BIOPROCESSING
GENERIC SCENARIO
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I. INTRODUCTION

The following generic scenario applies to the production of genetically engineered microorganisms from fermentation processes. The estimates given for occupational exposure and environmental release are for the microorganism and are reported in units of CFU (colony-forming units). Generic scenarios are given for both large scale (>10,000 l) and small scale (<10 l). The estimates are to be used in cases where there is no data available from the submitter.

II. LARGE SCALE FERMENTATION

A. Process Description

See flow diagram in Appendix 1
Process Steps:

1. Growth in small flask
2. Seed fermentation (1,000 - 10,000 l)
3. Main fermentation (>10,000 l)
4. Filtration using rotary drum filter
5. Ultra filtration
6. Packaging

B. Environmental Release

1. Sources:

a. Air - fermentor off-gas, rotary drum filter. These emissions may be uncontrolled or be filtered prior to venting.

b. Water - Steam condensate streams from the steam sterilization of the fermentor vessel and filters, disposal of samples, residues in the rotary filter trough, and cleaning wastes from the ultra filter membrane. These wastes may contain viable microorganisms and are typically sent to a kill tank prior to discharge to either on-site wastewater treatment (WWT) or publicly owned treatment works (POTW).

c. Land releases - disposal of filter cake typically sent to a landfill or spread onto land.

2. Estimates

TABLE 1: SUMMARY OF RELEASES OF GENETICALLY-ENGINEERED BACTERIA FOR TYPICAL LARGE SCALE FERMENTATION PROCESS[1]

MEDIA	Minimally controlled[2] (CFU/day)	Contained System[3] (CFU/Day)	Days/yr
Air			
Vents	2×10^{8} to 1×10^{11}	2×10^{6} to 1×10^{9}	350
Rotary Drum Filter	250	250	350
Water (to wwt)	7×10^{13}	7×10^{9}	90
Solids	7×10^{15}	7×10^{11}	90

Source: (Reilly, 91)

Notes:

1. Based on a 70,000 l batch volume and a maximum cell density of 10^{10} CFU/ml for bacteria.

2. Assume no treatment of fermentor off-gas. Assume a 2-log reduction relative to the maximum cell density of the fermentation broth resulting from inactivation.

3. Assume use of in-line filters to treat vent gases and a 99% removal efficiency under normal operating conditions. Assume an overall 6-log reduction relative to the maximum cell density of the fermentation broth resulting from inactivation steps.

C. Occupational Exposure

1. Activities - pipetting in the lab, sampling, inoculation, changing air filters.

2. Number of Workers - 30 based on 10 workers/shift and 3 shifts.

3. Days/yr = 250

4. Daily Inhalation Exposure = 650 to 1200 CFU/day (Reilly, 91)

III. SMALL SCALE FERMENTATION

A. Process Description

Process Steps

1. Colonies of bacteria strain are scraped from test tubes into a shake flask

2. When the culture reaches stationary phase (10^8 - 10^9 cells/ml), the culture will be used as the inoculum for shake flask runs at the desired final volume.

3. Cells are centrifuged from suspension, washed with saline solution and centrifuged to form a cell paste.

4. The cell paste is packed in sealed hard plastic centrifuge bottles, which are kept on ice.

5. The bottles are shipped on ice in styrofoam chests.

B. Environmental Release

Wastewater:

1. Liquid waste stream from centrifuge = 10^9 CFU/bt (Assumes the centrifuge operates at 99% efficiency.)

2. Residue in shake flasks and sample bottles = 10^6 CFU/bt

Source: Bree, 88

C. Occupational Exposure

Number of workers = 5 (Bree, 88)

Activities: Inoculation of test tube slants and shake flasks, sampling, plating, analysis, transferring the suspension to the centrifuge and transferring the cell paste to the plastic bottles.

Inhalation Exposure Levels: 500 CFU/day (Bree, 88)

REFERENCES

1. Bree M. May 1988. Engineering Report: Exposure and Release Assessment for PMN 88-1275 through 1278. Economics and Technology Division, Office of Toxic Substances, USEPA.

2. Reilly B. March 1991. Analysis of Environmental Releases and Occupational Exposure in Support of Proposed TSCA 5 (h)(4) Exemption. Economics and Technology Division, Office of Toxic Substances, US EPA.
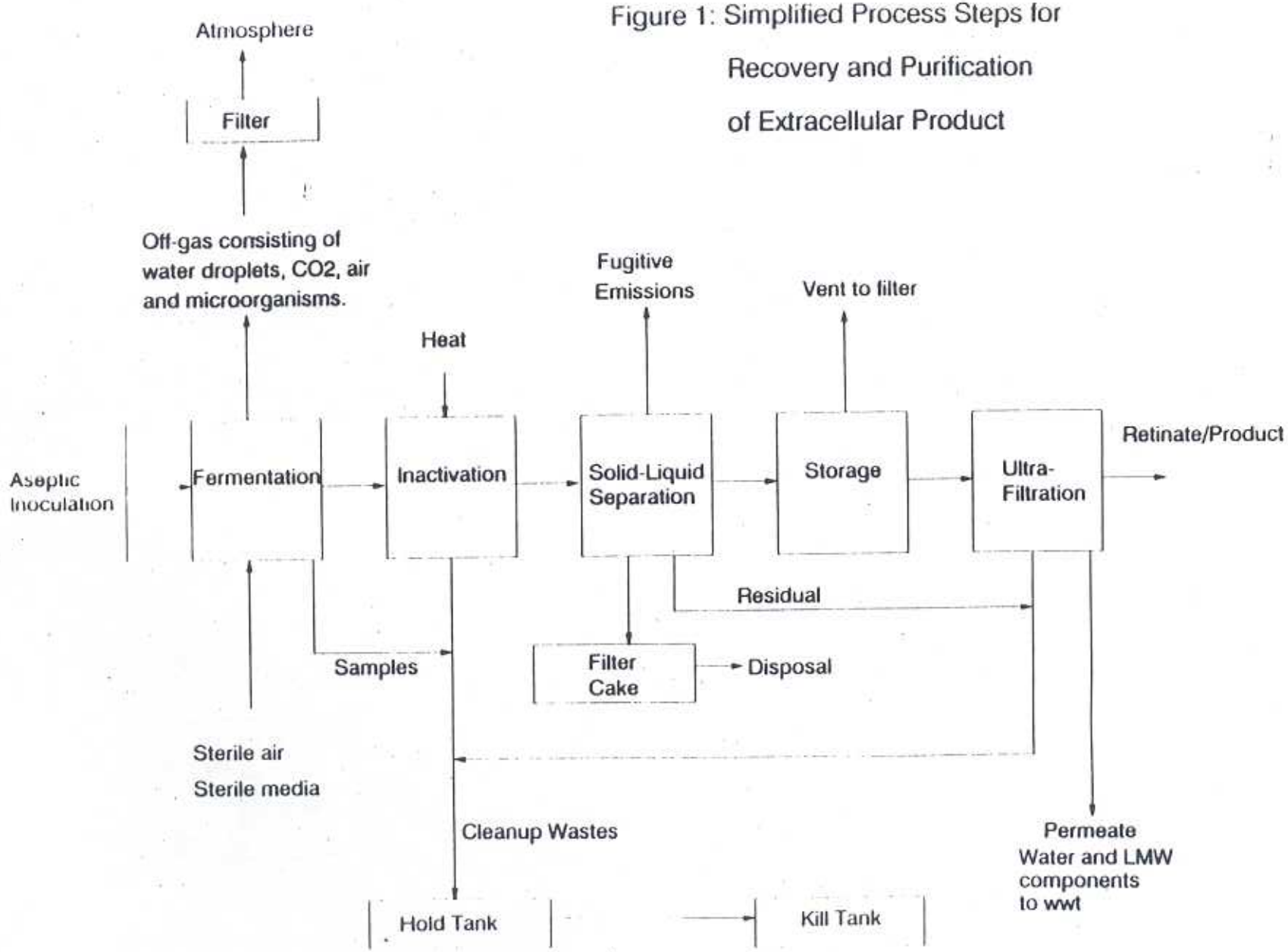
Figure 1: Simplified Process Steps for Recovery and Purification of Extracellular Product