Chapter III. Culture media in industrial microbiology

In the context of industrial production, several critical factors should be evaluated when selecting raw materials for microbial growth and production:

- Cost of Raw Materials: Analyzing the financial implications associated with sourcing and utilizing raw materials.
- Availability: Assessing the accessibility and supply stability of the required raw materials.
- Transportation Costs: Evaluating the logistical expenses related to the movement of raw materials to production facilities.
- Waste Disposal: Considering the environmental impact and ease of waste disposal associated with raw materials.

- Quality Uniformity: Ensuring consistent quality and the feasibility of standardizing raw materials for reliable production outcomes.
- Chemical Composition: Confirming that the raw materials possess the appropriate chemical characteristics necessary for optimal microbial growth.
- Presence of Precursors: Identifying the availability of essential precursors that facilitate metabolic processes in microorganisms.
- Alignment with Production Needs: Ensuring that the selected raw materials meet the specific requirements for the effective growth and production of microorganisms.

Table 4.1 Average composition of microorganisms (% dry weight)

Component	*Bacteria*	*Yeast*	*Molds*
Carbon	48 (46-52)	48 (46-52)	48 (45-55)
Nitrogen	12.5 (10-14)	7.5 (6-8.5)	6 (4-7)
Protein	55 (50 –60)	40 (35-45)	32 (25-40)
Carbohydrates	9 (6-15)	38 (30-45)	49 (40-55)
Lipids	7 (5-10)	8 (5-10)	8 (5-10)
Nucleic Acids	23 (15-25)	8 (5-10)	5 (2-8)
Ash	6 (4-10)	6 (4-10)	4 (4-10)
Minerals (same for all three organisms)			
Phosphorus	1.0 - 2.5		
Sulfur, magnesium	0.3 - 1.0		
Potassium, sodium	0.1 - 0.5		
Iron	0.01 - 0.1		
Zinc, copper, manganese	0.001 – 0.01		

The carbon substrate has a dual role in biosythesis 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:

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

Some values are given in Table 4.3. Thus for bacteria

TABLE 4.3. *Cellular yield coefficients (Y) of bacteria on different carbon substrates* (*data from* Abbott and Clamen, 1973)

Substrate	Cellular yield coefficient (g biomass dry wt. g^{-1} substrate)
Methane	0.62
n-Alkanes	1.03
Methanol	0.40
Ethanol	0.68
Acetate	0.34
Malate	0.36
Glucose (molasses)	0.51

Source of carbon and energy

Glucose, organic acids, alcohols, hydrocarbons, lipids……

1 g of glucose ⟶ 0.5 g dry cells (all MO)

Nitrogen source

Ammonia or other nitrogenous salts….

625 mg N ⟶ 5 g cells/L (bacteria)

Nitrogenous elements which are not synthesized by the cell are added to the culture medium

TABLE 4.9. *Best nitrogen sources for some secondary metabolites*

Product	Main nitrogen source(s)	Reference
Penicillin	Corn-steep liquor	Moyer and Coghill (1946)
Bacitracin	Peanut granules	Inskeep *et al.* (1951)
Riboflavin	Pancreatic digest of gelatine	Malzahn *et al.* (1959)
Novobiocin	Distillers' solubles	Hoeksema and Smith (1961)
Rifomycin	Pharmamedia	Sensi and Thiemann (1967)
	Soybean meal, $(NH_4)_2SO_4$	
Gibberellins	Ammonium salt and natural plant nitrogen source	Jefferys (1970)
Butirosin	Dried beef blood or haemoglobin with $(NH_4)_2SO_4$	Claridge *et al.* (1974)
Polyenes	Soybean meal	Martin and MacDaniel (1977)

Mineral elements

P, S, Mg and Fe.....(major)

Mn, Br, Zn, Cu and Mo.....(trace elements)

Growth factors

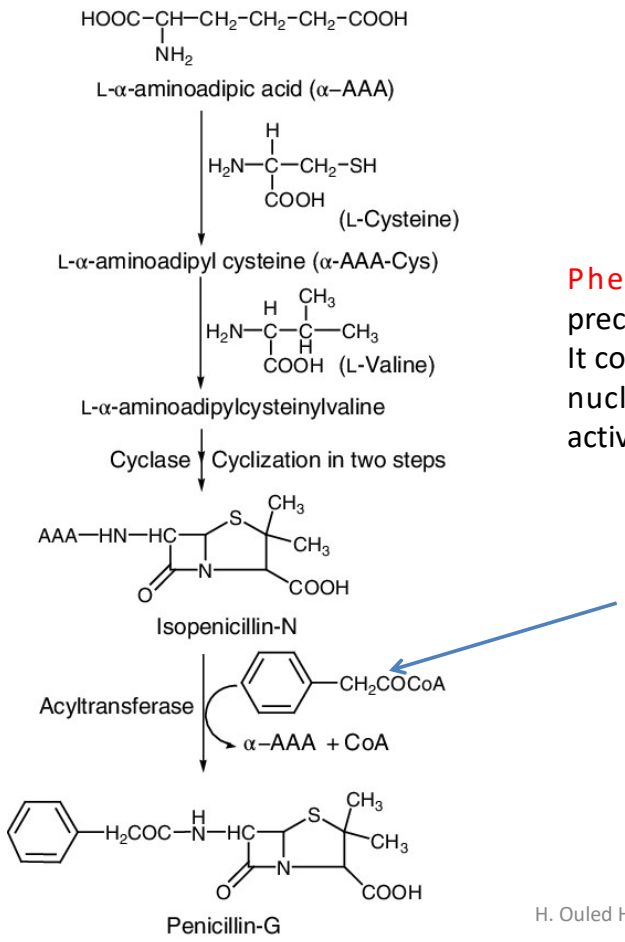
Vitamins, nucleic acids, amino acids

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

TABLE 4.12. *Precursors used in fermentation processes*

Precursor	Product	Micro-organism
Phenylacetic-acid related compounds	Penicillin G	*Penicillium chrysogenum*
Phenoxy acetic acid	Penicillin V	*Penicillium chrysogenum*
Chloride	Chlortetracycline	*Streptomyces aureofaciens*
Chloride	Griseofulvin	*Penicillium griseofulvin*
*Propionate	Riboflavin	*Lactobacillus bulgaricus*
Cyanides	Vitamin B12	*Proprianobacterium, Streptomyces* spp.
β-Iononones	Carotenoids	*Phycomyces blakesleeanus*
α-Amino butyric acid	L-Isoleucine	*Bacillus subtilis*
D-Threonine	L-Isoleucine	*Serratia marcescens*
Anthranilic acid	L-Tryptophan	*Hansenula anomala*



Phenylacetic acid serves as a side-chain precursor in the penicillin biosynthetic pathway. It contributes to the formation of the penicillin nucleus, which is essential for the antibiotic activity of penicillin G.

The main constituents of culture media used in industrial processes

Source	Raw material
Carbon and energy	Molasses Whey Cereal grains Agricultural wastes (corncobs)
Nitrogen	Corn steep liquor Soy flour Slaughterhouse waste Vinasse Ammonia and ammonium salts, nitrates
Vitamins	Raw preparations of plant and animal products
Iron, trace elements	Crude inorganic chemical derivatives
Buffers	Chalk or crude carbonates Phosphate for fertilizers
Antimousse	Higher alcohols Silicones, natural esters, lard, vegetable oils, etc.

Examples of some formulations of culture media used in MI

Amylase (Underkofler, 1966)

Ground soybean meal	1.85%
Autolysed Brewers yeast fractions	1.50%
Distillers dried solubles	0.76%
NZ-amine (enzymatic casein hydrolysate)	0.65%
Lactose	4.75%
$MgSO_4 \cdot 7H_2O$	0.04%
Hodag KG-1 antifoam	0.05%

Penicillin (Perlman, 1970)

Glucose or molasses (by continuous feed)	10% of total
Corn-steep liquor	4–5% of total
Phenylacetic acid (by continuous feed)	0.5–0.8% of total
Lard oil (or vegetable oil) antifoam by continuous addition	0.5% of total
pH to 6.5 to 7.5 by acid or alkali addition	

Examples of raw materials

- **Corn steep liquor**

It is a byproduct of the wet milling process of corn. It is a viscous concentrate composed of soluble corn materials, rich in amino acids, vitamins, and minerals. This nutrient-dense byproduct serves as a significant component in various growth media formulations.

Table 4.2 Approximate composition of corn steep liquor (%)

Lactose	3.0-4.0
Glucose	0.-0.5
Non-reducing carbohydrates (mainly starch)	1.5
Acetic acid	0.05
Glucose lactic acid	0.5
Phenylethylamine	0.05
Amino aids (peptides, mines)	0.5
Total solids	80-90
Total nitrogen	0.15-0.2%

- **Vinasses** (distiller's solubles)

Vinasse is a residual product derived from the distillation of alcoholic liquids, particularly within the sugar industry.

It represents the final by-product of the distillation process involving plant biomass, primarily in the production of ethanol from sugar and starch crops, as well as cellulosic biomass.

In its liquid form, vinasse comprises approximately 93% water and 7% solids. Notably, for each liter of alcohol produced in the sugar cane sector, approximately 15 liters of vinasse can be generated. In its dry state, vinasse is also utilized as a food additive.

Table 4.3 Composition of maize distillers soluble

	%
Moisture	5
Protein	27
Lipid	9
Fibre	5
Carbohydrate	43
Ash (mainly K, Na, Mg, CO_3, and P)	11

- **Cottonseed flour (Pharmamedia)**

Cottonseed meal is a byproduct generated after the processes of cotton ginning, seed grinding, and oil extraction. This remaining substance is commonly utilized as animal feed and as an ingredient in fertilizers. Marketed under the trade name "PROFLO," it appears as a yellow powder derived from cotton seed embryos.

In addition to its use in animal nutrition, cottonseed meal serves as a raw material in the manufacture of tetracycline and semi-synthetic penicillins. It is notably high in protein, comprising approximately 56% (v/v), 24% carbohydrates, 5% oil, and 4% ash. Furthermore, it is rich in essential minerals such as calcium, iron, chloride, phosphorus, and sulfate.

TABLE 4.8. *The composition of Pharmamedia (Traders Protein, Southern Cotton Oil Company, Division of Archer Dariels Midland Co.)*

Component	Quantity
Total solids	99%
Carbohydrate	24.1%
Reducing sugars	1.2%
Non reducing sugars	1.2%
Protein	57%
Amino nitrogen	4.7%
Components of amino nitrogen	
Lysine	4.5%
Leucine	6.1%
Isoleucine	3.3%
Threonine	3.3%
Valine	4.6%
Phenylalanine	5.9%
Tryptophan	1.0%
Methionine	1.5%
Cystine	1.5%
Aspartic acid	9.7%
Serine	4.6%
Proline	3.9%
Glycine	3.8%
Alanine	3.9%
Tyrosine	3.4%
Histidine	3.0%
Arginine	12.3%
Mineral components	
Calcium	2530 ppm
Chloride	685 ppm
Phosphorus	13 100 ppm
Iron	94 ppm
Sulphate	18 000 ppm
Magnesium	7360 ppm
Potassium	17 200 ppm
Fat	4.5%
Vitamins	
Ascorbic acid	32.0 mg kg^{-1}
Thiamine	4.0 mg kg^{-1}
Riboflavin	4.8 mg kg^{-1}
Niacin	83.3 mg kg^{-1}
Pantothenic acid	12.4 mg kg^{-1}
Choline	3 270 mg kg^{-1}
Pyidoxine	16.4 mg kg^{-1}
Biotin	1.5 mg kg^{-1}
Folic acid	1.6 mg kg^{-1}
Inositol	10 800 mg kg^{-1}

- **Soy flour**

Soybean (*Glycine max*) is an annual legume extensively cultivated worldwide, particularly in tropical and subtropical regions. Prior to oil extraction, the seeds are subjected to heat treatment. The extracted oil has various applications, including use in food products, as an anti-foaming agent in industrial fermentations, and in the production of margarine.

The residual material from this process is dried soybean meal, which contains approximately 11% nitrogen and 30% carbohydrates, making it suitable for animal feed.

The nitrogen in soybean meal is more complex than that found in corn liquor and is not readily accessible to most microorganisms, with the exception of actinomycetes. This complexity renders it particularly valuable in the fermentation processes for the production of antibiotics such as tetracycline and streptomycin.

Glycine max (soy)

- **Molasses**

Molasses is a significant source of sugar and is utilized in various fermentation industries, including the production of alcoholic beverages, acetone, citric acid, glycerin, and yeast.

It is a byproduct of the sugar industry. There are two primary types of molasses, differentiated by the source of sugar:

one is derived from the tropical crop, sugar cane (*Saccharum officinarum*),

while the other is produced from the temperate crop, sugar beet (*Beta vulgaris*).

Table 4.4 Average composition of beet and cane molasses

	Beet Molasses % (W/W)	*Cane Molasses % (W/W)*
Water	16.5	20.0
Sugars:	53.0	64.0
Sucrose	51.0	32.0
Fructose	1.0	15.0
Glucose	-	14.0
Raffinose	1.0	-
Non-sugar (nitrogeneous Materials, acids, gums, etc.)	19.0	10.0
Ash	11.5	8.0

Other raw materials

Sulphite liquor (By-product of the paper industry)
Alcohol
Acetic acid
Methanol
Methane
Crude oil

Growth factors

Table 4.5 Some sources of growth factors

Growth factor	*Source*
Vitamin B	Rice polishing, wheat germ, yeasts
Vitamin B_2	Cereals, corn steep liquor
Vitamin B_6	Corn steep liquor, yeasts
Nicotinamide	Liver, penicillin spent liquor
Panthothenic Acid	Corn steep liquor
Vitamin B_{12}	Liver, silage, meat

Examples of some sources of components from industrial environments

1. Sugars

(These are polysaccharides requiring hydrolysis before use)

- ✓ Cassava (cassava) (euphorbiaceae cultivated in South America) *Manihot esculenta*
- ✓ Sweet potato *Ipomoea batatas*
- ✓ Yam *Dioscorea spp*
- ✓ Cocoyam *Colocasia* and *Xanthosoma*
- ✓ Millets (various cereal grasses grown in Asia and Africa) *Pennisetum americanum, Setaria italica, Panicum miliaceum, Echinochloa frumentacea* and *Eleusine corcana*
- ✓ *Oryza sativa* rice
- ✓ Sorghum (grasses native to India) *Sorghum bicolor*
- ✓ Jerusalem artichokes *Helianthus tuberosus*

Cassava (manioc) (euphorbiacaes cultivated in South America)
Manihot esculenta

Starch

SCP, ethanol, beer

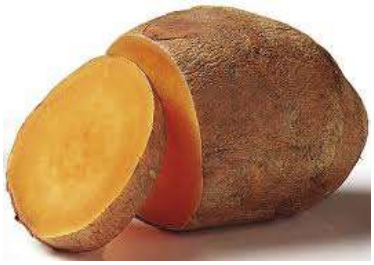
Sweet potato (*Ipomoea batatas*) or simply **potato** is a perennial plant of the convolvulaceae family widely cultivated in tropical and subtropical regions for its edible tubers.

→ syrup

↓

Butanol, acetone, ethanol

Yam *Dioscorea spp*



Yam flour and yam flakes.

Cocoyam *Colocasia and Xanthosoma*

Cocoyam starch is used in the pharmaceutical industry

Millets (various cereal grasses grown in Asia and Africa) *Pennisetum americanum*, *Setaria italica*, *Panicum miliaceum*, *Echinochloa frumentacea* and *Eleusine corcana*

Beer

Sorghum (grasses native to India) *Sorghum bicolor*

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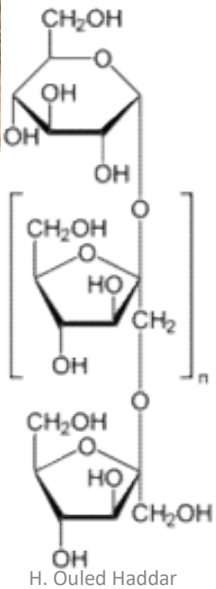
Helianthus tuberosus (Jerusalem artichokes)

inulin



2.Proteins

- ✓ ***Peanut flour (groundnut)*** *meal Arachis hypogea*

Oil (anti-foam) and press cake (animal feed, protein source)

- ✓ ***Blood Meal***
- ✓ ***Fish Meal***