

Enzymes such as lysozyme (a carbohydrase) can be used to lyse cell walls of bacteria. Gram-positive bacteria are far more susceptible to enzymatic lysis than gram-negative bacteria. The cells may be treated with EDTA or by freezing and thawing before treatment with lysozyme. Ethylenediaminetetraacetic acid (EDTA) is a chelating agent; high levels of EDTA will extract divalent ions that are part of the cell envelope. Enzymatic hydrolysis is an expensive method and is not very widely used in industry.

Actively growing cells can be treated with an antibiotic, such as penicillin or cycloserine, that interferes with cell-wall synthesis and, coupled with the correct osmotic conditions, can lead to cell disruption.

After cells have been lysed and the products are released into the medium, cell debris can be separated by ultracentrifugation or ultrafiltration, and soluble products are recovered using the following methods.

11.4. SEPARATION OF SOLUBLE PRODUCTS

Most microbial products, such as antibiotics, organic acids, solvents, amino acids, and extracellular enzymes, are soluble and extracellular. Various methods have been developed to recover such soluble products, including extraction, adsorption, ultrafiltration, and chromatography.

11.4.1. Liquid–Liquid Extraction

Liquid extraction is commonly used to separate inhibitory fermentation products such as ethanol and acetone–butanol from a fermentation broth. Antibiotics are also recovered by liquid extraction (using amylacetate or isoamylacetate). Ideally, the liquid extractant should be nontoxic, selective, inexpensive, and immiscible with fermentation broth and should have a high distribution coefficient for the product.

The extraction of a compound from one phase to the other is based on solubility differences of the compound in one phase relative to the other. When a compound is distributed between two immiscible liquids, the ratio of the concentrations in the two phases is known as the distribution coefficient:

$$K_D = \frac{Y_L}{X_H} \quad (11.27)$$

where Y_L and X_H are concentrations of the solute in light and heavy phases, respectively. In most, but certainly not all cases, the light phase will be the organic solvent and the heavy phase will be the aqueous fermentation broth.

Assuming that K_D is constant and the solvents are totally immiscible (that is, the mass flows of the light and heavy phases are conserved so that $L_0 = L_1 = L$ and $H_0 = H_1 = H$), a mass balance on the extracted solute yields (Fig. 11.8a)

$$H(X_0 - X_1) = LY_1 \quad (11.28)$$

or

$$X_1 = X_0 - \frac{L}{H} Y_1 \quad (11.29)$$