TABLE 14.6 Physical Containment Requirements for Large-scale Fermentations Using Organisms Containing Recombinant DNA Molecules

Item no.	Description	BL1-LS	BL2-LS	BL3-LS
1	Closed vessel	×	×	×
2	Inactivation of cultures by validated procedure before removing from the closed system	×	×	×
3	Sample collection and addition of material in a closed system	×	×	×
4	Exhaust gases sterilized by filters before leaving the closed system	×	×	×
5	Sterilization by validated procedures before opening for maintenance or other purposes	×	×	×
6	Emergency plans and procedures for handling large losses	×	×	×
7	No leakage of viable organisms from rotating seals and other mechanical devices		×	×
8	Integrity evaluation procedure: monitors and sensors		×	×
9	Containment evaluation with the host organism before introduction of viable organisms		×	×
10	Permanent identification of closed system (fermenter) and identification to be used in all records		×	×
11	Posting of universal biohazard sign on each closed system and containment equipment when working with a viable organism		×	×
12	Posting of universal biohazard sign on entry doors when work is in progress			×
13	Operations to be in a controlled area:			×
	Separate specified entry			×
	Double doors, air locks			×
	Walls, ceiling, and floors to permit ready cleaning and decontamination			×
	Utilities and services (process piping and wiring) to be protected against contamination			×
	Handwashing facilities and shower facilities in close proximity			×
	Area designed to preclude release of culture fluids outside the controlled area in the event of an accident			×
	Ventilation: movement of air, filtration of air			×

With permission, from R. J. Giorgiou and J. J. Wu, *Trends in Biotechnology 4:*198 (1986).

tion of hazardous wastes such as benzoates or trichloroethylene (see Fig. 14.8 for an example). The same concepts form the basis for gene therapy.

The reader may ask why genetically engineered organisms should be used instead of natural isolates. The potential advantages over natural isolates are as follows:

- Can put an "odd-ball" pathway under the control of a regulated promoter. The investigator can turn on the pathway in situations where the pathway might normally be suppressed (e.g., degradation of a hazardous compound to a concentration lower than necessary to induce the pathway in the natural isolate).
- High levels of enzymes in desired pathways can be obtained with strong promoters; only low activity levels may be present in the natural isolate.
- Pathways moved from lower eucaryotes to bacteria can be controlled by a single promoter; in lower eucaryotes, each protein has a separate promoter.