Yeasts are the preferred organisms for industrial-scale ethanol production. Different species can be utilized, depending on the composition of the raw material utilized. *S. cerevisiae*, particularly suitable for fermentation of hexoses, has been the major organism used so far. *Kluyveromyces fragilis* or *Candida* sp. can be utilized when lactose or pentoses, respectively, are the available substrates. Other alternative organisms capable of producing ethanol such as *Zymomonas mobilis, Pachysolen* sp., are not used in industrial production. However, *Zymomonas* species have significant advantages over yeast and may be used at industrial scale in the future. Other pentose and hexose fermenting organisms such as *Clostridium hermosaccharolyticum* and *Thermoanaerobacter ethanolicus* are thermophilic and provide significant advantages for ethanol fermentations and separation. However, they produce some undesirable end products and yield dilute ethanol solutions. Genetic engineering has transformed *E. coli* into a very efficient ethanol producer, reaching ethanol concentrations up to 43% (vol/vol).

Raw materials can constitute up to 70% of the cost of ethanol production. Therefore, the selection of inexpensive materials has an important impact on process economics. The material selected should be readily and economically available in the fermentation plant. In Brazil cane sugar is extensively used, while in the United States sugar prices make sugar a nonviable raw material. Instead, corn is the most utilized material in the United States for the production of industrial ethanol. Most microorganisms require readily available sugar compounds for fermentation. Such sugar compounds are present in inexpensive raw materials, as sugar cane, sweet sorghum, or sugar beet juices and molasses. Whey is also utilized in commercial fermentation. Starch- and cellulose-containing materials, such as grains, fruits, vegetables, wood, biomass, waste paper, and agricultural wastes, can also be used, but they need to be hydrolyzed before fermentation. Starch is enzymatically saccharified before fermentation. Acid hydrolysis of starch is not recommended, as it can result in nonfermentable or inhibitory by-products. Hydrolysis of cellulosic materials can be difficult, expensive, and inefficient. However, interest exists on increasing their utilization in an effort to recycle wastes.

Yeast convert hexoses to ethanol and carbon dioxide by glycolysis, as shown by the following reaction:

$$C_6H_{12}O_6 \longrightarrow 2C_2H_5OH + 2CO_2 \tag{A.1}$$

The theoretical ethanol yield over glucose is 0.51 g/g, and the growth yield over glucose is 0.12 g/g. Usually by-products such as glycerol, succinic acid, and acetic acid are produced, and the actual yield is about 90–95% of the theoretical yield. Optimal temperature and pH values for yeast are 30° to 35°C and 4–6, respectively. For thermophilic organisms optimal temperature may range from 50° and 60°C. Ethanol production is triggered by anaerobic conditions. Trace amounts of oxygen (0.05–0.1 mm Hg) are required by yeast for lipid biosynthesis and maintenance of cellular processes.

The feed solution should be balanced in terms of nitrogen, phosphorus, minerals, and some trace elements. Glucose medium is usually supplemented with NH_4Cl, KH_2PO_4, $MgSO_4$, $CaCl_2$, and yeast extract. In industry, only some ammonium and phosphate salts need to be added to diluted molasses. Glucose concentration in feed solution has an important effect on the rate and extent of ethanol production. Glucose concentrations above 100 g/l are inhibitory for yeast.