MICROBIAL FERMENTATION - GENERAL PROCESS IN DETAIL

"In its simplest form, the bioprocess can be seen as just mixing micro organisms with a nutrient broth and allowing the components to react. . . . More advanced and sophisticated processes operating at large scale need to control the entire system so that the bioprocess can proceed efficiently and be easily and exactly repeated with the same amounts of raw materials and inoculum to produce precisely the same amount of product." (**1**) Fermentation of recombinant cell lines begins with genetic engineering of microbes classified "generally recognized as safe" (GRAS), such as *E. coli*, *Bacillus subtilis*, *Streptomyces* species, and *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* yeasts.

Industrial microbiology: small and large scale **production** of commercial products, and also to carry out complex chemical transformations. Microbes become biocatalysts (Fig.1).

Biocatalysts are very valuable and specific producing strains are stored in "national" collections. ATTC - American Type Culture Collection, DSMZ – Deutsche Sammlung Von Mikroorganismenund Zellkulturen

Bacterial Fermentation: The most common microbial source for recombinant protein production is *E. coli* because the most is known about its genetics, and thus it is the species with which pioneering molecular biologists were familiar (**2**). *B. subtilis* and its relatives have been used, mainly because of their greater tendency to secrete proteins into their environment. Many of those native proteins have industrial uses as well, but unfortunately some of them are proteases, enzymes that break up other proteins (such as the foreign therapeutic molecules the bacteria may be genetically engineered to make). So the ultimate product yields can be lower than companies would like. Various *Streptomyces* species are under study in recombinant fermentation, but so far they have demonstrated low expression levels. *Pseudomonas fluorescens* may have greater potential. But *E. coli* "remains one of the most attractive because of its

ability to grow rapidly and at high density on inexpensive substrates, its well-characterized genetics, and the availability of an increasingly large number of cloning vectors and mutant host strains"

Fungal Fermentation: For about $50–100/gram of final product, you can make insulin or a subunit vaccine, among other things, by fermenting genetically engineered yeast. Although their product yields may be relatively low (around 5%), they can be as high as 40% of total soluble protein (6), and yeasts offer certain advantages over bacteria as a recombinant expression system. Chief among these is their ability as eukaryotic organisms to perform certain post translational modifications on the proteins they make. For many of the same reasons that *E. coli* is popular among bacterial production systems, *S. cerevisiae* (baker's or brewer's yeast) is the fungal species most commonly found in fermentation processes, recombinant or not. It is the one with which people have the most experience (thousands of years' worth) and thus the best understanding (the full genome was sequenced in 1996).

Alcohol and Alcoholic beverages are produced by yeast from the fermentation of sugar to ethyl alcohol and CO2. Wine is produced from grape juice (Figure). Beer from fermentation of malted grain (**brewing**). **Distilled beverages** from the distillation of fermented solutions. Commodity alcohol is used as a gasoline additive (**gasohol**) and as an industrial solvent.

Fig. Alcohol and Alcoholic beverages

Commercial yeast, *Saccharomyces cerevisiae*, is produced in large-scale aerated fermentors using molasses with controlled continuous feeding of the sugar substrate.

Industrial uses of yeast and yeast products:

Figure: Yeast production

S. cerevisiae has been genetically engineered to produce a wide range of proteins: antigens to heptitis B, influenza, and polio; human growth hormone and insulin; antibodies and antibody fragments; human growth factors; interferon and interleukin; blood components such as human serum albumin; and tissue plasminogen activator (3). Other species that have been studied include *S. pombe* and *Pichia pastoris.*

FERMENTATION TECHNOLOGY

Microbiologists define fermentation as "*any process for the production of a product by means of the mass culture of a microorganism*" (Pumphrey & Julien, 1996). Now a day widely used to produce enzymes, amino acids, and human and animal therapeutics through for recombinant technology. The common type of fermentation are batch, fed-batch, or continuous (perfusion). Amongst which batch mode is the most common type of fermentation found in industry.

Batch fermentation is a closed system: Microbes are added to a sterilized nutrient solution in the fermentor, then allowed to incubate. Nothing more is added except oxygen (most microorganisms used in biotechnology are aerobic species), an antifoam agent (to prevent bubble formation), and acid or base to control the solution pH. Consequently, the mixture changes constantly as a result of cellular metabolism, with waste products accumulating and biomass increasing over time. Four growth phases follow inoculation: a lag phase (microorganisms physicochemically equilibrating with their environment), a log phase (cells have adapted to their new surroundings and begin doubling their number logarithmically), a stationary phase (available food has been used up and growth slows or stops; this is when metabolites or recombinant proteins are best harvested), and a death phase (their energy depleted, the cells die off). Doubling times during the log phase vary according to the size and complexity of the microbe: bacteria double in less than an hour, yeasts in one or two hours, filamentous fungi in two to six hours.

Fed-batch fermentation is "an enhancement of the closed batch process" (**9**) in which nutrients are added in increments as growth progresses. Certain ingredients are present at inoculation, and they continue to be added in small doses throughout production. Continuous fermentation is an open system. Sterile nutrient solution is continuously introduced while an equal amount of waste products are removed. Cell growth may or may not be adjusted: *In the chemostat in the steady state, cell growth is controlled by adjusting the concentration of one substrate. In the turbidistat, cell growth is kept constant by using turbidity to monitor the biomass concentration and the rate of feed of nutrient solution is appropriately adjusted.* The nutrients involved can include carbohydrates, fatty acids, amino acids, and sources of nitrogen and sulfur. Sugars such as glucose, lactose, sucrose, and starch provide carbs and nitrogen. Vitamins, minerals, or growth factors may be necessary for some microbe species. Stirring and mixing adds an air supply, removes carbon dioxide, and distributes nutrients, but an antifoam chemical agent is necessary to keep excess bubbles from forming. Fermentation is a multiphasic reaction in which gaseous components (N2, O2, and CO2) must be mixed continuously with the liquid medium and solid cells. For optimal yields, the whole process must be carried out at a constant temperature.

During fermentation, cellular growth and metabolism produce heat through the energy released by the chemical reactions involved. Unless the resulting increase in overall temperature within the fermentor is counteracted, cells could die or at least fail to produce. Mechanical agitation of the medium also adds heat to the system. So a cooling system is necessary and must be closely monitored and controlled.

Fermentors in the biotechnology industry are "complex, aseptic, integrated systems involving varying levels of advanced computer inputs. According to guidelines provided by WHO and FDA regulations, for good manufacturing practices require contamination control to make an end product that is both safe and effective.

Characteristics of Large-Scale Fermentations:

Fermentation (generic term) is carrier out in a Fermentor (a vat) by a Fermenter (microbe). At laboratory Scale –5 – 10 L. while Production scale up to 500,000 L.

Table: Fermentor sizes for various industrial processes

Large-scale industrial **fermentation** presents several engineering problems while the microbial process must be continuously monitored to ensure yield and no contamination. ***Fermentation scale-up*** is an art in itself –optimization of laboratory scale to production scale. Industrial fermentations will be anaerobic and aerobic processes (Figure 30.4), aeration in the latter being critical, and requires special attention to stirring (impellor)/ bubbling (sparger). Both require heat removal systems (eg. **Ethanol – Kyowa Hakko 240 m3).**

Biotech processes must be monitored and well controlled which necessitates the use of both on-line sensors (measuring pH, CO_2, and dissolved O_2 levels, for example) that require calibration and special treatment and off-line analysis (periodic assays that test for nucleic acids and enzymes, the quality and amount of product present, and so on). On-line sensors are preferred, of course, because of their immediacy. A problem identified quickly can be corrected; some problems identified too late may be the death of a whole batch. Many vendor companies are working to develop more and better sensors for bioprocess monitoring.

Bioprocesses typically proceed through three stages: laboratory scale, pilot scale, and production scale (1). Initially, basic work is performed using Petri dishes, Erlenmeyer flasks, and other simple laboratory culture techniques. Next, pilot-plant studies help process developers determine the optimal operating conditions at a volume of 5–200 L. The final stage transfers those conditions into 500-L to 1000-L (or bigger) fermentors for commercial manufacturing.