MEDIA FOR INDUSTRIAL FERMENTATION

All microorganisms require water, sources of energy, carbon, nitrogen, mineral elements, and possibly vitamins plus oxygen if aerobic

Criteria

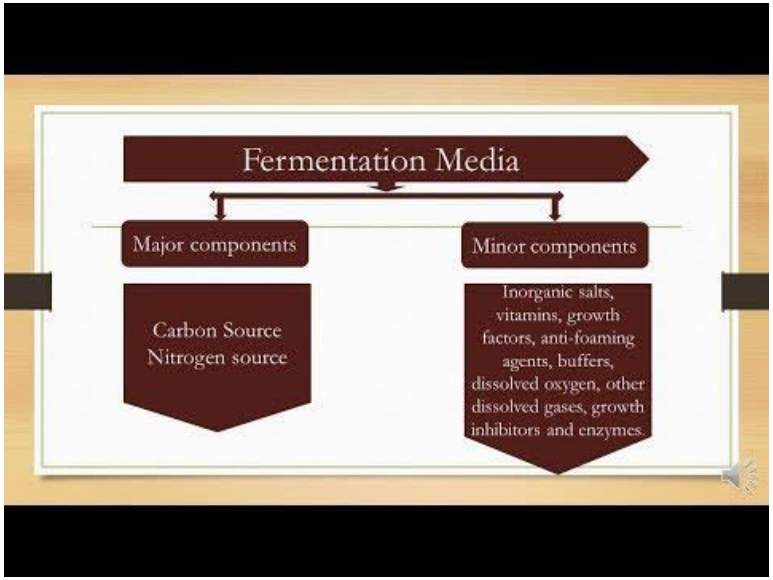
1. It will produce the maximum yield of product or biomass per gram of substrate used.
2. It will produce the maximum concentration of product or biomass.
3. It will permit the maximum rate of product formation.
4. There will be the minimum yield of undesired products.
5. It will be of a consistent quality and be readily available throughout the year.
6. It will cause minimal problems during media making and sterilization.
7. It will cause minimal problems in other aspects of the production process particularly aeration and agitation, extraction, purification, and waste treatment

Medium Formulation

- The constituents of a medium must satisfy the elemental requirements for cell biomass and metabolite production
- must be an adequate supply of energy for biosynthesis and cell maintenance

$$\text{carbon and energy source} + \text{nitrogen source} + O_2 + \text{other requirements} \rightarrow \text{biomass} + \text{products} + CO_2 + H_2O + \text{heat}$$

This equation should be expressed in quantitative terms, which is important in the economical design of media if component wastage is to be minimal



A knowledge of the elemental composition of a process microorganism is required for the solution of the elemental balance equation.

Element Composition of Bacteria, Yeasts, and Fungi (% by Dry Weight)

Element	Bacteria (Luria, 1960; Herbert, 1976; Aiba, Humphrey, & Millis, 1973)	Yeasts (Aiba et al., 1973; Herbert, 1976)	Fungi (Lilly, 1965; Aiba et al., 1973)
Carbon	50–53	45–50	40–63
Hydrogen	7	7	
Nitrogen	12–15	7.5–11	7–10
Phosphorus	2.0–3.0	0.8–2.6	0.4–4.5
Sulfur	0.2–1.0	0.01–0.24	0.1–0.5
Potassium	1.0–4.5	1.0–4.0	0.2–2.5
Sodium	0.5–1.0	0.01–0.1	0.02–0.5
Calcium	0.01–1.1	0.1–0.3	0.1–1.4
Magnesium	0.1–0.5	0.1–0.5	0.1–0.5
Chloride	0.5	—	—
Iron	0.02–0.2	0.01–0.5	0.1–0.2

The carbon substrate has a dual role in biosynthesis and energy generation. The carbon requirement for biomass production under aerobic conditions may be estimated from the cellular yield coefficient (*Y) which is defined as*

$$\frac{\text{Quantity of cell dry matter produced}}{\text{Quantity of carbon substrate utilized}}$$

Constituents of medium

- Water
- Carbon source / Nitrogen source / Sources of phosphorous and sulfur / Minor and trace elements / Vitamins such as biotin and riboflavin
- Oxygen: even some anaerobic fermentations require initial aeration, e.g. beer fermentations
- Buffers or controlled by acid and alkali additions
- Antifoam agents
- Precursor, inducer or inhibitor compounds

Water

- Water is the major component of almost all fermentation media
- Needed for ancillary services such as heating, cooling, cleaning, and rinsing
- The mineral content of the water is very important in brewing, and most critical in the mashing process

Water also influenced the siting of breweries and the types of beer produced .

The reuse or efficient use of water is normally of high priority which reduces capital and operating costs

Energy Source

- Energy for growth from oxidation of medium components or from light
- Most of the industrial microorganisms are chemo-organotrops
- Energy source may be the carbon source such as carbohydrates, lipids and proteins
- Hydrocarbons and methanol can also be used

Carbon Sources

- Rate which carbon is metabolized can influence the final product/secondary metabolites
- High concentration of metabolized sugars and fast growth of microorganisms are associated with low productivity of secondary metabolites
- Overcome by using readily metabolized sugar as lactose or carbon catabolite regulator
- Purified carbon source also influence the fermentation
- Method of media preparation, particularly sterilization may affect the suitability of carbohydrates.

- Carbohydrates
- Oils and fats
- Hydrocarbon and their derivatives

Carbohydrates:

Starch from maize, cassava, cereals, potatoes

Barley grains – malt.

Sucrose from sugar cane and sugar beet.

Molasses – high volume, low-value product.

Lactose and crude lactose

Corn steep liquor.

Nitrogen Sources

- microorganisms can utilize inorganic or organic sources of Nitrogen
- Inorganic nitrogen may be supplied as ammonia gas, ammonium salts, or nitrates
- Ammonia has been used for pH control and as the major nitrogen source in a defined medium
- Ammonium salts such as ammonium sulfate will usually produce acid conditions as the ammonium ion is utilized and the free acid will be liberated.
- Organic nitrogen may be supplied as amino acid, protein or urea, or in a complex media as yeast extract

FACTORS INFLUENCING THE CHOICE OF N SOURCE

- Control mechanism exist by which NO_3 reductase an enzyme involved in the conversion of NO_3 to NH_4 ion, is repressed in the presence of NH_3.
- NH3 also regulates the production of alkaline & neutral proteases.
- Antibiotic production may be inhibited by a rapidly utilized N source (NH_4, NO_3).
- Antibiotic production only begins to increase in the culture broth after most of the N consumed.
- Production of polyene antibiotic, soybean has been considered a good N source b/c of the balance of the nutrients, low P content & slow hydrolysis.
- Some complex N material cant be utilized by a micro-organism & create problems in downstream processing & effluent treatment. This can be an important factor in the final choice of substrate.

Minerals

In many media, magnesium, phosphorus, potassium, sulfur, calcium, and chlorine are essential components, and because of the concentrations required, they must be added as distinct components. Others such as cobalt, copper, iron, manganese, molybdenum, and zinc are also essential but are usually present as impurities in other major ingredients.

The Range of Typical Concentrations of Mineral Components (g dm^{-3})

Component	Range
[a]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

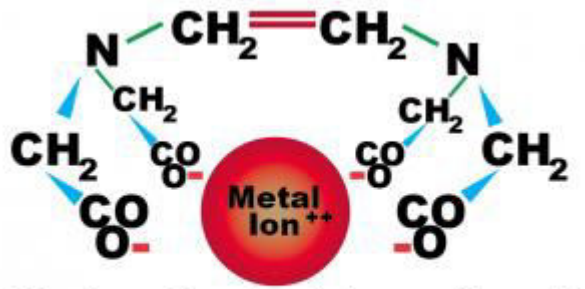
[a]Complex media derived from plant and animal materials normally contain a considerable concentration of inorganic phosphate.

CHELATORS

- Many media cannot be prepared or autoclaved without the formation of a visible precipitate of insoluble metal phosphates

- The problem of insoluble metal phosphate(s) may be eliminated by incorporating low concentrations of chelating agents such as ethylene diamine tetraacetic acid (EDTA), citric acid, polyphosphates, etc., into the medium

- It is important to check that a chelating agent does not cause inhibition of growth of the microorganism which is being cultured.



Ethylenediaminetetraacetic acid (EDTA) chelates a metal ion

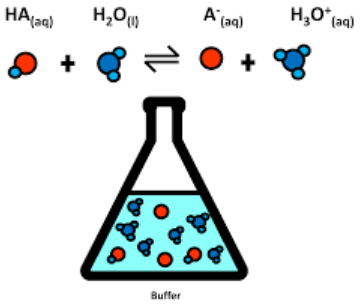
Growth Factors

- Some microorganisms cannot synthesize a full complement of cell components and therefore require preformed compounds called growth factors.
- The growth factors most commonly required are vitamins, but there may also be a need for specific amino acids, fatty acids, or sterols.
- When there is a vitamin deficiency it can often be eliminated by careful blending of materials (Rhodes & Fletcher, 1966).
- Calcium pantothenate has been used in one medium formulation for vinegar production (Beaman, 1967).
- The effects of biotin as a growth factor in succinic acid production by *Actinobacillus succinogenes showing* it's importance to succinate productivity at low biotin concentrations.
- A second growth factor (5-aminolevulinate) was also examined and similar results to biotin supplementation were obtained.

- It was shown that *M. methylotrophus* could be grown in a medium containing 86% recycled supernatant plus additional fresh nutrients to make up losses.

- This approach made it possible to reduce the costs of media components, media preparation, and storage facilities

NUTRIENT RECYCLE



BUFFERS

- The control of pH may be extremely important if optimal productivity is to be achieved.
- The pH may also be controlled externally by the addition of ammonia or sodium hydroxide and sulfuric acid
- phosphates which are part of many media also play an important role in buffering.
- However, high phosphate concentrations are critical in the production of many secondary metabolites

PRECURSORS AND METABOLIC REGULATORS TO MEDIA

Some chemicals, when added to certain fermentations, are directly incorporated into the desired product.

Phenylacetic acid

Corn-steep liquor

When certain inhibitors are added to fermentations, more of a specific product may be produced, or a metabolic intermediate which is normally metabolized is accumulated.

Precursors Used in Fermentation Processes

Precursor	Product	Microorganism	References
Phenylacetic-acid related compounds	Penicillin G	*Penicillium chrysogenum*	Moyer and Coghill (1947)
Phenoxy acetic acid	Penicillin V	*Penicillium chrysogenum*	Soper, Whitehead, Behrens, Corse, and Jones (1948)
Chloride	Chlortetracycline	*Streptomyces aureofaciens*	Van Dyck and de Somer (1952)
Chloride	Griseofulvin	*Penicillium griseofulvin*	Rhodes et al. (1955)
[a]Propionate	Riboflavin	*Lactobacillus bulgaricus*	Smiley and Stone (1955)
Cyanides	Vitamin B12	*Proprianobacterium, Streptomyces* spp.	Mervyn and Smith (1964)
β-Tononones	Carotenoids	*Phycomyces blakesleeanus*	Reyes, Chichester, and Nakayama (1964)
α-Amino butyric acid	L-Isoleucine	*Bacillus subtilis*	Nakayama (1972b)
D-Threonine	L-Isoleucine	*Serratia marcescens*	
Anthranilic acid	L-Tryptophan	*Hansenula anomala*	
Nucleosides and bases	Nikkomycins	*Streptomyces tendae*	Vecht-Lifshitz and Braun (1989)
Dihydronovobionic acid	Dihydronovo-biocin	*Streptomyces* sp.	Walton, McDaniel, and Woodruff (1962)
p-Hydroxycinnamate	Organomycin A and B	*Streptomyces organonensis*	Eiki, Kishi, Gomi, and Ogawa (1992)
DL-α-Amino butyric acid	Cyclosporin A	*Tolypocladium inflatum*	Kobel and Traber (1982)
L-Threonine	Cyclosporin C		
Tyrosine or *p*-hydroxy-phenylglycine	Dimethylvanco-mycin	*Nocardia orienlalis*	Boeck, Mertz, Wolter, and Higgens (1984)

[a]*Yields are not so high as by other techniques.*

Specific and General Inhibitors Used in Fermentations

Product	Inhibitor	Main Effect	Microorganism	References
Glycerol	Sodium bisulfite	Acetaldehyde production repressed	*Saccharomyces cenutsiae*	Eoff et al. (1919)
Tetracycline	Bromide	Chlortetracycline formation repressed	*Streptomyces aureofaciens*	Le petit (1957)
Glutamic acid	Penicillin	Cell wall permeability	*Micrococcus glutamicus*	Phillips and Somerson (1960)
Citric acid	Alkali metal/ phosphate, pH below 2.0	Oxalic acid repressed	*Aspergillus niger*	Batti (1967)
Valine	Various inhibitors	Various effects with different inhibitors	*Breuibacterium roseum*	Uemura, Sugisaki, and Takamura (1972)
Rifamycin B	Diethyl barbiturate	Other rifamycins inhibited	*Nocardia mediterranei*	Lancini and White (1973)
7-Chloro-6 de-methyltetracycline	Ethionine	Affects one-carbon transfer reactions	*Streptomyces aureofaciens*	Neidleman, Bienstock, and Bennett (1963)

INDUCERS

- Enzymes which are of industrial importance are inducible
- Enzymes are synthesized in the presence of inducers
- Some inducers are substrates or substrate analogues
- Sometimes intermediate or products may be used as inducers

Some examples of industrially important enzyme inducers

Enzyme	Inducer	Micro-organism	Reference
α-Amylase	Starch Maltose	*Aspergillus* spp. *Bacillus subtilis*	Windish and Mhatre (1965)
Pullulanase	Maltose	*Aerobacter aerogenes*	Wallenfels *et al.* (1966)
α-Mannosidase	Yeast mannans	*Streptomyces griseus*	Inamine *et al.* (1969)
Penicillin acylase	Phenylacetic acid	*Escherichia coli*	Carrington (1971)
Proteases	Various proteins	*Bacillus* spp. *Streptococcus* spp. *Streptomyces* spp. *Asperigillus* spp. *Mucor* spp.	Keay (1971) Aunstrup (1974)
Cellulase	Cellulose	*Trichoderma viride*	Reese (1972)
Pectinases	Pectin (beet pulp, apple pomace, citrus peel)	*Aspergillus* spp.	Fogarty and Ward (1974)
Nitralase	Isovaleronitrile	*Rhodococcus rhodochrous*	Kobayashi *et al.* (1992)

Oxygen requirement

Very important in controlling growth rate & metabolic production. Medium may influence:

Fast metabolism: culture may become oxygen limited b/c sufficient oxygen can't be made available in the fermeter if certain substrate such as rapidly metabolized sugars which lead to a high oxygen demand are available in high conc.

Rheology: the individual components of the medium can influence the viscosity of the final medium & its subsequent behavior with respect to aeration & agitation.

Antifoams

High degree of aeration and agitation will result in foam formation.

Foaming reduces oxygen transfer. Air bubbles entrapped in the foam and again and again they recirculate in the medium. This will result in oxygen depleted bubbles residing in the system

To control foam antifoam agents are added.

Causes of foaming in microbial fermentation

- Microorganisms in the medium are active and produce bubbles.
- medium is rich in raw materials, high in content of organic nitrogen, and easy to generate foam.
- in the fermentation process, there is a trend of temperature rising and rapid foaming operation.
- In the process of microbial production, stirring is intense, which brings air into oil and causes foaming.
- The influence of environmental factors around microorganisms leads to foaming.
- Misoperation of microorganisms results in foaming.
- foam is produced during the mixing process.

The hazards of foaming in microbial fermentation:

- excessive foam will affect the results of the observation of the surface conditions of the medium.
- The presence of bubbles may change the structure of culture medium and affect subsequent fermentation.
- the foam will affect the stability of the microbial community.
- The foam will reduce the oxygen transfer system and affect the fermentation level.
- when the bubble is too much, a lot of spills are generated, and the fermentation liquid escapes from the exhaust pipe or shaft seal to increase the chance of infection.
- when the foam is serious, ventilation can not be carried out, and the respiration of bacteria is impeded, resulting in abnormal metabolism or autolysis of bacteria.

Ideal antifoam

- Disperse readily
- Active at low concentration
- Prevent new foam formation
- Not be metabolized by microorganism
- Non-toxic
- Not cause problem during extraction and purification
- Cheap
- No effect on oxygen transfer
- Heat sterilizable

Controlling Foaming

- Alcohols; stearyl and octyl decanol
- Esters
- Fatty acids and derivatives
- Silicones
- Sulphonates
- Oxazaline, polypropylene glycols