Fermentation Media

- Dr. Ekta Khare
Department of Microbiology,
School of Lifesciences & Biotechnology,
CSJM University, Kanpur

Fermentation

- Fermentation is the process in which a substance breaks down into a simpler substance by using microorganism.
- According to the industrial microbiologist, fermentation is a process to produce a products by the mass culture of microorganisms.

TYPES OF MEDIA

The media used in fermentation processes may be

Synthetic or Crude

1.Complex media

It is a complex or natural media such as molasses, meat extract etc , are not completely define chemically however they are the media of choice in industrial fermentations.

2.Synthetic media

Synthetic media is prepaird by using chemicals or using naturaly available substances for carbon source , nitrogen source, vitamins , growth factor and hormones.

Media Formulation

- Most fermentations required

1. liquid media, often referred to as broth
2. solid-substrate for fermentation

- In most industrial fermentation processes there are several stages where media are required.

1. Several inoculum (starter culture) propagation steps
2. Pilot-scale fermentations
3. The main production fermentation.

The initial step in media formulation is the examination of the overall process based on the stoichiometry for growth and product formation.

Carbon source + Nitrogen Source + Oxygen + Other requirements ➡ Biomass + Products + CO_2 + H_2O + Heat

- Where biomass or primary metabolites are the target product, the objective is to provide a production medium that allows optimal growth of the microorganism.
- For secondary metabolite production, such as antibiotics, provide an initial period of cell growth, followed by conditions optimized for secondary metabolite production. At this point the supply of one or more nutrients (carbon, phosphorus or nitrogen source) may be limited and rapid growth stopped.
- Most fermentations, except those involving solid substrates, require large quantities of water in which the medium is formulated.
- General media requirements include a carbon source, which in virtually all industrial fermentations provides both energy and carbon units for biosynthesis, and sources of nitrogen, phosphorus and sulphur.
- Other minor and trace elements must also be supplied, and some microorganisms require added vitamins, such as biotin and riboflavin.
- Aerobic fermentations are dependent on a continuous input of molecular oxygen, and even some anaerobic fermentations require initial aeration of media, e.g. beer fermentations
- Media incorporate buffers, or the pH is controlled by acid and alkali additions, and antifoam agents may be required.
- For some processes, precursor, inducer or inhibitor compounds must be introduced at certain stages of the fermentation.
- The main elemental composition of microbial cells is the dry weight is 48% C, 7% H, 32% O and 14% N.

- Certain media nutrients or environmental conditions may affect not only the physiology and biochemistry, but also the morphology of the microorganism.
- The media adopted also depend on the scale of the fermentation.
- For small-scale laboratory fermentations pure chemicals are often used in well-defined media.
- Industrial-scale fermentations primarily use cost-effective complex substrates, where many carbon and nitrogen sources are derived from natural plant and animal materials.

The main factors that affect the final choice of individual raw materials

1. Cost and availability
2. Easy of handling in solid or liquid forms, along with associated transport and storage costs, e.g. requirements for temperature control.
3. Sterilization requirements and any potential denaturation problems.
4. Formulation, mixing, complexing and viscosity characteristics that may influence agitation, aeration and foaming during fermentation and downstream processing stages.
5. The concentration of target product attained, its rate of formation and yield per gram of substrate utilized.
6. The levels and range of impurities, and the potential for generating further undesired products during the process.
7. Overall health and safety implications.

The most frequently used substrates for industrial fermentation are

Medium component	Defined Component	Un-defined component
Carbon source	Glucose, Fructose, Glycerol, Xylose, Sucrose, Starch	Molasses, Meat extract, Peptone, Plant extracts and Materials (Cellulosic, lignoncellulosic and hemicellulosic materials, Starch complex, etc...)
Nitrogen source	Ammonium and Nitrate Salts	Yeast extract, Amino acid complex, Casein
Phosphate	Mono and di-phosphate salts	In traces of complex C- and N sources
Sulphur	Ammonium and Magnesium sulphate	In traces of complex C- and N sources
Magnesium	Mainly Magnesium sulphate In	In traces of complex C- and N sources
Mn, Mo, Fe, Zn, etc..	In form of Inorganic salts	In traces of complex C- and N sources
Vitamins and Growth factors	Added in pure form of vitamin and growth factors preparation	Yeast extract, and may found also as traces in some C- and N-sources

CARBON SOURCES

- A carbon source is required for all biosynthesis leading to reproduction, product formation and cell maintenance.
- In most fermentations it also serves as the energy source.
- Carbon requirements may be determined from the biomass yield coefficient (Y), an index of the efficiency of conversion of a substrate into cellular material.
- $$Y_{carbon(g/g)} = \frac{\text{Biomass Produced (g)}}{\text{Carbon Substrate Utilized (g)}}$$
- For commercial fermentations the determination of yield coefficients for all other nutrients is usually essential.
- Various organisms may exhibit different yield coefficient for the same substrate due to the pathway by which the compound is metabolized.
- For example; *Saccharomyces cerevisiae* grown on glucose has biomass yield coefficient of 0.56 g/g under aerobic condition and 0.12 g/g under anaerobic condition.
- Carbohydrates are traditional carbon and energy sources for microbial fermentations, although other sources may be used, such as alcohols, alkanes and organic acids. Animal fats and plant oils may also be incorporated into some media, often as supplements to the main carbon source.

Molasses

- Pure glucose and sucrose are rarely used for industrial scale fermentations, primarily due to cost. Molasses, a by product of cane and beet sugar production, is a cheaper and more usual source of sucrose.
- It is a dark coloured viscous syrup containing 50–60% (w/v) carbohydrates, primarily sucrose, with 2% (w/v) nitrogenous substances, along with some vitamins and minerals.
- Overall composition varies depending upon the plant source, the location of the crop, the climatic conditions under which it was grown and the factory where it was processed.
- Hydrol molasses, can also be used. This byproduct of maize starch processing primarily contains glucose.

- Sugarcane molasses (sucrose 48%)
- Sugar beet molasses (sucrose 33%)
- Molasses also contains
  - nitrogenous substances
  - Vitamins
  - Trace elements

Malt Extract

- Aqueous extracts of malted barley can be concentrated to form syrups that are particularly useful carbon sources for the cultivation of filamentous fungi, yeasts and actinomycetes.
- Malt extract contain about 90% carbohydrate on dry weight basis. This comprises 20% hexoses (glucose and small amounts of fructose), 55% disaccharides (mainly maltose and traces of sucrose).
- In addition, these products contain a range of branched and unbranched dextrins (15–20%), which may or may not be metabolized, depending upon the microorganism.
- Malt extracts also contain some vitamins and approximately 5% nitrogenous substances, proteins, peptides and amino acids.
- Sterilization of media containing malt extract must be carefully controlled to prevent over-heating.
- The constituent reducing sugars and amino acids are prone to generating Maillard reaction products when heated at low pH.
- These are brown condensation products resulting from the reaction of amino groups of amines, amino acids and proteins with the carbonyl groups of reducing sugars, ketones and aldehydes.
- Not only does this cause colour change, but it also results in loss of fermentable materials and some reaction products may inhibit microbial growth.

Starch & Dextrins

- These polysaccharides are not as readily utilized as monosaccharides and disaccharides, but can be directly metabolized by amylase-producing microorganisms, particularly filamentous fungi.
- Extracellular enzyme of fungi hydrolyse the substrate to a mixture of glucose, maltose or maltotriose to produce a sugar.
- Maize starch is most widely used, but it may also be obtained from other cereal and root crops.
- It is widely used in fermentation to converted into sugar syrup which contain mainly glucose.
- Starch firstly gelatinized and then hydrolysed by dilute acids or amylolytic enzymes, often microbial glucoamylases that operate at elevated temperatures.

Sulphite waste Liquor

- It is a by product in the manufacture of wood pulp by the suphite process.
- The Sugar containing wastes derived from the paper pulping which possess mixture of hexoses (80%) and pentoses (20%).
- Hexoses include glucose, mannose and galactose, whereas the pentose sugars are mostly xylose and arabinose.
- Usually the liquor requires processing before use as it contains sulphur dioxide.
- The low pH is adjusted with calcium hydroxide or calcium carbonate, and these liquors are supplemented with sources of nitrogen and phosphorus.

Cellulose

- Cellulose is predominantly found as lignocellulose in plant cell walls, which is composed of three polymers: cellulose, hemicellulose and lignin.
- Lignocellulose is available from agricultural, forestry, industrial and domestic wastes.
- Few microorganisms can utilize it directly, as it is difficult to hydrolyse.
- It is potentially a very valuable renewable source of fermentable sugars once hydrolysed, particularly in the bioconversion to ethanol for fuel use.

Whey

- Whey is an aqueous by product of the dairy industry. This material is expensive to store and transport.
- Therefore, lactose concentrates are often prepared for later fermentation by evaporation of the whey, following removal of milk proteins for use as food supplements.
- Lactose is generally less useful as a fermentation feedstock than sucrose, as it is metabolized by fewer organisms, for example i.e. *S. cerevisiae,* does not ferment lactose.
- This disaccharide was formerly used extensively in penicillin fermentations and it is still employed for producing ethanol, single cell protein, lactic acid, xanthan gum, vitamin B12 and gibberellic acid.

Fats & Oils

- Hard animal fats that are mostly composed of glycerides of palmitic and stearic acids are rarely used in fermentations.
- Plant oils (primarily from cotton seed, linseed, maize, olive, palm, rape seed and soya) and occasionally fish oil, may be used as the primary or supplementary carbon source, especially in antibiotic production.
- Plant oils are mostly composed of oleic and linoleic acids, but linseed and soya oil also have a substantial amount of linolenic acid.
- The oils contain more energy per unit weight than carbohydrates.

Corn Steep Liquor

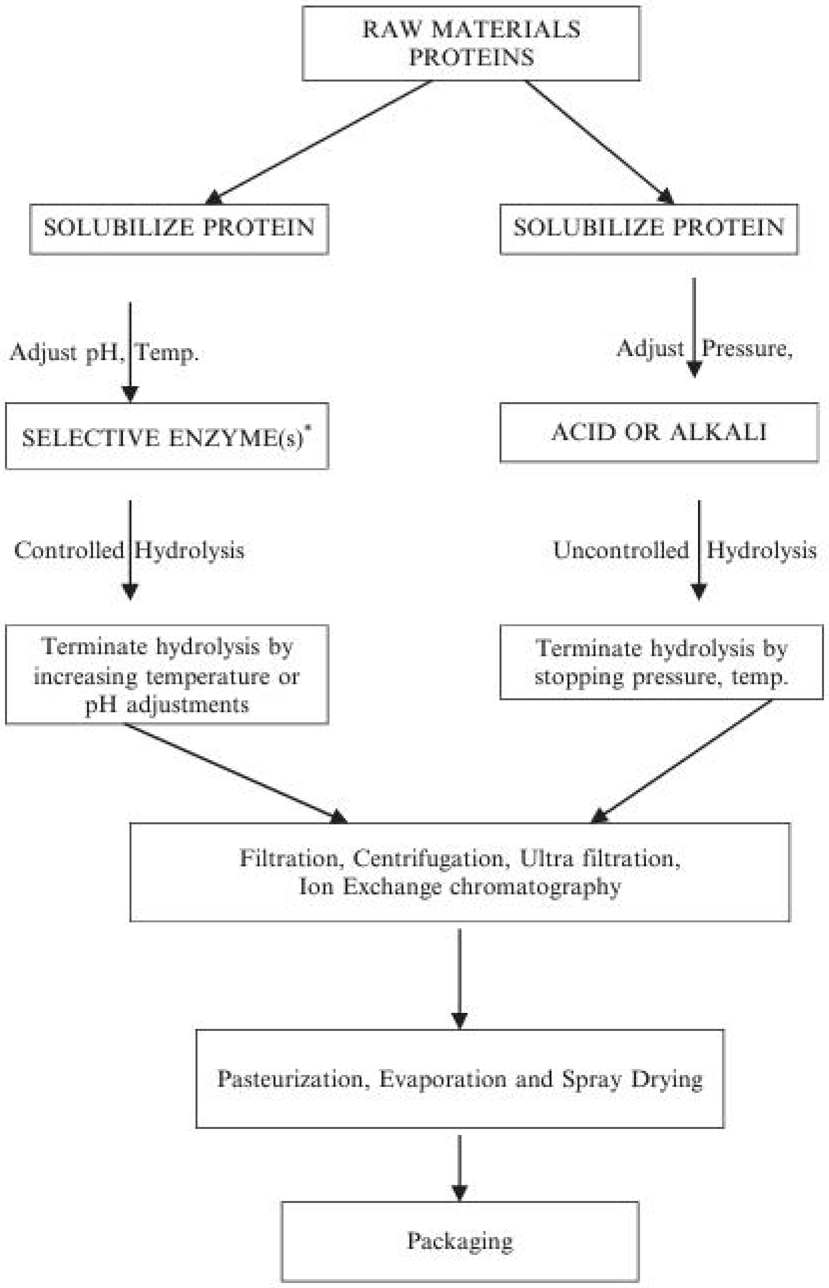
- Corn steep liquor is generated as a byproduct in the extraction of starch from corn by wet-milling process (soaking corn kernels in water to extract starch).
- The starch dissolves and the liquid component is separated from the solid residue.
- The resulting corn steep liquor contains solubilized protein components from the endosperm of corn and is relatively rich in essential nutrients such as N, P, K, amino acids, vitamins, and organic acids that are beneficial for plant growth.
- It is a viscous concentrate containing maltotriose (4.0%), lactic acid (4.8%), dextrose (1.2%), maltobiose (0.64%), glycerol (0.35%), acetic acid (0.06%), proteins (40%) and nitrogen free extract (16%).
- It also contains amino acids and vitamins which make it an excellent and inexpensive nitrogen source for fermentation processes.
- The cost of corn steep liquor is many times less than other nitrogen sources like yeast extract.
- Corn steep liquor is traditionally used for production of many microbial products including penicillin, ethanol, β-d-galactosidase or lactic acid.
- This by-product is mainly utilized as nitrogen source, but it also contains sugars, amino acids, ash and vitamins.

Yeast Extract

- Yeast extract is a product prepared mainly from waste brewer's yeast, which is rich in nucleotides, proteins, amino acids, sugars and a variety of trace elements, and has the advantages of low production cost and abundant supply of raw material.
- Yeast extract is usually defined as the water-soluble extract produced from yeast waste streams (*e.g.*, baker's yeast, brewer's yeast, *Candida utilis*, *Candida tropicalis*, and *Kluyveromyces marxianus*) following disruption of the cell membrane by various means.
- Yeast extract production begins with fresh yeast and consists of 3 steps: fermentation, breaking of the cell wall andseparation.
- Yeast extract by mechanical crushing and found the protein content to be as high as 64.1% (dw), the fat content at only 1.32% (dw), and the RNA content at 4%. Meanwhile, the essential amino acids accounted for 40% of total amino acid, while those with flavor-enhancing functions (glutamic acid, aspartic acid, glycine, and alanine) accounted for 34% of total amino acid.

Protein Hydrolysates

- Protein hydrolysates, otherwise commonly known as peptones or peptides, are used in a wide variety of products in fermentation and biotechnology industries.
- The term “peptone” was first introduced in 1880 by Nagelli for growing bacterial cultures. However, later it was discovered that peptones derived from the partial digestion of proteins would furnish organic nitrogen in readily available form.
- Ever since, p- tones, which are commonly known as protein hydrolysates, have been used not only for growth of microbial cultures, but also as nitrogen source in commercial fermentions using animal cells and recombinant microorganisms for the production of value added products such as therapeutic proteins, hormones, vaccines, etc.
- The predominant manufacturing method of protein hydrolysates for applications in biotechnology is by enzymes. However, protein hydrolysates made by acid and alkaline hydrolysis of proteins are also commercially available.
- The most commonly used animal protein hydrolysates in biotechnology applications are casein, whey and meat obtained from different organs.
- Widely used plant derived proteins are from soy and wheat however; recently rice, pea and cottonseed proteins have been introduced commercially



*Preservative for longer Hydrolysis