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Fermentation processes: Historical development, the range and components and criteria for selection of industrially important microorganisms



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Industrial Fermentation Products & Producer Organisms

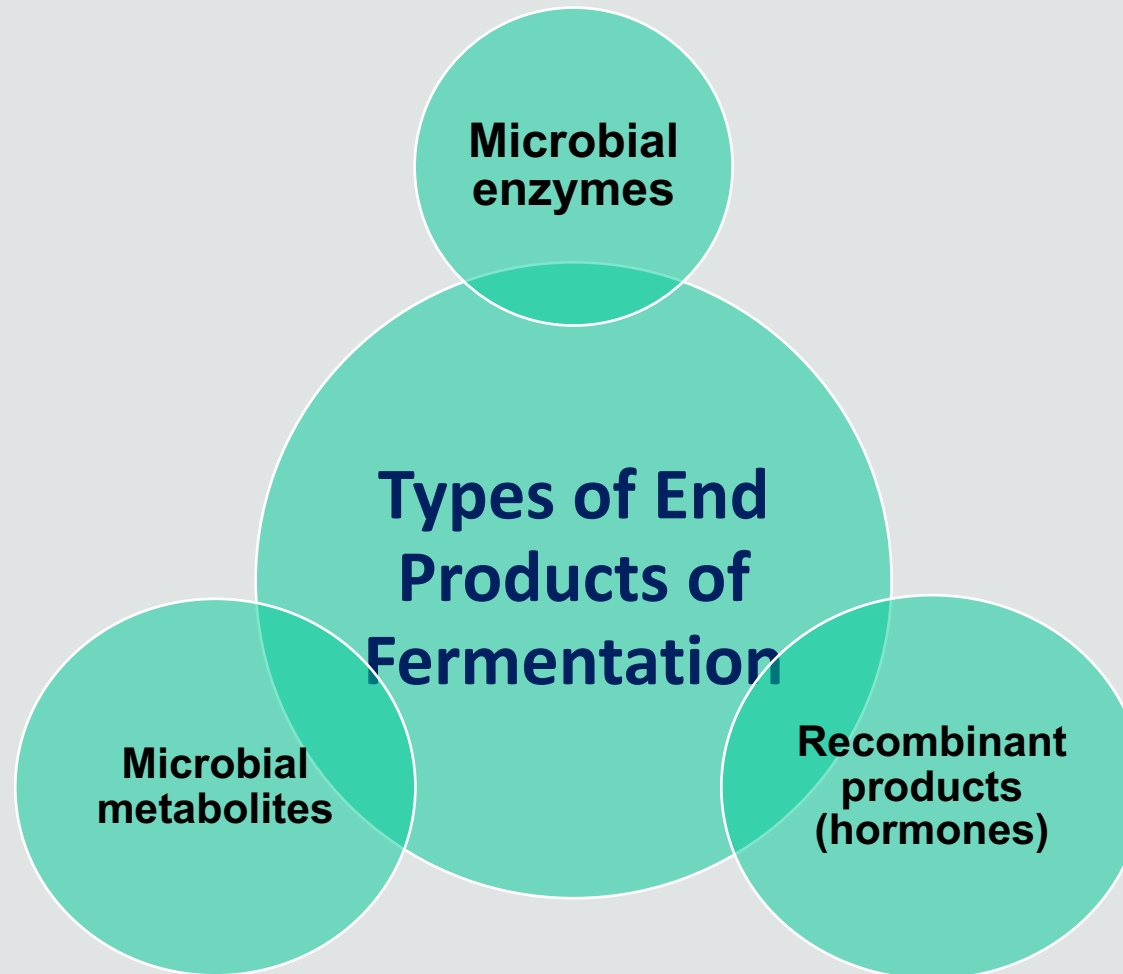
The word fermentation originated from fact that early at the start of wine fermentation gas bubbles are released continuously to surface giving impression of boiling.

- a) First meaning relates to microbial physiology. Fermentation is type of metabolism of carbon source in which energy is generated by substrate level phosphorylation and in which organic molecules function as final electron acceptor generated during breakdown of carbon-containing compounds or catabolism.
- b) Fermentation' is any process in which micro-organisms are grown on a large scale, even if final electron acceptor is not an organic. Production of penicillin, ethanol or alcoholic beverages.
- c) A fermented food is one, processing of which microorganisms play a major part. Microorganisms determine nature of food through producing flavor components.



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Fermentation products can be broadly divided into two categories:

1.

- **High volume, low value products**
- **Food and beverage fermentation products**

2.

- **Low volume, high value products**
- **Fine chemicals and pharmaceuticals**



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Products of Primary Metabolism:

- Inter-related group of reactions within microorganism which are associated with growth and maintenance of life.
- Primary metabolism is same in all living things and is concerned with release of energy, and synthesis of proteins, nucleic acids and other cell constituents.
- When primary metabolism is stopped, organism dies.
- Products of primary metabolism are associated with growth and maximum production occurs in logarithmic phase of growth in a batch culture.
- Primary catabolic products: Ethanol, lactic acid, butanol
- Anabolic products: Amino-acids, enzymes, nucleic acids.
- Single-cell proteins would also be regarded as primary products.



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Examples of industrial products resulting from primary metabolism

<i>Anabolic Products</i>	<i>Catabolic Products</i>
1. Enzymes	1. Ethanol and ethanol-containing products, e.g. wines
2. Amino acids	2. Butanol
3. Vitamins	3. Acetone
4. Polysaccharides	4. Lactic acid
5. Yeast cells	5. Acetic acid (vinegar)
6. Single cell protein	
7. Nucleic acids	
8. Citric acid	



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Secondary Metabolism Characteristics:

- No apparent function in organism.
- Produced in response to restriction in nutrients.
- Appears to be restricted to some species of microorganisms.
- Have unusual chemical structures and several closely related metabolites may be produced by same organism in wild-type strains.
- Ability to produce a particular secondary metabolite is easily lost.
- Owing to ease of loss of ability to synthesize secondary metabolites, secondary metabolite production is controlled by plasmids rather than by the organism's chromosomes.



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Product	Organism	Use/Importance
<i>Antibiotics</i>		
Penicillin	<i>Penicillium chrysogenum</i>	Clinical use
Streptomycin	<i>Streptomyces griseus</i>	Clinical use
<i>Anti-tumor Agents</i>		
Actinomyin	<i>Streptomyces antibioticus</i>	Clinical use
Bleomycin	<i>Streptomyces verticulus</i>	Clinical use
<i>Toxins</i>		
Aflatoxin	<i>Aspergiulus flavous</i>	Food toxin
Amanitine	<i>Amanita sp</i>	Food toxin
<i>Alkaloids</i>		
Ergot alkaloids	<i>Claviceps purpurea</i>	Pharmaceutical
<i>Miscellaneous</i>		
Gibberellic acid	<i>Gibberalla fujikuroi</i>	Plant growth hormone
Kojic acid	<i>Aspergillus flavus</i>	Food flavor
Muscarine	<i>Clitocybe rivalosa</i>	Pharmaceutical
Patulin	<i>Penicillium urticae</i>	Anti-microbial agent

Examples of Industrial products of microbial secondary metabolism



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Important criteria in choice of organism:

- Nutritional characteristics of the organism
- Optimum temperature of the organism
- Reaction of organism with equipment to be employed and suitability of organism to type of process to be used
- Stability of organism and its amenability to genetic manipulation
- Productivity of the organism, measured in its ability to convert substrate into product and to give high yield of product per unit time
- Ease of product recovery from the culture
- Irrespective of origins of an industrial microorganism



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Isolation de novo of Organisms Producing Metabolites of Economic Importance :

- **Enrichment with the substrate utilized by the organism**
- **Enrichment with toxic analogues of the substrate utilized by organism being sought**
- **Testing microbial metabolites for bioactive activity**
- **Testing for anti-microbial activity**
- **Testing for enzyme inhibition**
- **Testing for morphological changes in fungal test organisms**
- **Conducting animal tests on microbial metabolites**



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Strain Improvement

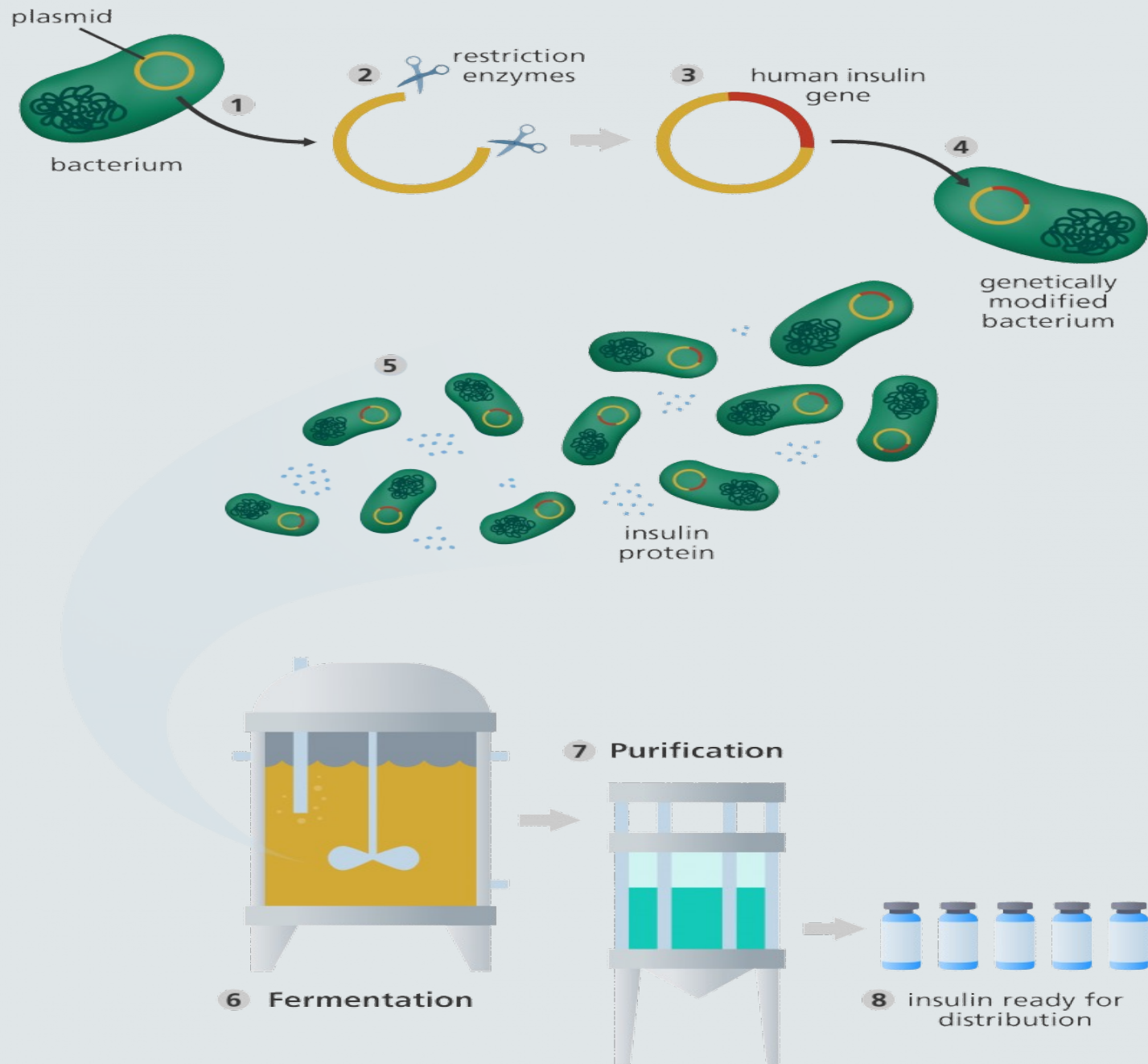
Operations in fermentor may also be improved by its use of a more productive medium, better environmental conditions, better engineering control of fermentor processes, or it may genetically improve productivity of microbial strain. Strain improvement appears to be one single factor with greatest potential for contributing to greater profitability.

- 1. Regulating activity of enzymes secreted by organisms**
- 2. Increasing permeability of organism so that microbial products can find their way more easily outside cell.**
- 3. Selecting suitable producing strains from a natural population**
- 4. Manipulation of existing genetic apparatus in producing organism**
- 5. Introducing new genetic properties into organism by recombinant DNA**
- 6. Genetic engineering**



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Examples of targets for strain improvement:

- 1. Rapid growth**
- 2. Genetic stability**
- 3. Non-toxicity to humans**
- 4. Large cell size, for easy removal from the culture fluid**
- 5. Ability to use cheaper substrates**
- 6. Elimination of the production of compounds that may interfere with downstream processing**
- 7. Permeability alterations to improve product export rates**
- 8. Metabolite resistance**
- 9. Production of additional enzymes and compounds to inhibit contaminant microorganisms**



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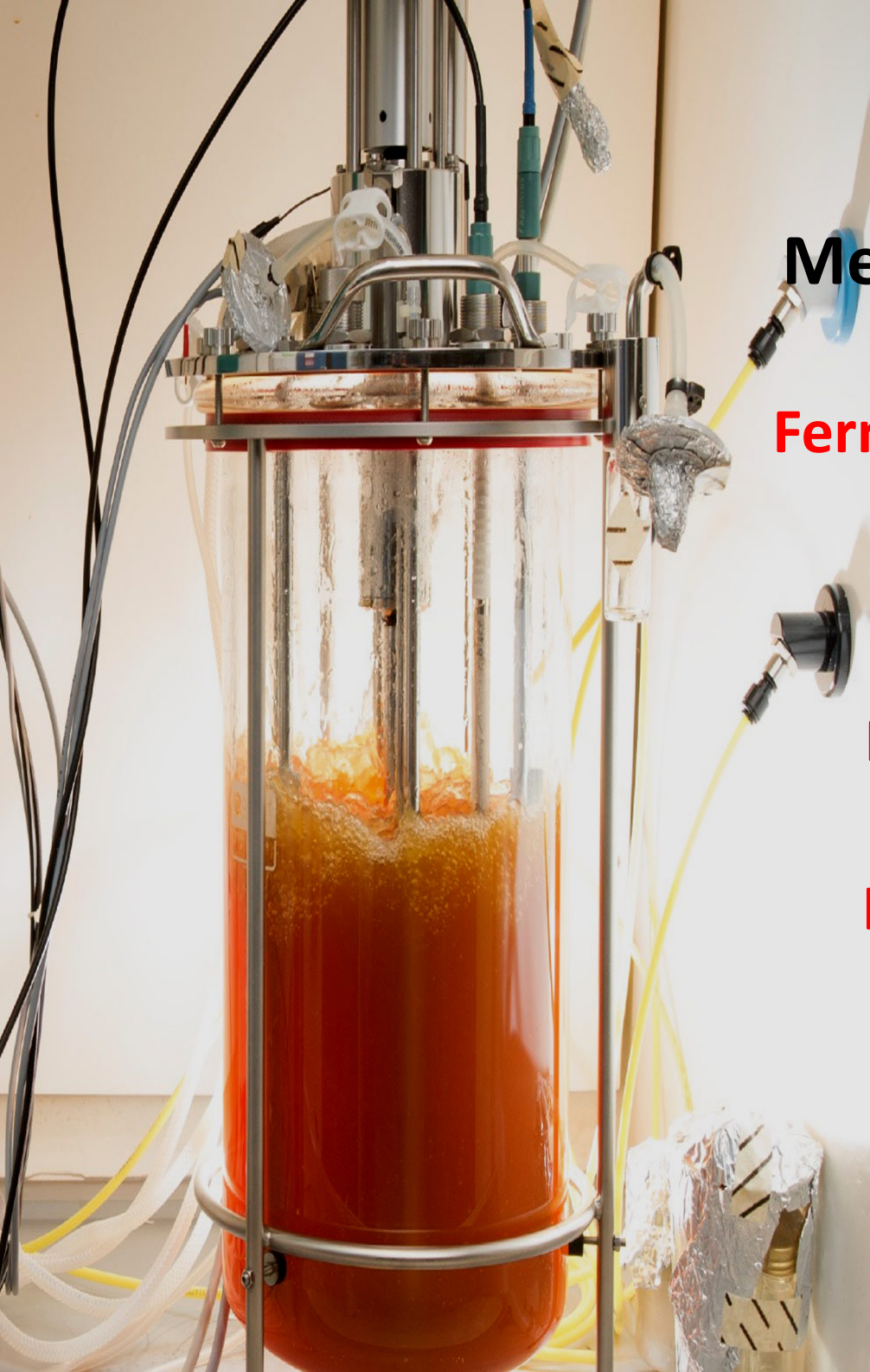
Manipulation of genome of industrial organisms in strain improvement:

Methods not involving foreign DNA

- Conventional mutation

Methods involving DNA foreign to organism

- Transduction
- Conjugation
- Transformation
- Protoplast fusion
- Genetic engineering



Media for Industrial Inoculums Development

Fermentation media must satisfy all nutritional requirements of microorganism and fulfill technical objectives of process. Nutrients should be formulated to promote synthesis of target product, either cell biomass or a specific metabolite.



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Main factors that affect final choice of individual raw materials

Cost of the material

Ready availability of the raw material

Transportation costs

Ease of disposal of wastes resulting from the raw material

Uniformity in quality of raw material

Adequate chemical composition of medium

Presence of relevant precursors

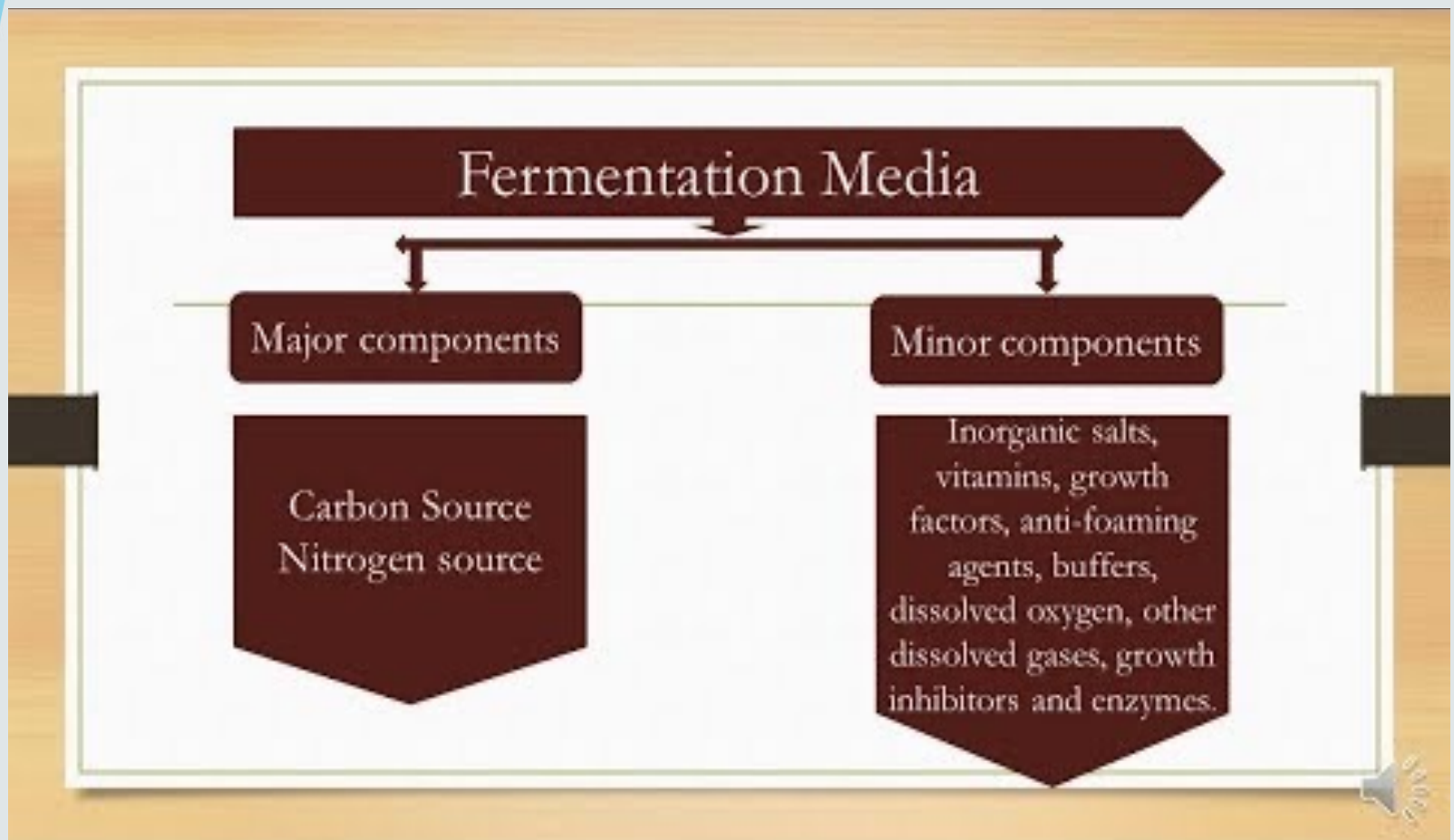


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Components of media for industrial inoculums development:

Media should support metabolic process of microorganisms and allow bio-synthesis of the desired products.





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Carbon sources

- 1. Required for biosynthesis leading to reproduction, product formation and cell maintenance.**
- 2. Serves as energy source.**
- 3. Carbohydrates are traditional carbon and energy sources for microbial fermentations, although alcohols, alkanes and organic acids may be used.**



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Molasses:

1. Pure glucose and sucrose are rarely used for industrial scale fermentations.
2. Molasses, a byproduct of cane and beet sugar production, is a cheaper and more usual source of sucrose.
3. It is residue remaining after most of sucrose has been crystallized from plant extract.
4. Dark coloured, viscous syrup containing 50–60% (w/v) carbohydrates, primarily sucrose, with 2% (w/v) nitrogenous substances, some vitamins and minerals.





Malt Extract:

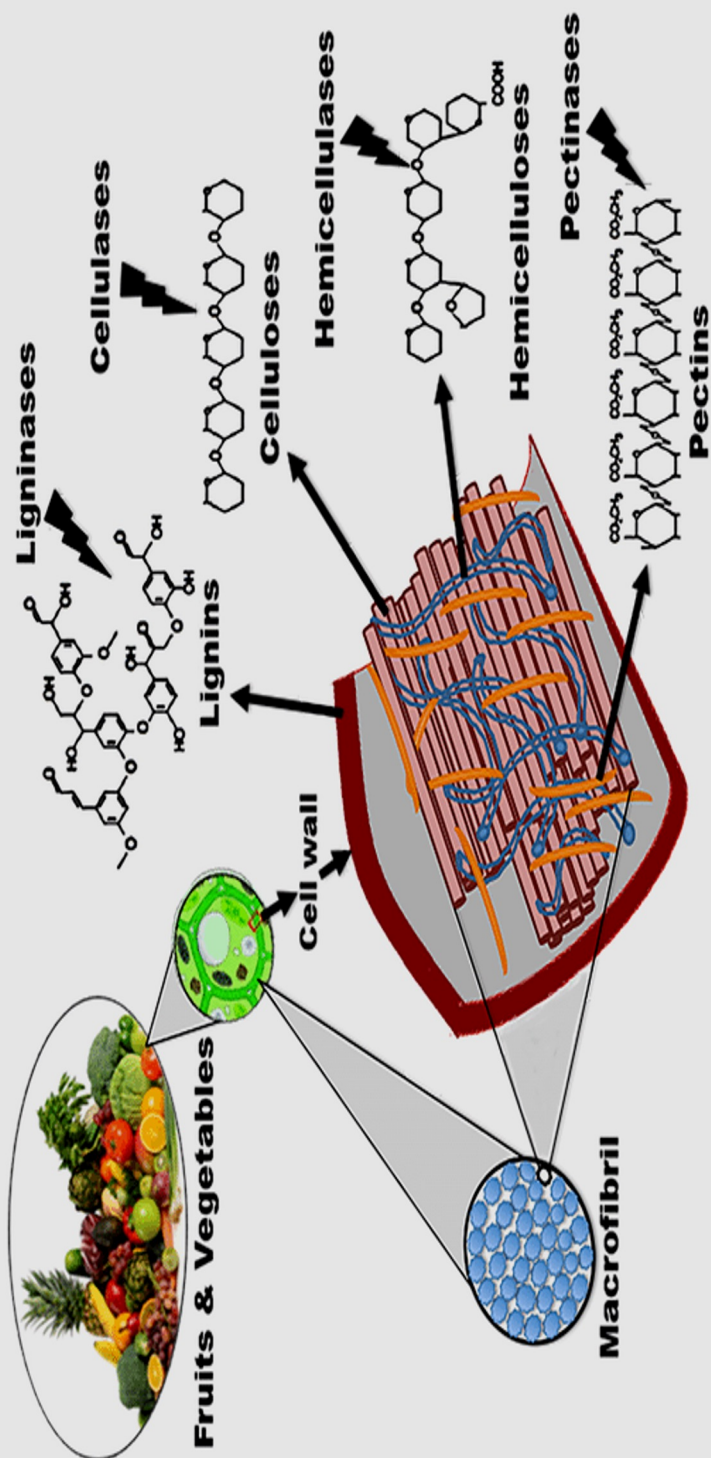
1. Aqueous extracts of malted barley can be concentrated to form syrups that are useful carbon sources for cultivation of filamentous fungi, yeasts and actinomycetes.
2. Malt extracts contain approx. 90% carbohydrate, on dry weight basis. This comprises 20% hexoses (glucose and small amounts of fructose), 55% disaccharides (mainly maltose and traces of sucrose), along with 10% maltotriose.
3. Contain some vitamins and approx. 5% nitrogenous substances, proteins, peptides and amino acids.



Starch and Dextrins:

1. Polysaccharides not readily utilized as monosaccharides and disaccharides, but can directly metabolized by amylase-producing microorganisms (filamentous fungi).





Sulphite Waste Liquor:

1. Sugar containing wastes derived from paper pulping industry are used for cultivation of yeasts.
2. Waste liquors from coniferous trees contain 2–3% (w/v) sugar, which is a mixture of hexoses (80%) and pentoses (20%).
3. Hexoses include glucose, mannose and galactose, pentose sugars are mostly xylose and arabinose.

Cellulose :

1. Predominantly found as lignocellulose in plant cell walls, composed of 3 polymers: cellulose, hemicellulose and lignin.
2. Lignocellulose is available from agricultural, forestry, industrial and domestic wastes.
3. Valuable renewable source of fermentable sugars once hydrolyzed, particularly in bioconversion to ethanol for fuel use.

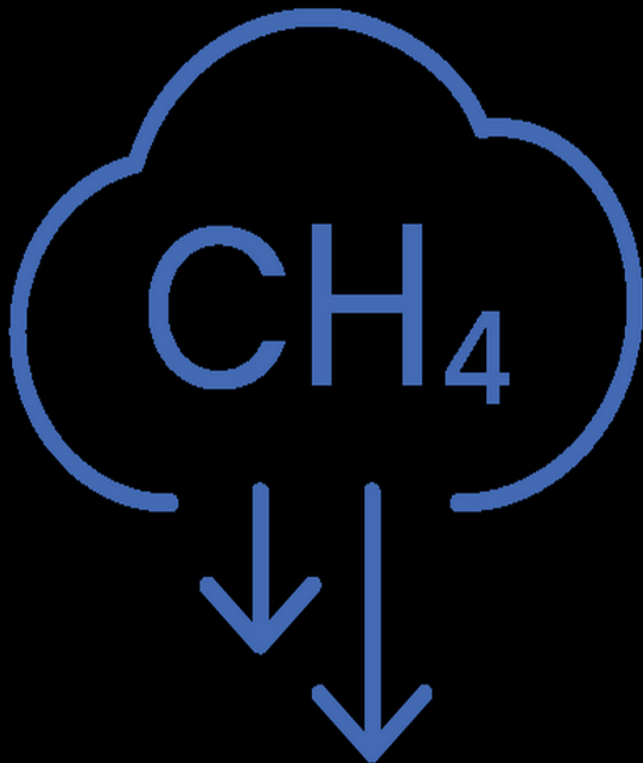


Whey:

1. Aqueous byproduct of dairy industry.
2. Expensive to store and transport.
3. Lactose concentrates are prepared for later fermentation by evaporation of whey, following removal of milk proteins for use as food supplements.
4. Lactose is less useful as fermentation feedstock as it is metabolized by few organisms.
5. *S. cerevisiae* does not ferment

Alkanes and Alcohols:

1. n-Alkanes of chain length C10–C20 are readily metabolized by certain microorganisms.
2. Methane utilized as carbon source by few microorganisms, but its conversion product methanol is preferred for industrial fermentations.
3. Ethanol is less toxic than methanol and used as sole/co-substrate by microorganisms.





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Fats and Oils

- 1. Hard animal fats that are mostly composed of glycerides of palmitic and stearic acids are rarely used in fermentations.**
- 2. Plant oils (primarily from cotton seed, linseed, maize, olive, palm, rape seed and soya) and occasionally fish oil, may be used as primary or supplementary carbon source, especially in antibiotic production.**



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Nitrogen sources

- 1. Industrial microbes can utilize both inorganic and organic nitrogen sources.**
- 2. Inorganic nitrogen may be supplied as ammonium salts, often ammonium sulphate and di-ammonium hydrogen phosphate or ammonia.**
- 3. Ammonia can also be used to adjust pH of fermentation.**
- 4. Organic nitrogen sources include amino acids, proteins and urea.**
- 5. Nitrogen is supplied in crude forms that are essentially byproducts of corn steep liquor, yeast extracts, peptones and soya meal.**



Corn steep liquor:

1. Byproduct of starch extraction from maize.
2. Exact composition of liquor varies depending on quality of maize and processing conditions.
3. Concentrated extracts generally contain about 4% (w/v) nitrogen, including wide range of amino acids, along with vitamins and minerals.



Peptones:

1. Too expensive for large-scale industrial fermentations.
2. Prepared by acid or enzyme hydrolysis of high protein materials: meat, casein, gelatin, keratin, peanuts, soy meal, cotton seeds, etc.



Everything you need
to know about

Yeast Extract



Yeast extract:

1. Produced from waste baker's and brewer's yeast, or other strains of *S. cerevisiae*.
2. Alternate sources are *Kluyveromyces marxianus* grown on whey and *Candida utilis* cultivated using ethanol, or wastes from wood and paper processing.



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Water

- 1. All fermentation processes, except solid-substrate fermentations, require vast quantities of water.**
- 2. Provides trace mineral elements and is important for ancillary equipment and cleaning.**
- 3. Before use, removal of suspended solids, colloids and microorganisms is usually required.**
- 4. When water supply is “hard”, it is treated to remove salts such as calcium carbonate.**



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Minerals

- 1. Normally, sufficient quantities of cobalt, copper, iron, manganese, molybdenum, and zinc are present in water supplies and as impurities in other media ingredients.**
- 2. Occasionally, levels of calcium, magnesium, phosphorus, potassium, sulphur and chloride ions are too low to fulfill requirements and these may be added as specific salts.**



Vitamins and growth factors

- 1. Many bacteria can synthesize all necessary vitamins from basic elements.**
- 2. For other bacteria, filamentous fungi and yeasts, they must be added as supplements to fermentation medium.**
- 3. Most natural carbon and nitrogen sources also contain at least some of required vitamins as minor contaminants.**
- 4. Other necessary growth factors, amino acids, nucleotides, fatty acids and sterols are added either in pure form or, for economic reasons, as less expensive plant and animal extracts.**
- 5. Corn steep powder and malt sprouts are examples of media ingredients.**



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Precursors

1. Some fermentations must be supplemented with specific precursors, notably for secondary metabolite production.
2. Examples include phenylacetic acid or phenylacetamide added as side-chain precursors in penicillin production.
3. Threonine is used as precursor in isoleucine production by *Serratia marcescens* and anthranillic acid additions are made to fermentations of yeast *Hansenula anomala* during tryptophan production.



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Inhibitors

1. Inhibitors are used to redirect metabolism towards target product and reduce formation of other metabolic intermediates; others halt a pathway at certain point to prevent further metabolism of target product.
2. An example of an inhibitor specifically employed to redirect metabolism is sodium bisulphite, which is used in production of glycerol by *S. cerevisiae*.

Antifoams

- 1. Antifoams are necessary to reduce foam formation during fermentation.**
- 2. Foaming is largely due to media proteins that become attached to air–broth interface where they denature to form a stable foam.**
- 3. If uncontrolled, foam may block air filters, resulting in loss of aseptic conditions; fermenter becomes contaminated and microorganisms are released into environment.**
- 4. Natural antifoams include plant oils (soya, sunflower and rapeseed), deodorized fish oil, mineral oils.**



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Cell permeability modifiers

- 1. These compounds increase cell permeability by modifying cell walls and/or membranes, promoting release of intracellular products into fermentation medium.**
- 2. Compounds used for this purpose include penicillins and surfactants.**