5. Lack of understanding of environmental impact; no emissions.
6. Complexity of biomass power infrastructure compared to known well established coal and natural gas markers.

Advantages:

1. Renewable fuel, obtained from vegetable oils or animal fats.
2. Low toxicity, in comparison with diesel fuel.
3. Degrades more rapidly than diesel fuel, minimizing the environmental consequences of biofuel spills.
4. Lower emissions of contaminants: CO, particulate matter, polycyclic aromatic hydrocarbons, aldehydes.
5. Lower health risk, due to reduced emissions of carcinogenic substances.
6. No SO_2 emissions.
7. Higher flash point (100^0 C minimum).
8. May be blended with diesel fuel at any proportion; both fuels may be mixed during the fuel supply to vehicles.
9. Excellent properties as a lubricant.
10. It is the only alternative fuel that can be used in a conventional diesel engine, without modifications.
11. Used cooking oils and fat residues from meat processing may be used as raw materials.

Disadvantages:

1. Poorly made biodiesel can cause engine problems – gives out more nitrogen oxide emissions.
2. Slightly higher fuel consumption due to the lower calorific value of biodiesel.
3. Slightly higher nitrous oxide emissions than diesel fuel.
4. Higher freezing point than diesel fuel. This may be inconvenient in cold climates.
5. It is less stable than diesel fuel and therefore, long term storage (more than six months) of biodiesel is not recommended.
6. May degrade plastic and natural rubber gaskets and hoses when used in pure form, in which case replacement with Teflon components is recommended.
7. It dissolves the deposits of sediments and other contaminants from diesel fuel in storage tanks and fuel lines, which then are flushed away by biofuel into the engine, where they can cause problems in the valves and injection systems. In consequence, the cleaning of tanks prior to filling with biodiesel is recommended.

It must be noted that these disadvantages are significantly reduced when biodiesel is used in blends with diesel fuel.

Fossil fuels

The three main fossil fuels, namely –

1. Coal
2. Natural gas and
3. Oil

Approximately 93% of fossil fuel consumed throughout the world is for energy production, with only 7% being used by industry for the production of solvents, plastics and a host of other organic chemicals.

Biogas

Biogas: Biogas is the gaseous emission from anaerobic degradation of organic matter (from plants or animals) by a consortium of bacteria. It is principally a mixture of CH_4 and CO_2 along with other trace gases. Methane gas, the primary component of natural gas (98%), makes up 55-90% by volume of biogas, depending on the source of organic matter and conditions of degradation.

Typical composition of biogas:

Compounds	**%**
Methane	50-75
CO_2	25-50
Nitrogen	0-10

4. Chemistry and Biotechnology

Production of solvents

The commercially important organic solvents are:

1. Ethanol
2. Acetone
3. Butanol and
4. Glycerol

Ethanol

Biosynthesis of ethanol

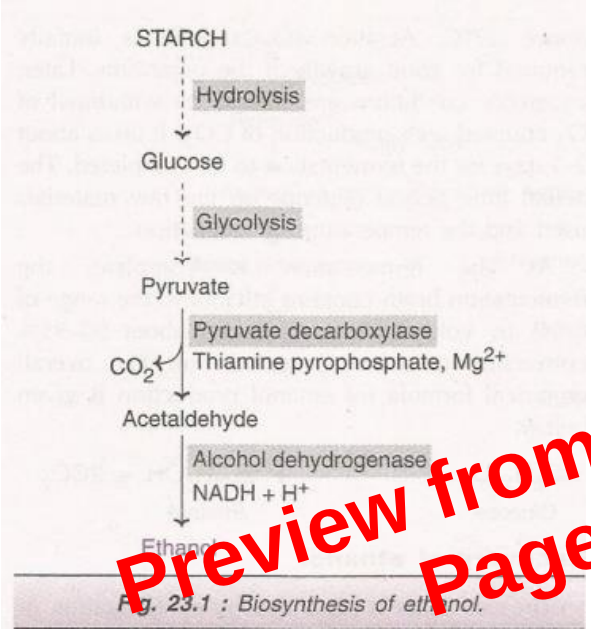

Fig. 23.1 : Biosynthesis of ethanol.

Production process of ethanol

8. Bioprocess engineering and downstream processing

Bioprocess technology: The word fermentation originates from a Latin verb fervere which literally means to boil. During the production of alcohol, the gas bubbles of CO_2 appear at the surface of the boiling liquid. Fermentation in a strict sense is a biological process that occurs in the absence of O_2 (anaerobic).

Bioprocess technology is a more recent usage to replace fermentation technology. Bioprocessing broadly involves a multitude of enzyme catalyzed reactions carried out by living cells (or cell-free systems) for industrial purpose. Some workers prefer to use bioprocess technology for industrial use of higher plant and animal cells while fermentation technology is confined to microbial use. This demarcation is however, not very rigid.

Bioreactors or fermenters: The heart of fermentation (or bioprocessing) technology is the fermenter or bioreactor. A bioreactor is basically a device in which the organisms (cells) are cultivated and motivated to form desired product(s). It is a containment system designed to give right environment for the optimal growth and metabolic activity of the organism.

A fermenter usually refers to the containment system for the cultivation of prokaryotic cells (bacteria, fungi), while a bioreactor grows the eukaryotic cells (mammalian, insect).

Traditional fermenters are open vats made up of wood or slate. In recent years, stainless steel bioreactors are in use. A high quality stainless steel that does not corrode or leak toxic metals into the growth medium is used. The size of a bioreactor is highly variable, ranging from 20 liters to 250 million liters or even more.

Types of bioreactors: Based on the designs, bioreactors are of the following types:

1. Continuous stirred tank bioreactors
2. Bubble column bioreactors
3. Airlift bioreactors
4. Fluidized bed bioreactors
5. Packed bed bioreactors
6. Plug flow reactors

In all types of bioreactors, the ultimate aim is to ensure that all parts of the system are subjected to the same conditions.

Downstream processing: As the fermentation is complete, it is necessary to recover the desired end product. The end products include antibiotics, amino acids, vitamins, organic acids, industrial enzymes and vaccines. The extraction and purification of a biotechnological product from fermentation is referred to as downstream processing (DSP) or product recovery. DSP is as complex and important as fermentation process. It often requires the expertise and technical skills of chemists, process engineers and bio-scientists.

In the present day biotechnology, the fermentation and downstream processing are considered as an integrated system. The methodology adopted for downstream processing depends on the nature of the end product, its concentration, stability and the degree of purification required, besides the presence of other products. The product recovery yield in general is expected to be higher, if the number of steps in DSP is lower.

The desired products for isolation by DSP are most frequently metabolites which may be present as follows:

1. **Intracellular metabolites:** These products are located within the cells e.g. vitamins and enzymes.
2. **Extracellular metabolites:** They are present outside the cells (culture fluids) e.g. most antibiotics (penicillin, streptomycin), amino acids, alcohol, citric acid, some enzymes (amylases, proteases).
3. **Both intracellular and extracellular:** Examples include vitamin B_{12}, flavomycin.

1. Adsorption
2. Entrapment
3. Encapsulation

Chemical methods:

1. Covalent binding
    - Support and
    - Cross linking

They are discussed below:

Physical methods:

1. **Adsorption:** Adsorption involves the physical binding of enzymes or cells on the surface of an inert support. The support materials may be inorganic (e.g. alumina, silica gel, calcium phosphate gel, glass) or organic (starch, carboxymethyl cellulose, DEAE cellulose, DEAE sephadex).
   Adsorption of enzyme molecules (on the inert support) involves weak forces such as van dar Waals force and hydrogen bonds. Therefore, the adsorbed enzymes can be easily removed by minor changes in pH, ionic strength or temperature.
   The support or carrier used may of different types such as:
    - Mineral support e.g. aluminum oxide, clay.
    - Organic support e.g. starch.
    - Modified sepharose and ion exchange resins.

Methods of adsorption:

a. **Static process:** Immobilization to carrier by allowing the solution containing enzyme to contact the carrier without stirring.
b. **Dynamic batch process:** Carrier is placed in the enzyme solution and mixed by stirring or agitation.
c. **Reactor loading process:** Carrier is placed in the reactor, and then the enzyme solution is transferred to the reactor with continuous agitation.
d. **Electrode position process:** Carrier is placed near to an electrode in an enzyme bath and then the current is put on, under the electric field the enzymes migrates to the carrier and deposited on its surface.

Advantages:

i. No pore diffusion limitation
ii. Easy to carry out.
iii. No reagents are required.
iv. Minimum activation steps are involved.
v. It is comparatively cheap method of immobilization.
vi. It is less disruptive to enzyme than chemical methods.

Disadvantages:

i. Deposition of enzymes from the carrier.
ii. Efficiency is less.

2. **Entrapment:** Enzymes can be immobilized by physical entrapment inside a polymer or gel matrix. Bonds involved in stabilizing the enzyme to the matrix may be covalent or non-covalent. Examples of commonly used matrices for entrapment:
    - Polysaccharide gels
    - Cellulose triacetate
    - Agar
    - Gelatin
    - Carrageenan
    - Alginate

Methods of entrapment:

a. **Enzyme inclusion in gels:** This is an entrapment of enzymes inside the gels.

10. Cell Culture

Animal cell culture basically involves the in vitro (in the laboratory) maintenance and propagation of animal cells in a suitable nutrient media. Thus, culturing is a process of growing cells artificially. Cell culture has become an indispensable technology in various branches of life sciences.

Terminology in cell culture

The term tissue culture is commonly used to include both organ culture and cell culture.

Organ culture: The culture of native cells (i.e. undisaggregated tissue) that retains most of the in vivo histological features is regarded as organ culture.

Cell culture: This refers to the culture of dispersed or disaggregated cells obtained from the original tissue or from a cell line.

Histotypic culture: The culturing of the cells for their re-aggregation to form a tissue-like structure represents histotypic culture.

Organotypic culture: This culture technique involves the recombination of different cell types to form a more defined tissue or an organ.

Primary culture: The culture produced by the fresh isolated cells or tissue taken from an organism is the primary culture. These cells are heterogenous and slow growing. They represent the tissue of their origin with regard to their properties.

Cell line: The sub-culturing of the primary culture gives rise to cell lines. The term continuous cell lines implies the indefinite growth of the cells in the subsequent sub-culturing. On the other hand, finite cell lines represent the death of cells after several subcultures.

Minimal requirements for the cell culture:

Infrastructure:

1. Clean and quite sterile area
2. Preparation facilities
3. Animal house
4. Microbiology laboratory
5. Storage facilities for glassware, chemicals, liquids, small equipment.

Equipment:

1. Laminar flow
2. Sterilizer
3. Incubator
4. Refrigerator and freezer (-20^0 C)
5. Balance
6. CO_2
7. Cylinder
8. Centrifuge
9. Inverted microscope
10. Water purifier
11. Hemocytometer
12. Liquid nitrogen freezer
13. Slow cooling device for freezing cells
14. Pipette washer
15. Deep washing sink
16. Air conditioned rooms

Culture:

Human embryonic stem cells (hESCs) are generated by transferring cells from a pre-implantation stage embryo into a plastic laboratory culture dish that contains a nutrient broth known as culture medium.

↓

	Totipotent	Pluripotent	Multipotent
Relative potency	High	Medium	Low
Cell types capable of generating	Differentiate into any cell type	Differentiate into cells from any of the three germ layers	Differentiate into a limited range of cell types
Terminology	Toti = Whole	Pluri = Many	Multi = Several
Examples	Zygote, early morula	Embryonic stem cells, Induced pluripotent stem cells	Haematopoietic stem cells, neural stem cells, mesenchymal stem cells
Found	Early cells of fertilised egg	Inner mass cells of the blastocyst	In many tissues
Expression of pluripotency genes	+++	++	+
Expression of lineage-specific genes	+	++	+++
Pros of use in research	Easy to isolate and grow	Easy to isolate and grow	Less ethical issues, less chance of immune rejection if taken from same patient
Cons of use in research	Ethical issues	Ethical issues, teratoma formation	Hard to isolate, limited differentiation, scarce

The cells divide and spread over the surface of the dish.

↓

However, if the plated cells survive, divide and multiply enough to crowd the dish, they are removed gently and plated into several fresh culture dishes.

↓

The process of re-planting or sub-culturing the cells is repeated many times and for many months. Each cycle of sub-culturing the cells is referred to as a passage.

↓

Once the cell line is established, the original cells yield millions of embryonic stem cells.

↓

Embryonic stem cells have proliferated in cell culture for six or more months without differentiating, are pluripotent, and appear genetically normal are referred to as an embryonic stem cell line.

↓

At any stage in the process, batches of cells can be frozen and shipped to other laboratories for further culture and experiments.

Short Notes

Resazurin test

Resazurin dye is a chemical that changes colour in response to the number of bacteria in a liquid. It is a blue dye, itself weekly fluorescent until it is irreversibly reduced to the pink colored and highly red fluorescent resorufin. It is used as an oxidation-reduction indicator in cell viability assays for both aerobic and anaerobic respiration.

Material required:

1. Milk sample
2. Resazurin color or solution 0.05%

Apparatus:

1. All purpose lovibond comparator
2. One resazurin color disk from blue to white
3. Water path, thermostatically controlled to maintain temperature of 37^0 C.
4. Test tube 10 ml
5. Pipette 10 ml and 1 ml.

Procedure:

The sample is milked thoroughly by inverting from one to another container
↓
10 ml of milk sample is poured into previously sterilized test tube
↓
1 ml of resazurin solution is added quickly in the test tube
↓
The milk is mixed and then dyed thoroughly by inverting 2-3 times
↓
Test tubes are placed into water bath at the temperature of 37.5^0 C only for two minutes
↓
The tubes are then removed from the water bath
↓
Compared the color to test tube with standard disc until the colors are matched under comparator
↓
The number of disc or color of disk is recorded. If color falls between two disc numbers half value is recorded.

Result:

Color of sample	Bacterial content	Drinking quality of milk
Blue-purple	Very low	Good
Mauve	Low	Satisfactory
Pink	Medium	Poor
White	High	Unsatisfactory