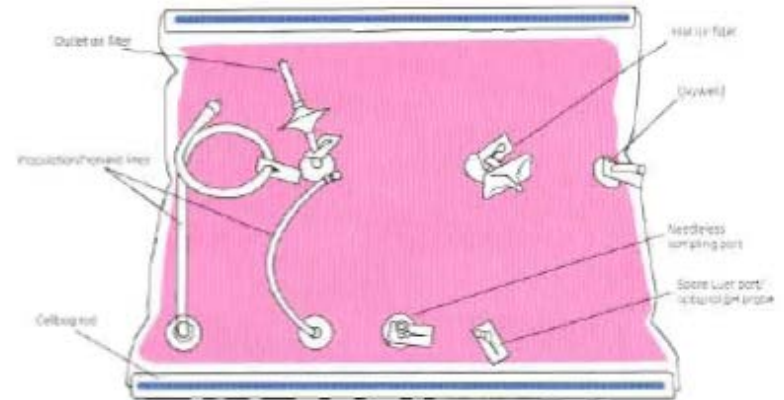
Stirred Rigid Wall Bioreactors



Agitated Flexible Bag Bioreactors



Non Agitated Flexible Bag Bioreactors

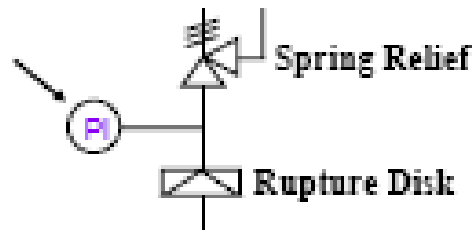


Vessel Integrity and Pressure Relief

Rupture Disk

Tell-tale pressure gauge

Protects spring device against corrosion, plugging.



Protects spring reliefs: corrosion
plugging

Absolute protection, no weeping: toxicants
flammables

Problem: Piece of rupture disk might break off and plug spring relief

Nunc Cell Factory



Single Use Bioreactors for DOE Studies



Can contain up to 36 bioreactors



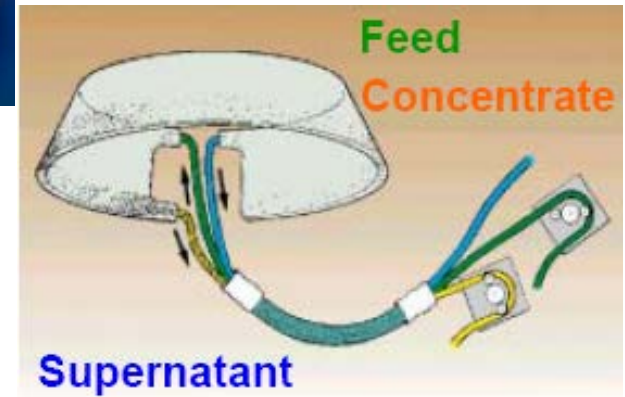
Clarification

- Centrifugation
- Depth Filtration
- Microfiltration
 - Separation of Particulate
 - Filters rated by pore size
- Ultrafiltration
 - Separation of Macromolecules
 - Filters Rated by MW Cutoff

Centrifugation



CENTRITECH III Lab System
For Mammalian Cell Harvesting
to Develop Seed Stocks



Depth Filter Applications

- Post fermentation cell/cell debris clarification
- Pre chromatography clarification
- Post chromatography debris clarification
- Concentrated protein clarification
- DNA, virus removal

Single Use Depth Filters

Zeta Plus™ Single-Use Depth Filtration for Cell Clarification Scale-up Capsule Filters

- Designed for intermediate scale-up studies and lab scale protein production
- Available in three configurations with 170 cm², 340 cm² and 1020 cm² effective filtration area
- Translucent plastic shell allows easy detection of the liquid level during capsule filling
- Multiple capsule filters can be easily configured for both series and parallel process setups
- Innovative design results in minimal fill volume and minimal hold up volume after post-use blow down



Fast.



Easy.



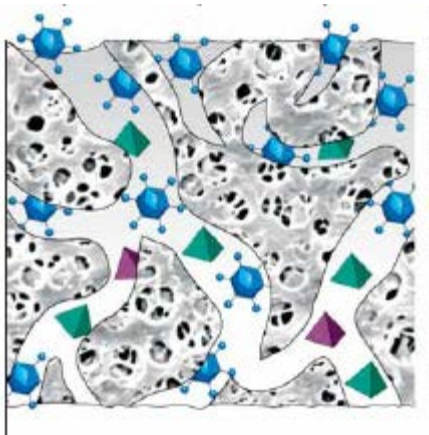
Clean.

Chromatography Applications

- Virus removal
- DNA removal
- Virus purification
- Protein purification

Viral Purification Chromatography

Purification of Adenovirus,
AAV, and Lentivirus Vectors

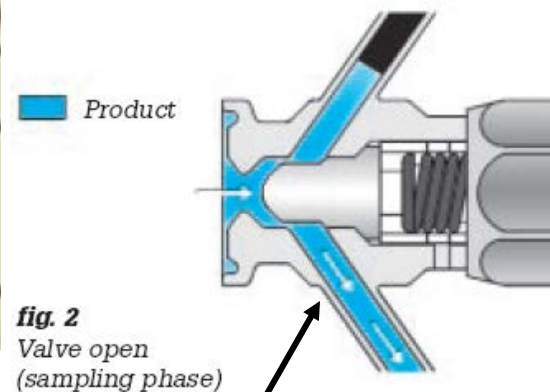
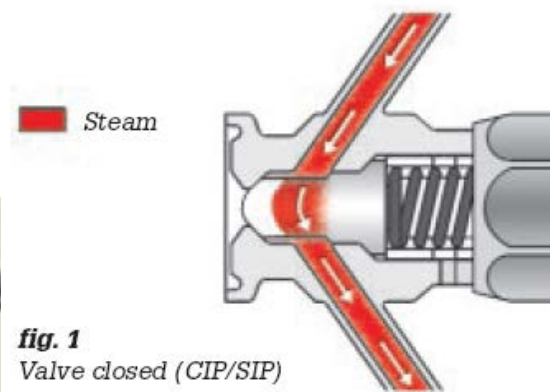




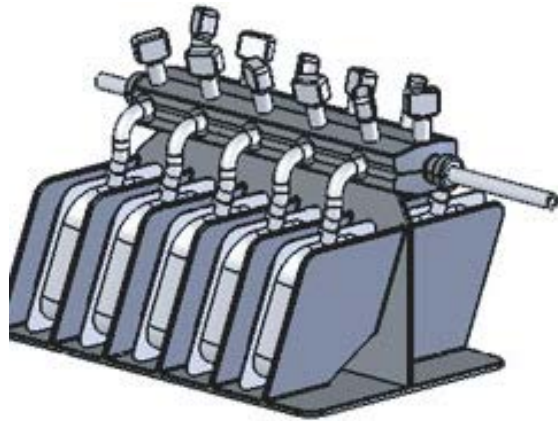
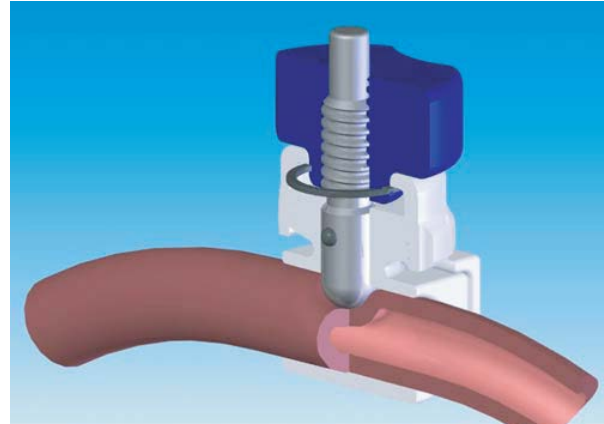
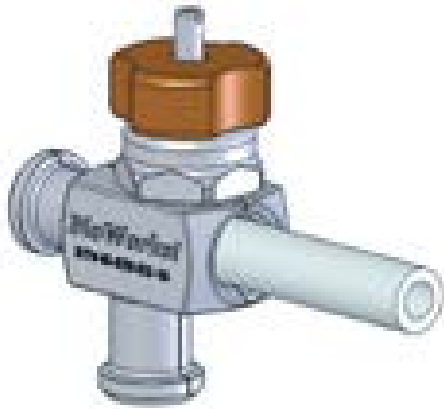
Other SUB Areas



Sampling



Sampling



Fittings



Tubing Welder



Millipore

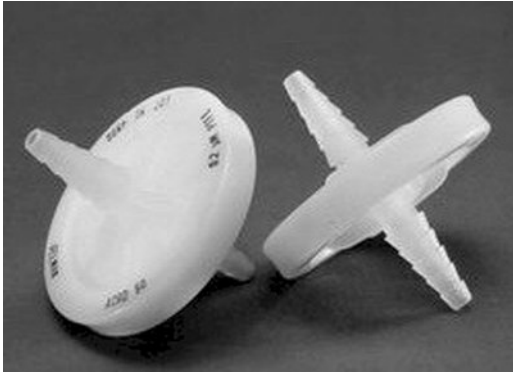


Pall



Colder

Vent Filtration



**Hydrophobic vs.
Hydrophilic**



**Heated vs.
Ambient Housings**



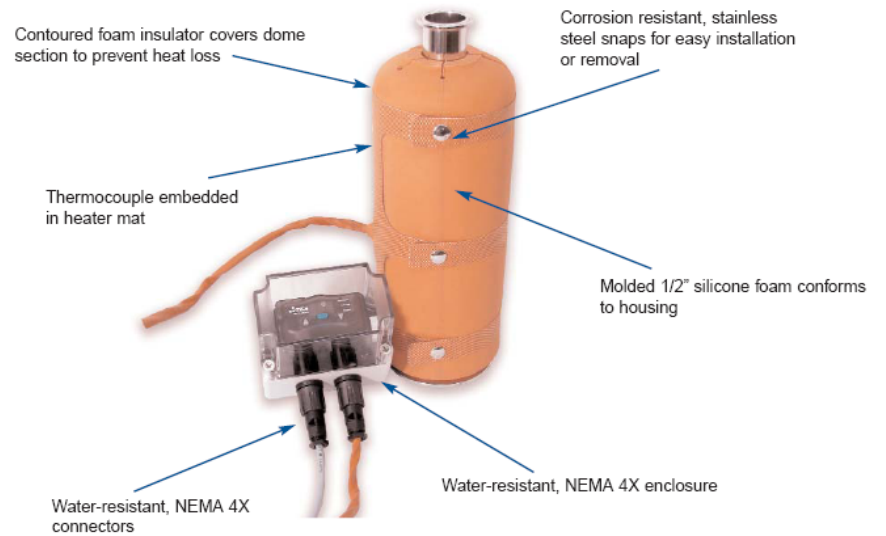
Vent Filtration



Vent Filtration



Heater Features





Performing a Process Risk Assessment



Risk Assessment Parameters

Construction Containment Risks :

- ✓ Flexible Bioreactors
- ✓ Agitated Bioreactors
- ✓ Conventional Sensors
- ✓ Disposable Sensors

Operational Containment Risks :

- ✓ Inoculation
- ✓ Substraction and Addition
- ✓ Using Conventional Sensors
- ✓ Transport and disposal
- ✓ Software failures

Risk Assessment Reference



VROM-Inspectie
*Ministerie van
Infrastructuur en Milieu*

SINGLE-USE BIOREACTORS

Risk Evaluation -
Checklist

Ministry of Infrastructure and
Environment
VROM-Inspectorate
The Netherlands

P.J.M. de Wildt
Inspector

<http://bggo.rivm.nl/paginas/doc-ig.htm>

