industrial fermentation.

Fermentation Technology

industrial fermentation is the large-scale cultivation of microbes or other single cells to produce a commercially valuable substance. We have just discussed the most familiar examples: the anaerobic food fermentations used in the dairy, brewing, and winemaking industries. Much of the same technology, with the frequent addition of aeration, has been adapted to make other industrial products, such as insulin and human growth hormone, from **genetically modified microorganisms**. Industrial fermentation is also used in biotechnology to obtain useful products from genetically modified plant and animal cells. For example, animal cells are used to make monoclonal antibodies.

Vessels for industrial fermentation are called **bioreactors**; they are designed with close attention to aeration, pH control, and temperature control. There are many different designs, but the most widely used bioreactors are of the continuously stirred type (Figure 1). The air is introduced through a diffuser at the bottom (which breaks up the incoming airstream to maximize aeration), and a series of impeller paddles and stationary wall baffles keep the microbial suspension agitated. Oxygen is not very soluble in water, and keeping the heavy microbial suspension well aerated is difficult. Highly sophisticated designs have been developed to achieve maximum efficiency in aeration and other growth requirements, including medium formulation. The high value of the products of genetically modified microorganisms and eukaryotic cells has stimulated the development of newer types of bioreactors and computerized controls for them.

Bioreactors are sometimes very large, holding as much as 500,000 liters. When the product is harvested at the completion of the fermentation, this is known as **batch production**. There are other designs of fermentors. For **continuous flow production**, in which the substrates (usually a carbon source) are fed continuously past immobilized enzymes or into a culture of growing cells, spent medium and desired product are continuously removed.

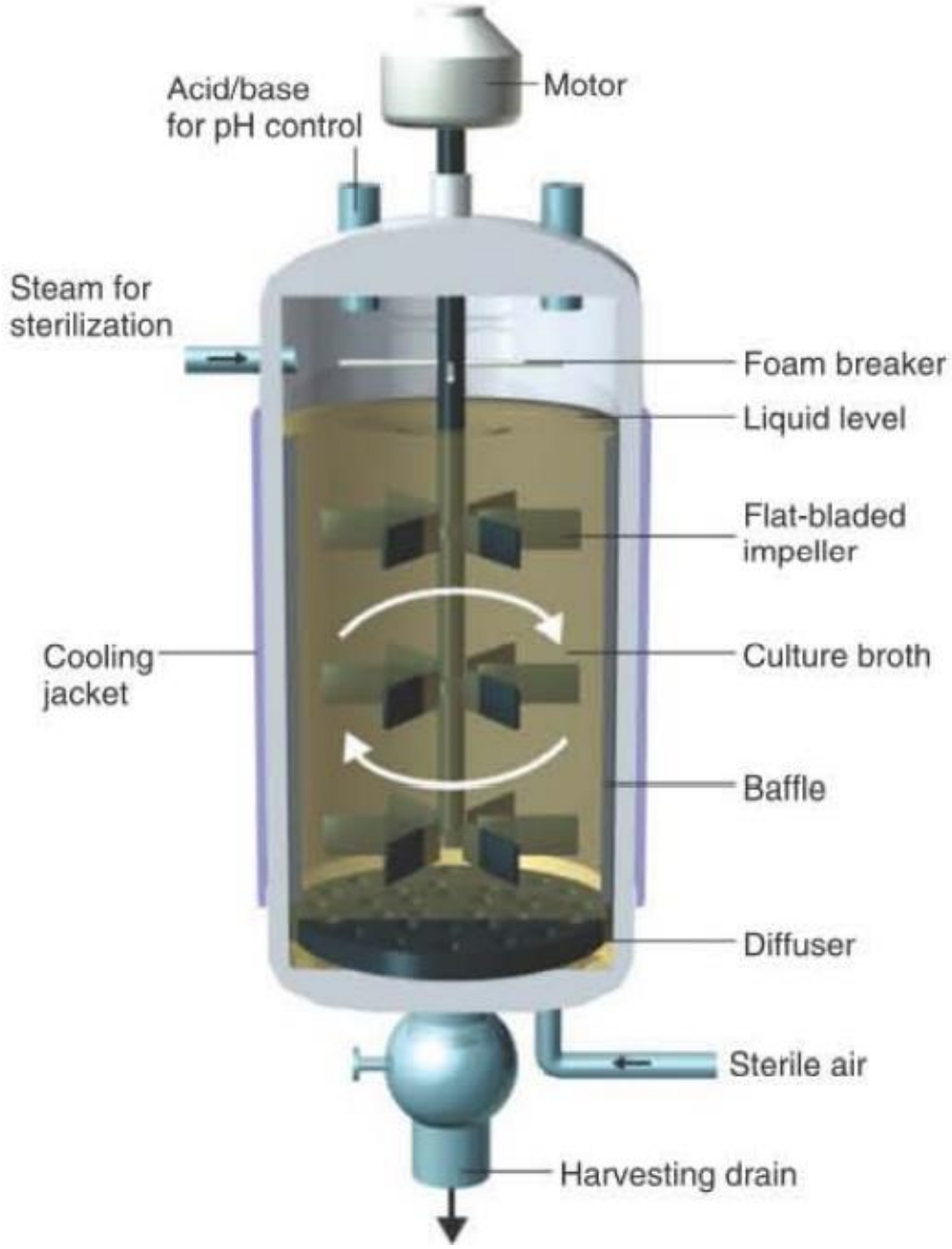
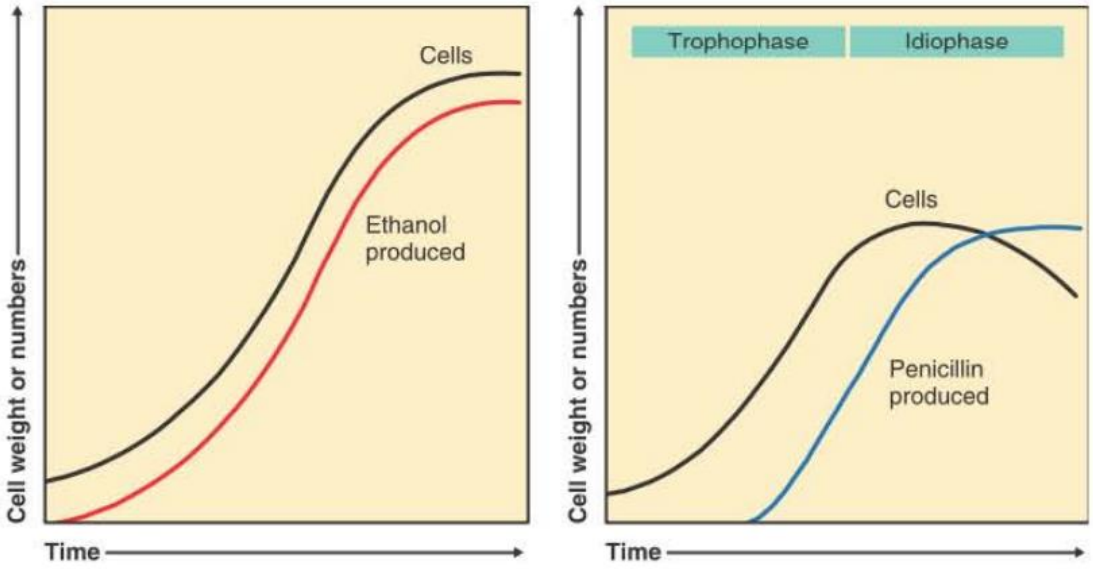


Figure 1: Bioreactors for industrial fermentations.

Generally speaking, the microbes in industrial fermentation produce either primary metabolites, such as ethanol, or secondary metabolites, such as penicillin. A primary metabolite is formed essentially at the same time as the new cells, and the production curve follows the cell population curve almost in parallel, with only minimal lag (Figure 2 a). Secondary metabolites are not produced until the microbe has largely completed its logarithmic growth phase, known as the **trophophase**, and has entered the stationary phase of the growth cycle (Figure 2 b). The

following period, during which most of the secondary metabolite is produced, is known as the **idiophase**. The secondary metabolite may be a microbial conversion of a primary metabolite. Alternatively, it may be a metabolic product of the original growth medium that the microbe makes only after considerable numbers of cells and a primary metabolite have accumulated.



(a) A primary metabolite, such as ethanol from yeast, has a production curve that lags only slightly behind the line showing cell growth.

(b) A secondary metabolite, such as penicillin from mold, begins to be produced only after the logarithmic growth phase of the cell (trophophase) is completed. The main production of the secondary metabolite occurs during the stationary phase of cell growth (idiophase).

Strain improvement is also an ongoing activity in industrial microbiology. (A microbial strain differs physiologically in some significant way. For example, it has an enzyme to carry out some additional activity or lacks such an ability, but this difference is not enough to change its species identity). A well-known example is that of the mold used for penicillin production. The original culture of *Penicillium* did not produce penicillin in large enough quantities for commercial use. A more efficient culture was isolated from a moldy cantaloupe from a Peoria, Illinois, supermarket. This strain was treated variously with UV light, X rays, and nitrogen mustard (a chemical mutagen). Selections of mutants, including some that arose spontaneously, quickly increased the production rates by a factor of more than 100. Today, the original penicillin-producing molds produce, not the original 5 mg/L, but 60,000 mg/L. Improvements in fermentation techniques have nearly tripled even this yield.

Immobilized Enzymes and Microorganisms

In many ways, microbes are packages of enzymes. Industries are increasing their use of free enzymes isolated from microbes to manufacture many products, such as high-fructose syrups, paper, and textiles. The demand for such enzymes is high because they are specific and do not produce costly or toxic waste products. And, unlike traditional chemical processes that require heat or acids, enzymes work under moderate conditions and are safe and biodegradable. For most industrial purposes, the enzyme must be immobilized on the surface of some solid support or otherwise manipulated so that it can convert a continuous flow of substrate to product without being lost.

Continuous flow techniques have also been adapted to live whole cells, and sometimes even to dead cells (Figure 3). Whole-cell systems are difficult to aerate, and they lack the single enzyme specificity of immobilized enzymes. However, whole cells are advantageous if the process requires a series of steps that can be carried out by one microbe's enzymes. They also have the advantage of allowing continuous flow processes with large cell populations operating at high reaction rates. Immobilized cells, which are usually anchored to microscopically small spheres or fibers, are currently used to make high-fructose syrup, aspartic acid, and numerous other products of biotechnology.

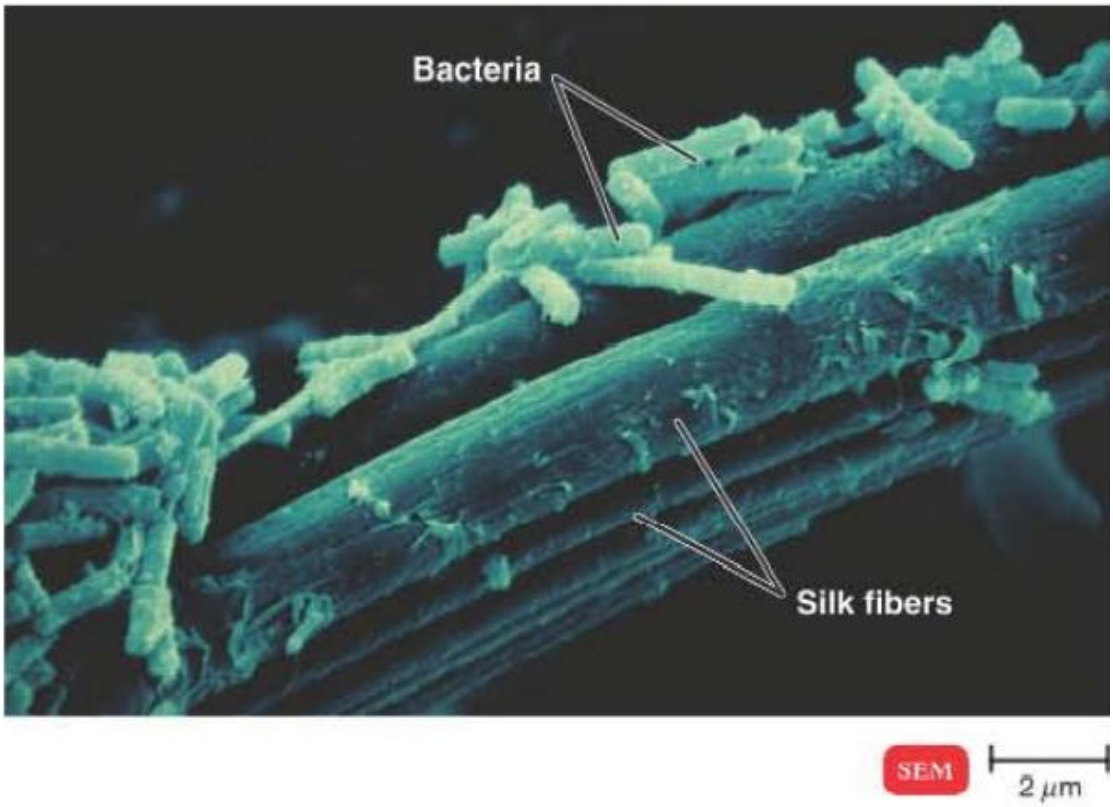


Figure 3: Immobilized cells. In some industrial processes, the cells are immobilized on surfaces such as the silk fibers shown here. The substrate flows past the immobilized cells