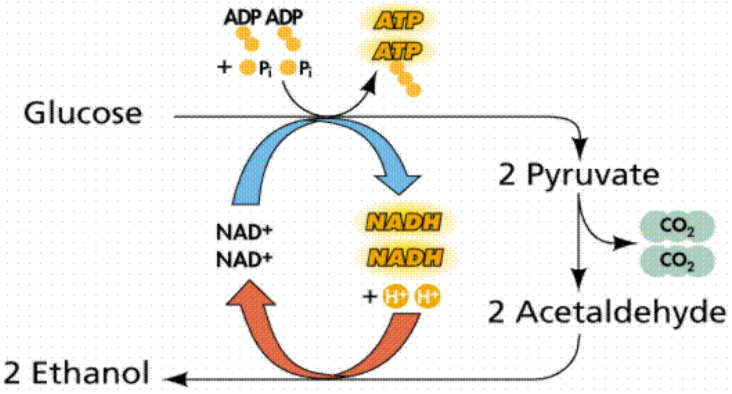
Development of media for industrial fermentation

By
SELVAPRAKASH N
2015008040
B.Sc (Food Nutrition&Dietetics)
HSC&RI –TNAU,Madurai-625104

Introduction

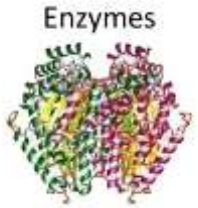


- Fermentation is a metabolic process that consumes sugar in the absence of oxygen.
- Products –acids ,gases or alcohol.
- Occurs in bacteria and yeast
- Science of fermentation –Zymology
- Producing energy by the degradation of organic nutrients anaerobically .
- Rate of fermentation depends –con of micro organisms,cells,cellularcomponents,enzymes,tempe rature,pH.
- Product recovery - con.of microoragnisms

Types of industrial fermentation

- Production of Biomass-scp,bakers yeast
- Production of extra cellular metabolites-(primary and secondary metabolites)

- Production of intra cellular metabolites-(enzymes)



- Transformation of substrate-

food fermentation

- All micro-organisms require water, sources of energy, carbon, nitrogen, mineral element and vitamin and oxygen in their growth medium.

- On a small scale - device a medium containing pure compounds satisfy the growth .But may be unsuitable for use in a large scale process.
- sources -cheap nutrient to create a medium.

Criteria for media selection

- Produce maximum yield of product
- Produce the maximum concentration of biomass
- Will permit maximum rate of product formation
- Minimum yield of undesirable product

- Will be consistent quality and available throughout the year.
- Cause minimal problems during medium sterilization.
- Aspects of production process such as aeration,agitation,downstream processing,waste treatment.

Fermentation media

- Laboratory medium may not be ideal in a large fermenter with a low gas transfer pattern.
- A medium –high viscosity - higher power input for effective stirring.
- pH variation, foam formation, oxidation reduction potential & morphological form of the organism.
- Undefined complex natural materials have been used in fermentation process because they are much cheaper.
- Batch variation-nutritionally differ each batch gives final yield

Medium formulation

- It is an essential stage in the design of successful of
  - ✓ laboratory experiments
  - ✓ pilot scale development
  - ✓ manufacturing process.
- The constituents of a medium must satisfy the elemental recruitments for cell biomass and metabolite production & must be an adequate supply of energy for cell biomass and cell maintenance.

Medium formulation

carbon and energy source + nitrogen source + O_2 + other requirements → biomass + products + CO_2 + H_2O + heat

Types of media

- Synthetic media
- Semi-synthetic media
- Complex media

Synthetic media

- Fully chemically defined.
- components known and specific concentration of each of the components.
- Media are quite simple containing carbon source ,nitrogen source, and a range of salts.
- Useful in research and laboratory situations where experimental is accuracy.

Semi synthetic media

- Largely chemically defined but one or more poorly specified components of variable but a controlled composition .

  ex .Yeast extract,beef tract.

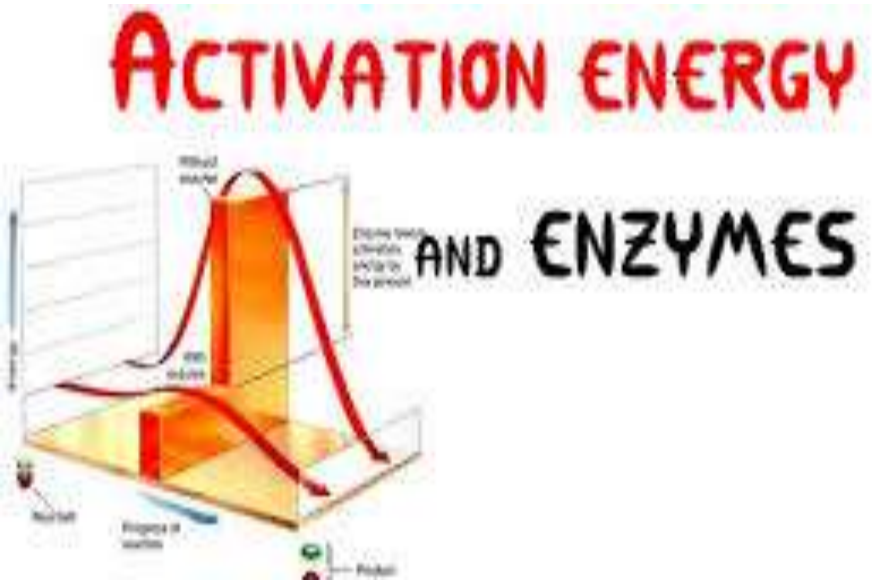
- This type of media are useful in research and laboratory situations –particular organism growth.
- Plant ,animal ,fish and microbial extracts routinely been used in the past to supply vitamins and essential growth factors for specific organisms.

Crude media /complex media

- Largely composed of substances that are usually of plant or animal origin, and that have defined and variable composition.
- These materials vary from batch to batch, and composition is influenced by time of year, location of origin, and small changes in production methods.
- Seasonal availability may change appreciably.

Energy requirements

- Carbon sources
- Nitrogen sources
- Vitamins and minerals
- Antifoams
- Inducers
- Chelators
- Cell Permeability Modifiers
- Vitamins and growth regulators



Carbon sources

- Required for all biosynthesis leading to reproduction, product formation and cell maintenance.
- The energy source
- Requirement biomass yield coefficient ,an index of the efficiency of conversion of a substrate into cellular material.
- carbon(g/g) = biomass produced(g) / carbon substrate utilized (g)
- Various organisms -different yield coefficients for the same substrate-pathway -compound is metabolized
- Differences -within an individual organism
- *Saccharomyces cerevisiae* -glucose biomass yield coefficients of 0.56 and 0.12 g/g under aerobic and anaerobic conditions, respectively.

Factors influencing the choice of carbon source

- Fast growth due to High concentrations rapidly metabolized sugars –produced secondary metabolites.
- Main product of a fermentation process often determine the choice of carbon source.
- Ethanol and SCP production 60-70% production cost ,the selling price depends upon type of carbon source used.

Sources

- Molasses
- Carbohydrates
- Oils and fats
- Hydrocarbons and their derivatives
- cellulose

Molasses

- Pure glucose and sucrose are rarely used for industrial-scale fermentations, primarily due to cost.
- Molasses-by-product of cane and beet sugar production- is a cheaper and more usual source of sucrose.
- Molasses -concentrated syrups or mother liquors recovered at any one of several steps in the sugar refining process with different names depending on the step from which it is recovered
- Blackstrap molasses from sugar cane is normally the cheapest and most used sugar source for industrial fermentation.

Molasses

- This material is the residue remaining after most of the sucrose has been crystallized from the plant extract.
- It is a dark-coloured viscous syrup containing 50-60% (w/v) carbohydrates, primarily sucrose, with 2% 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.
- The carbohydrate concentration may be reduced during storage by contaminating microorganisms.

Refinery blackstrap molasses

- It is a similar product obtained from the recrystallization refining of Crude sucrose.
- High test or invert molasses contains approximately 70-75% sugar.
- It is produced after whole cane juice is partially inverted to prevent sugar crystallization.
- It is preferable to blackstrap molasses as it has lower levels of non fermentable solids and lower shipping charges (on a concentration basis).
- It is only produced during sugarcane overproduction and availability may be questionable.

- Beet molasses: produced in a similar process -sugarcane.
- It may be limiting in biotin for yeast growth and a small amount of cane molasses may need to be added in these fermentations.
- Hydrol molasses: by-product of maize starch processing primarily contains glucose (60%) and a relatively high salt concentration.

Malt Extract

- Malted barley -concentrated to form syrups that are particularly useful carbon sources for the cultivation of filamentous fungi, yeast and actinomycetes.
- Extract preparation is essentially the same as for malt wort production in beer brewing.
- The composition of malt extracts varies to some extent, but they usually contain approximately 90% carbohydrate, on a dry weight basis.
- 20% hexoses (glucose and small amounts of fructose), 55% disaccharides (maltose and traces of sucrose), 10% maltotriose, a trisaccharide.,Dextrins (15-20%)

Malt extract

- Malt extracts -vitamins and 5% nitrogenous substances, proteins, peptides and amino acids.
- Sterilization of media containing malt extract must be carefully controlled to prevent overheating.
- The constituent reducing sugars and amino acids are prone to generating Maillard reaction products when heated at low pH.
- Brown condensation products resulting from the reaction of amino groups of amines, amino acids and proteins with the carboxyl groups of reducing sugars, ketones and aldehydes.
- Colour change- loss of fermentable materials and reaction products may inhibit microbial growth.

Starch and Dextrins

- Polysaccharides are not as readily [illegible] monosaccharides and disaccharides, but can be directly metabolized by amylase-producing microorganisms, particularly filamentous fungi.
- Extracellular enzymes hydrolyze the substrate to a mixture of glucose, maltose or maltotriose to produce a sugar.
- To allow use in a wide range of fermentations, the starch is usually converted into sugar syrup, containing mostly glucose.

Sulphite Waste Liquor

- Derived from the paper pulping industry after wood for paper manufacture is digested to cellulose pulp.
- It can be used as a dilute fermentation medium for ethanol production by *S. Cerevisiae* and the growth of *Torula utilis* for feed.
- The liquors derived from deciduous trees contain mainly pentoses.
- Usually the liquor requires processing before use as it contains sulphur dioxide or calcium hydroxide or calcium carbonate which need to be stripped or removed by precipitation with lime.
- These liquors also require supplementation with sources of nitrogen and phosphorous

Cellulose

- Found as lignocellulose in plant cell walls
- Composed of three polymers: cellulose, hemicellulose and lignin
- Available from agricultural, forestry, industrial and domestic wastes.
- Few microorganisms can utilize it directly, as it is difficult to hydrolyze
- Mainly used in solid-substrate fermentations to produce various mushrooms.

Whey

- An aqueous by-product of the dairy industry
- Expensive to store and transport
- Lactose is generally less useful as a fermentation food stock than sucrose, as it is metabolized by fewer organisms. *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 and Oils

- Hard animal fats
- Plant and animal oils-cotton seed, linseed, maize, olive, palm, rape seed ,soya and occasionally fish oil
- 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.
- Quantity larger

Nitrogen Sources

- Industrial microbes- inorganic and organic nitrogen sources
- Inorganic nitrogen -ammonium salts, often ammonium sulphate and diammonium hydrogen phosphate, or ammonia.
- Ammonia can also be used to adjust pH of the fermentation.
- Organic nitrogen -amino acids, proteins and urea.
- By-products of other industries, such as corn steep liquor, yeast extracts, peptones and soya meal.

Corn steep liquor

- By-product of starch extraction from maize and its first use in fermentations was for penicillin production in the 1940s.
- Concentrated extracts generally contain about 4% (w/v) nitrogen.
- Corn steep liquor replaced by potato starch production.

Yeast Extracts

- Produced from waste baker's and brewer's yeast.
- *Kluveromyces marxianus* cultivated from whey and *Candida utilis* cultivated using ethanol, or wastes from wood and paper processing .
- 50-60 oC autolysis-cells disrupted by plasmolysis-filteration -55-60% solids.
- They contain amino acids, peptides, water-soluble vitamins and some glucose, derived from the yeast storage carbohydrates (trehalose and glycogen).

Peptones

- Too expensive for large scale industrial fermentations.
- Prepared by acid or enzyme hydrolysis of high protein materials: meat, casein, gelatine, keratin, peanuts, soy meal, cotton seeds.
- Amino acid compositions may vary depending upon the original protein source.
- Gelatine-derived peptones are rich in proline and Hydroxyproline, lack of sulphur-containing amino acids
- Keratin peptone is rich in both proline and cystine, lacks in lysine.

Soya Bean Meal

- Residues remaining after soya beans have been processed to extract the bulk of their oil are composed of 50% protein, 8% non-protein nitrogenous compound, 30% carbohydrates and 1% oil.
- This residual soya meal is often used in antibiotic fermentations because the components are only slowly metabolized.

Water

- All fermentation processes, except SSF, require vast quantities of water.
- Important for ancillary services like heating, cooling, cleaning and rinsing.
- Important factors to consider when assessing suitability of a water supply are: pH, dissolved salts and effluent contamination.
- Water is hard , it is treated to remove salts such as calcium carbonate.
- Iron and chlorine may also require removal from the water.
- Plant and animal cell culture, the water must be highly purified.

Minerals

- Major elements-Mg,P,K,S,Ca &Cl
- Minor elements-Co,Cu, Fe,Mn,Mb & Zn
- Ex :corn steep liquor contains a wide range of minerals that will usually satisfy the minor and trace mineral needs.
- Sec.meatabolite production process have a lower tolerance range to inorganic phosphate than vegetative growth.

TABLE 4.10. *The range of typical concentrations of mineral components* ($g\ dm^{-3}$)

Component	Range
*KH_2PO_4	1.0–4.0 (part may be as buffer)
$MgSO_4 \cdot 7H_2O$	0.25–3.0
KCl	0.5–12.0
$CaCO_3$	5.0–17.0
$FeSO_4 \cdot 4H_2O$	0.01–0.1
$ZnSO_4 \cdot 8H_2O$	0.1–1.0
$MnSO_4 \cdot H_2O$	0.01–0.1
$CuSO_4 \cdot 5H_2O$	0.003–0.01
$Na_2MoO_4 \cdot 2H_2O$	0.01–0.1

*Complex media derived from plant and animal materials normally contain a considerable concentration of inorganic phosphate.

Chelators

- Many media cannot be prepared without precipitation during autoclaving.
- Some chelating agents are added to form complexes with metal ions which are gradually utilised by microorganisms.
    - Examples :EDTA, Citric acid,phosphates.
- Phosphates are widely used as a chelating agent .
- Used as a required quantity otherwise it will inhibit the growth of microorganism.
-

Antifoams

- Antifoams are necessary to reduce foam formation during fermentation.
- Foaming is largely due to media proteins that become attached to the air-broth interface where they denature to form a stable foam "skin" that is not easily disrupted.
- If uncontrolled the foam may block air filters, resulting in the loss of aseptic conditions; the fermenter becomes contaminated and Microorganisms are released into the environment

Approaches to controlling foam production

- Use of a defined medium
- Modification of some of the physical parameters, e.G. pH, temperature, aeration and agitation (if the foam is due to media components),
- Use of chemical foam breakers
- Addition of chemical antifoams

Vitamins and Growth Factors

- Many bacteria can synthesize all necessary vitamins from basic elements
- Most natural carbon and nitrogen sources also contain at least some of the required vitamins as minor contaminants.
- 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.

Precursors

- Defined as “substances added prior to or simultaneously with the fermentation which are incorporated without any major change into the molecule of the fermentation product and which generally serve to increase the yield or improve the quality of the product”.
- D-threonine is used as a precursor in

L-isoleucine production by *Serratia marcesans*

- Anthranillic acid addition -fermentation of the yeast *Hansenula anomola during L-tryptophan production*

Inducers and Elicitors

- If product formation is dependent upon the presence of a specific inducer compound or a structural analogue, it must be incorporated into the culture medium or added at a specific point during the fermentation.
- Inducers are often substrates such as starches or dextrins for amylase.
- Plant cell culture the production of secondary metabolites, such as flavanoids and terpenoids can be triggered by adding elicitors.

Inhibitors

- Inhibitors are used to redirect metabolism towards the target product and reduce formation of other metabolic intermediates; others halt a pathway at a certain point to prevent further metabolism of the target product.
- Some GMMs contain plasmids bearing an antibiotic resistance gene, as well as the heterologous gene(s).
- The incorporation of this antibiotic into the medium used for the production of the heterologous product selectively inhibits any plasmid-free cells that may arise.

Cell Permeability Modifiers

- These compounds increase cell permeability by modifying cell walls and/or membranes, promoting the release of intracellular products into the fermentation medium.
- Compounds used for this purpose include penicillins and surfactants.
- They are frequently added to amino acid fermentations, including processes for producing L-glutamic acid using the genera *Corynebacterium and Brevibacterium.*

Oxygen

- Supplied in the form of air containing about 21% (v/v) oxygen
- Oxygen requirements may vary widely depending upon the carbon source.
- The specific oxygen uptake rate of a microorganism increases with increase in the dissolved oxygen concentration up to a certain point referred to as the critical level.
- Maximum biomass production is achieved by satisfying the organism's maximum specific oxygen demand by maintaining the dissolved oxygen concentration greater than the critical level.

Animal cell culture media

- Animal cell culture media are normally based on complex basal media.
- Eagle's cell culture medium, which contains glucose, mineral salts, vitamins and amino acids.
- Mammalian cells a serum is usually added, such as foetal calf serum, calf serum, newborn calf serum or horse serum.
- Sterilization of formulated animal culture media and media constituents is also more problematic as many components are thermo labile, requiring filter sterilization

Plant cell culture media

- Plant cell culture are usually chemically defined.
- Contain an organic carbon source, a nitrogen source, mineral salts and growth hormones.
- Sucrose is frequently incorporated as the carbon source.
- Nitrate is the usual nitrogen source, often supplemented with ammonium salts.
- Combination and concentration of plant hormones provided depend upon the specific fermentation.
- Auxins are usually supplied, along with cytokinins to promote cell division.

References

- Fermentation principles media formulation
- **Principles of fermentation technology by**

  Second edition P.F.Stanbury,A.Whitaker &S.J.Hall
- **Practical FermentationTechnology by**

  Brian Mcneil & Linda M. Harvey


Questions?


THANK
YOU!