living organism. A wide range of host backgrounds (i.e., combinations of specific mutations) is available, as well as vectors and promoters. This large knowledge base greatly facilitates sophisticated genetic manipulations. The well-defined vectors and promoters greatly speed the development of an appropriate biological catalyst.

The relatively high growth rates for *E. coli* coupled with the ability to grow *E. coli* to high cell concentrations (> 50 g dry wt/l) and with the high expression levels possible from specific vector–promoter combinations (about 25% to 50% or more of total protein) can lead to extremely high volumetric productivities. Also, *E. coli* will grow on simple and inexpensive media. These factors give *E. coli* many economic advantages.

An important engineering contribution was the development of strategies to grow cultures of *E. coli* to high cell densities. The buildup of acetate and other metabolic by-products can significantly inhibit growth. Controlled feeding of glucose so as to prevent the accumulation of large amounts of glucose in the medium prevents overflow metabolism and the formation of acetate. Glucose feeding can be coupled to consumption rate if the consumption rate can be estimated on-line or predicted.

However, *E. coli* is not a perfect host. The major problems result from the fact *E. coli* does not normally secrete proteins. When proteins are retained intracellularly and produced at high levels, the amount of soluble active protein present is usually limited due to either proteolytic degradation or insolubilization into *inclusion bodies.*

The production of large amounts of foreign protein may trigger a *heat-shock* response. One response of the heat-shock regulon is increased proteolytic activity. In some cases, intracellular proteolytic activity results in product degradation at a rate nearly equal to the rate of production.

More often, the target protein forms an inclusion body. Although the heterologous protein predominates in an inclusion body, other cellular material is also often included. The protein in the inclusion body is misfolded. The misfolded protein has no biological activity and is worthless. If the inclusion bodies are recovered from the culture, the inclusion bodies can be resolubilized and activity (and value) restored. Resolubilization can vary tremendously in difficulty from one protein to another. When resolubilization is straightforward and recoveries are high, the formation of inclusion bodies can be advantageous, as it simplifies the initial steps of recovery and purification. It is important that during resolubilization the protein be checked by several analytical methods to ensure that no chemical modifications have occurred. Even slight changes in a side group can alter the effectiveness of the product.

Other consequences of cytoplasmic protein production can be important. The intracellular environment in *E. coli* might not allow the formation of disulfide bridges. Also the protein will usually start with a methionine, whereas that methionine would have been removed in normal posttranslational processing in the natural host cell. If the product is retained intracellularly, then the cell must be lysed (broken) during recovery. Lysis usually results in the release of endotoxins (or pyrogens) from *E. coli.* Endotoxins are lipopolysaccharides (found in the outer membrane) and can result in undesirable side effects (e.g., high fevers) and death. Thus, purification is an important consideration.

Many of the limitations on *E. coli* can be circumvented with protein secretion and excretion. *Secretion* is defined here as the translocation of a protein across the inner membrane of *E. coli. Excretion* is defined as release of the protein into the extracellular compartment.