Al- Farabi Kazakh National University

Media for Industrial Fermentations

Lecture 4

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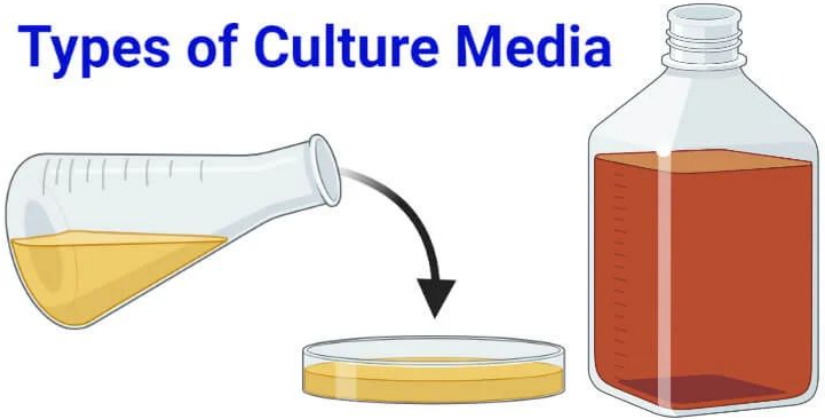
Types of Culture Media



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

On a small scale it is relatively simple to devise a medium containing pure compounds, but the resulting medium, although supporting satisfactory growth, may be unsuitable for use in a large scale process.

https://microbenotes.com/types-of-culture-media/

Criteria for Creating a Media

It will produce the maximum yield of product or biomass per gram of substrate used.

It will produce the maximum concentration of product or biomass.

It will permit the maximum rate of product formation.

There will be the minimum yield of undesired products.

It will be of a consistent quality and be readily available throughout the year.

It will cause minimal problems during media making and sterilization.

It will cause minimal problems in other aspects of the production process particularly aeration and agitation, extraction, purification, and waste treatment.

Nutrient Sources for Industrial Fermentation

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

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

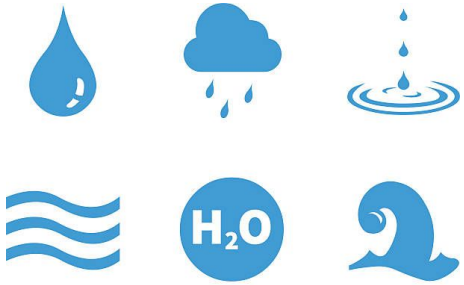
- Some microorganisms cannot synthesize specific nutrients, for example, amino acids, vitamins, or nucleotides.

- Once a specific growth factor has been identified it can be incorporated into a medium in adequate amounts as a pure compound or as a component of a complex mixture.

Table 4.2 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

Water



Water is the major component of almost all fermentation media, and is needed in many of the ancillary services such as heating, cooling, cleaning, and rinsing.

Clean water of consistent composition is therefore required in large quantities from reliable permanent sources. When assessing the suitability of a water supply it is important to consider pH, dissolved salts, and effluent contamination.

https://www.istockphoto.com/illustrations/drinking-water-icon

The mineral content of the water is very important in brewing, and most critical in the mashing process, and historically influenced the siting of breweries and the types of beer produced.

Hard waters containing high $CaSO_4$ concentrations are better for the English Burton bitter beers and Pilsen type lagers, while waters with a high carbonate content are better for the darker beers such as stouts.

Energy Sources

- Energy for growth comes from either the oxidation of medium components or from light.
- Most industrial microorganisms are chemoorganotrophs, therefore the commonest source of energy will be the carbon source such as carbohydrates, lipids, and proteins.
- Some microorganisms can also use hydrocarbons or methanol as carbon and energy sources

Carbon Sources

When the carbon source is metabolized can often influence the formation of biomass or production of primary or secondary metabolites.

The purity of the carbon source may also affect the choice of substrate. For example, metallic ions must be removed from carbohydrate sources used in some citric acid processes

COMMONLY USED CARBON SOURCES

- The most widely available carbohydrate is starch obtained from maize grain. It is also obtained from other cereals, potatoes, and cassava.
- Starch may also be readily hydrolyzed by dilute acids and enzymes to give a variety of glucose preparations (solids and syrups). Hydrolyzed cassava starch is used as a major carbon source for glutamic acid production
- Malt is the main substrate for brewing beer.
- Sucrose that obtained from sugar cane and sugar beet are used in fermentation media in a very impure form as beet or cane molasses.

Table 4.4 Carbon Catabolite Regulation of Metabolite Biosynthesis

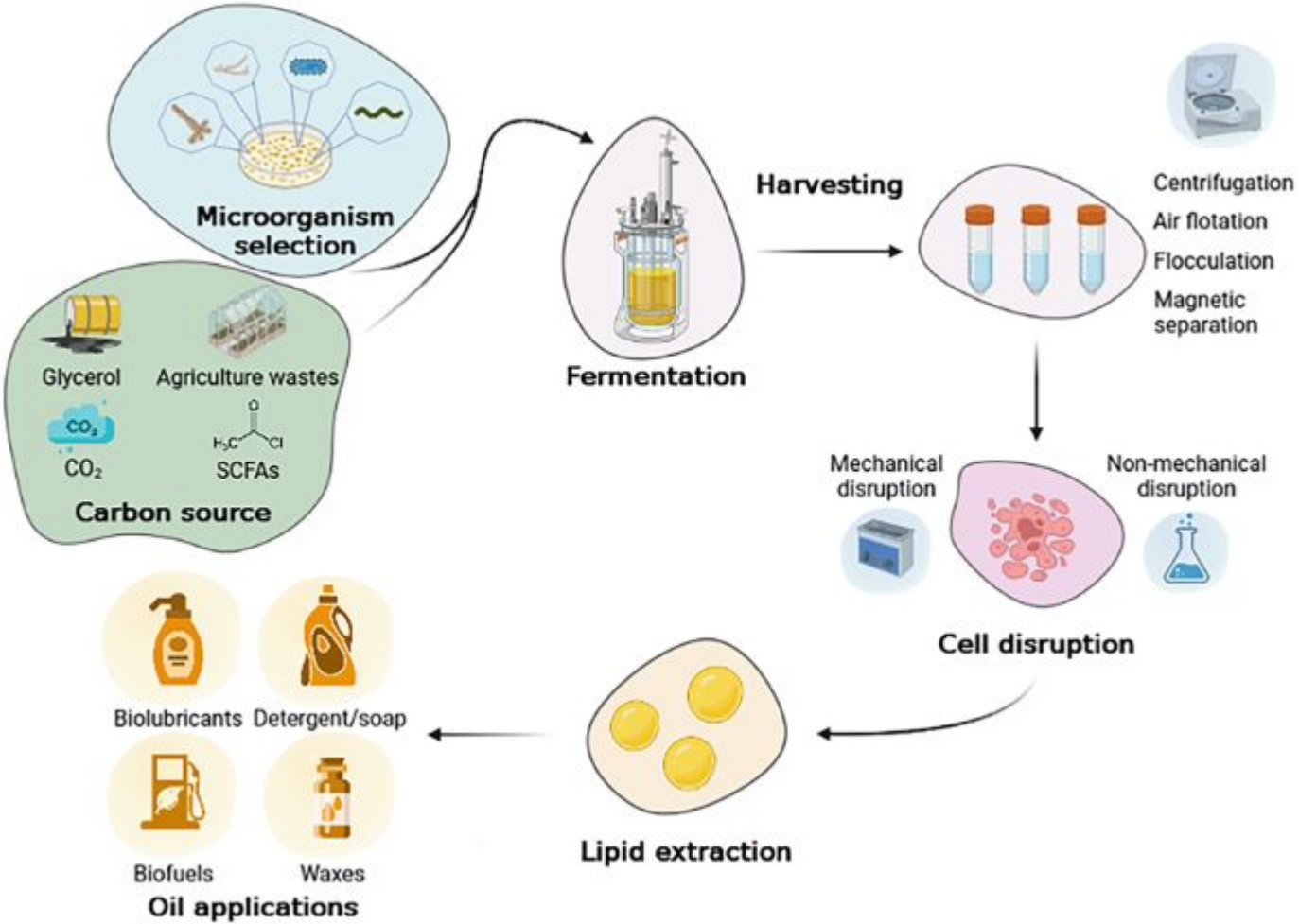
Metabolite	Microorganism	Interfering Carbon Source	References
Griseofulvin	*Penicillium griseofulvin*	Glucose	Rhodes (1963); Rhodes et al. (1955)
Penicillin	*P. chrysogenum*	Glucose	Pirt and Righelato (1967)
Cephalosporin	*Cephalosporium acremonium*	Glucose	Matsumura, Imanaka, Yoshida, and Taguchi (1978)
Aurantin	*Bacillus aurantinus*	Glycerol	Nishikiori et al. (1978)
α-Amylase	*B. licheniformis*	Glucose	Priest and Sharp (1989)
Bacitracin	*B. licheniformis*	Glucose	Weinberg (1967)
Puromycin	*Streptomyces alboniger*	Glucose	Sankaran and Pogell (1975)
Actinomycin	*S. antibioticus*	Glucose	Marshall, Redfield, Katz, and Weissback (1968)
Cephamycin C	*S. clavuligerus*	Glycerol	Aharonowitz and Demain (1978)
Neomycin	*S. fradiae*	Glucose	Majumdar and Majumdar (1965)
Cycloserine	*S. graphalus*	Glycerol	Svensson, Roy, and Gatenbeck (1983)
Streptomycin	*S. griseus*	Glucose	Inamine et al. (1969)
Kanamycin	*S. kanamyceticus*	Glucose	Basak and Majumdar (1973)
Novobiocin	*S. niveus*	Citrate	Kominek (1972)
Siomycin	*S. sioyaensis*	Glucose	Kimura (1967)

Oils and Fats

- Oils were first used as carriers for antifoams in antibiotic processes. Vegetable oils may also be used as carbon substrates, particularly for their content of the fatty acids: oleic, linoleic, and linolenic acid.

- The use of a microemulsion of rapeseed oil for oxytetracycline production by Streptomyces rimosus. More biomass was produced, oil utilization was increased threefold and oxytetracycline production was increased.

- Glycerol trioleate is used in some fermentations where substrate purity is an important consideration. Methyl oleate has been used as the sole carbon substrate in cephalosporin production.



https://link.springer.com/referenceworkentry/10.1007/978-94-007-6724-9_17-1#Fig1

Nitrogen Sources

- Most industrially used 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 for the commercial production of human serum albumin by *Saccharomyces cerevisiae*.

- Organic nitrogen may be supplied as amino acid, protein or urea, or in a complex media as yeast extract. Chemically defined amino acid media devoid of protein are necessary in the production of certain vaccines when they are intended for human use.

Minerals

- All microorganisms require certain mineral elements for growth and metabolism .

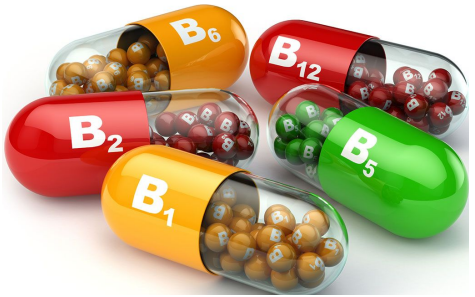
- In many media, magnesium, phosphorus, potassium, sulfur, calcium, and chlorine are essential components, and because of the concentrations required, they must be added as distinct components.

- Others such as cobalt, copper, iron, manganese, molybdenum, and zinc are also essential but are usually present as impurities in other major ingredients.

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.

For example, some production strains may require thiamine as a growth factors. Biotin is used as a growth factor in succinic acid production by *Actinobacillus succinogenes*



Buffers

The control of pH may be extremely important if optimal productivity is to be achieved. A compound may be added to the medium to serve specifically as a buffer, or may also be used as a nutrient source.

Many media are buffered at about pH 7.0 by the incorporation of calcium carbonate.

The pH may also be controlled externally by the addition of ammonia or sodium hydroxide and sulfuric acid.

Additives

Precursors

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

E.g Penicillin yields

Corn steep liquor contain phenylethylamine when incorporated, it yield benzyl penicillin.

Phenylacetic acid is widely used precursor in penicillin production.

Inhibitors

When certain inhibitors are added to fermentations more of specific product may be produced.

Inhibitors have also been used to affect cell wall structure and increase the permeability for release of metabolites.

The best example is the use of penicillin & surfactants in glutamic acid production.

Inducers

Induced enzymes are synthesized only in response to the presence in the environment of an inducer.

E.g maltodextrins will induce amylase & fatty acids induce lipase.

Oxygen Requirements

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

- ***Fast metabolism:*** culture may become oxygen limited because sufficient oxygen can't be made available in the fermenter if certain substrate such as rapidly metabolized sugars which lead to a high oxygen demand are available in high concentrations.

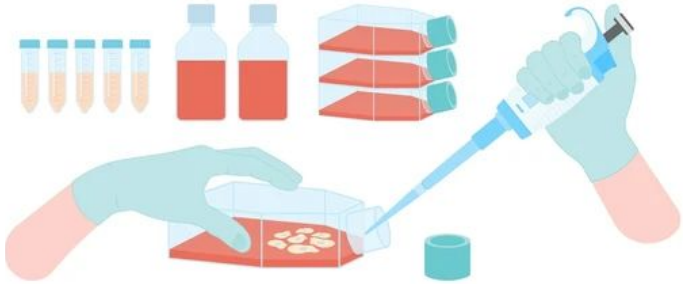
- ***Rheology:*** the individual components of the medium can influence the viscosity of the final medium & its subsequent behavior with respect to aeration & agitation.

- ***Antifoam:*** Many of the antifoams in use will act as surface active agents & reduce the oxygen transfer rate.

Animal Cell Media

The use of animal cell cultures in biotechnology was initially confined to the production of vaccines, with the polio injectable vaccine being the first to be produced in an animal cell culture system in 1955.

Media are the sources of nutrients for the cells in culture. They are rich in essential nutrients such as amino acids, glucose, ions, fructose, and hormones with or without serum.



Serum Containing Media

- Serum is a complex mixture of many small and large biomolecules with different, physiologically balanced growth-promoting and growth-inhibiting activities. Serum performs major functions which are hormonal factors; stimulating cell growth and functions; attachment and spreading factors; transport proteins carrying hormones, minerals, lipids etc.

- Albumin binds vitamins such as pyridoxal, fatty acids: such as oleic, linoleic, linolenic arachidonic, myristic and palmitic acids, and ions such as copper.

- *Examples* of types of serum available for culture are Fetal Bovine Serum, New Calf Serum, Donor Horse Serum, Porcine Serum etc.

Serum - Free Media

Biological industries have developed serum-free formulations for all commercially significant cell and tissue cultures. Examples are CHO Cell Culture Media, PER.C6 and 293 Media, Insect Cell Media, Immunology Media, Stem Cell Media, Hybridoma Media, Primary Cell Media.

The two categories of **serum free media** are:

(i) ***Chemically defined media:*** A chemically defined medium is one in which the exact chemical composition is known e.g. expression media.

(ii) ***Protein free media:*** The common examples are hybridoma serum free media, PFHM II is protein-free media.