2026

Ph.D. Process & Food Engineering
PFE 606 Bioprocess Engineering
Media Formulation

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4 Lecture

Medium formulation

Medium formulation is an essential stage in the design of successful fermentation process from initial laboratory experiments to pilot-scale development and full industrial-scale processes. To ensure a successful fermentation, the medium must be meticulously designed to satisfy three primary biological needs.

Requirement	Purpose	Key Constituents Involved
Cell Biomass	*To satisfy the basic elemental building blocks of the organism.*	*Carbon, Nitrogen, Hydrogen, and trace elements.*
Metabolite Production	*To provide the specific elements required for the synthesis of the desired product.*	*Precursors for antibiotics, vitamins, enzymes, or organic acids.*
Energy Supply	*To provide adequate energy for biosynthesis and the ongoing maintenance of the cell.*	*Primary carbon sources (sugars, lipids) that drive metabolic pathways.*

The first step to consider during media formulation is an equation based on the stoichiometry for growth and product formation.

For a general aerobic bioprocess:

Carbon and Energy source + Nitrogen Source + 0_2 + other requirements = Biomass + Products + CO_2 + H_20 + heat

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Elemental Composition of Microorganisms

The elemental makeup of microorganisms is a critical factor in media formulation for fermentation, as the nutrients provided must meet the biological requirements of the specific organism being cultured.

1. Major Elements (Macronutrients)

- **Carbon:** This is the most abundant element across all groups, making up **50–53%** of bacteria, **45–50%** of yeasts, and **40–63%** of fungi.
- **Nitrogen:** Bacteria typically have the highest nitrogen content (**12–15%**), while yeasts (**7.5–11%**) and fungi (**7–10%**) contain slightly less.
- **Hydrogen:** The hydrogen content remains remarkably consistent across bacteria and yeasts at approximately **7%**.

2. Secondary Elements

- **Phosphorus:** Essential for nucleic acids and ATP, phosphorus levels are highest in bacteria (**2.0–3.0%**) and vary most significantly in fungi (**0.4–4.5%**).
- **Potassium:** This element is found in relatively high concentrations in bacteria (**1.0–4.5%**) and yeasts (**1.0–4.0%**), but is lower in fungi (**0.2–2.5%**).
- **Sulfur:** Bacteria contain the highest sulfur concentration (**0.2–1.0%**), while yeasts have the lowest (**0.01–0.24%**).

3. Trace and Other Elements

- **Magnesium:** All three groups share a similar requirement range for magnesium, typically between **0.1% and 0.5%**.
- **Sodium & Calcium:** These are present in trace amounts, though bacteria generally show a higher requirement for sodium (**0.5–1.0%**) compared to yeasts and fungi.
- **Iron:** While essential, iron is found in very low percentages, generally ranging from **0.01% to 0.5%** across all species.
- **Chloride:** Only recorded for bacteria in this dataset at a steady **0.5%**.

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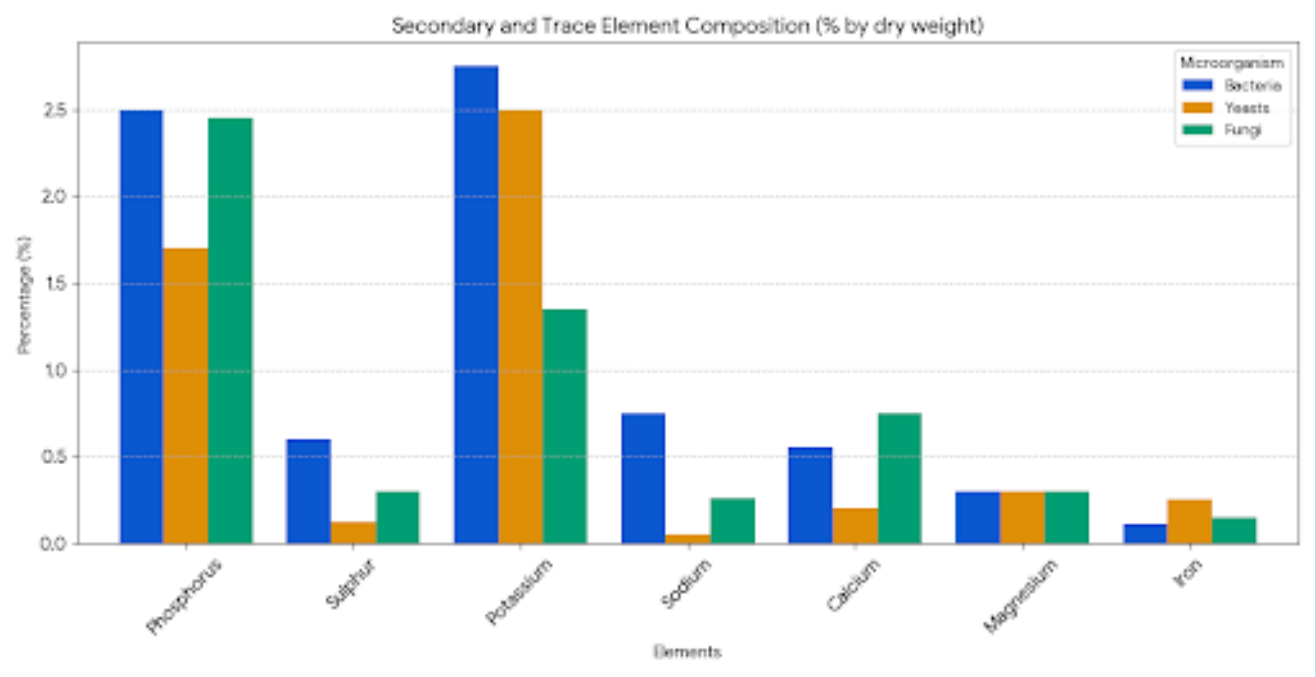

Importance of media

1. The growth medium is formulated to maintain microbial growth for economic production of our target product.
2. The design of media should be based on a good understanding of the relationship between microbial growth kinetics and product formation.
3. The Choice of medium affect the activity at both Up-stream and Down-stream processes.
4. A knowledge of the elemental composition of a choice of microorganism is required for the solution of the elemental balance equation.

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3 Lecture

THE COMPONENT OF A FERMENTATION PROCESS

Fermentation process may be divided into six basic component parts

Step	Phase	Action	Purpose & Key Details
1	Preparation	Media Formulation	Designing the nutrient mix (carbon, nitrogen, minerals) needed for both seed (inoculum) and large-scale production.
2	Preparation	Sterilization	Eliminating contaminating microorganisms from the medium, the fermenter vessel, and all piping/equipment.
3	Upstream	Inoculum Development	Scaling up a pure, active culture from a small vial to a large enough volume to "start" the production vessel.
4	Upstream	Fermentation	Growing the organism in the main fermenter under strict control (pH, temp, O_2) to maximize product yield.
5	Downstream	Extraction & Purification	Separating the desired product from the biomass and medium, followed by refining it to the required purity.
6	Post-Process	Effluent Disposal	Treating and safely disposing of chemical or biological waste generated during the process.

Microorganisms used in Fermentation

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Microorganisms used in fermentation are primarily classified into three main biological groups: Bacteria, Yeasts, and Molds (Fungi).

Group	Classification / Type	Representative Species	Primary Products
Bacteria	Lactic Acid Bacteria (LAB)	*Lactobacillus bulgaricus*, *Streptococcus thermophilus*, *Lactococcus lactis*	Yogurt, Cheese, Sauerkraut, Lactic acid.
	Acetic Acid Bacteria	*Acetobacter aceti*, *Gluconobacter* spp.	Vinegar (Acetic acid), Kombucha.
	Butyric Acid Bacteria	*Clostridium acetobutylicum*, *C. butyricum*	Acetone, Butanol, Butyric acid.
	Propionic Acid Bacteria	*Propionibacterium freudenreichii*	Swiss cheese (CO_2 holes), Propionic acid.
	Proteolytic/Alkaline	*Bacillus subtilis*, *Bacillus licheniformis*	Natto, Soy sauce, Protease enzymes.
Yeasts	Alcoholic Fermenters	*Saccharomyces cerevisiae*	Bread (leavening), Beer, Wine, Bioethanol.
	Bottom-Fermenters	*Saccharomyces pastorianus* (formerly *S. carlsbergensis*)	Lager beers.
	Industrial/Specialty	*Candida utilis*, *Pichia pastoris*	Single-cell protein (SCP), Recombinant proteins.
Molds	Filamentous Fungi	*Aspergillus niger*, *Aspergillus oryzae*	Citric acid, Soy sauce, Miso, Sake, Amylase.
	Cheese Ripening Molds	*Penicillium roqueforti*, *Penicillium camemberti*	Blue cheese (Roquefort), Camembert, Brie.

Methods of inoculation

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Inoculation refers to the purposeful introduction of microorganisms into a sterile culture medium.

Method	Primary Purpose	Step-by-Step Procedure	Best Tool to Use
Streak Plate (General)	To obtain pure, isolated colonies from a mixed sample.	Sterilize loop. ⇩ Pick a small sample. ⇩ Streak back and forth on a section of the agar. ⇩ Re-sterilize loop. ⇩ Drag once from the previous section and streak a new area. ⇩ Repeat until 3–4 areas are covered.	Inoculation Loop
1. Spiral Streak	To spread a sample uniformly or for quick propagation.	Place the loop at the center of the plate. ⇩ Move in a continuous outward circular motion toward the edge.	Inoculation Loop
2. X-mas Tree (Radiant)	To handle diluted specimens and check for colony morphology.	Streak the inoculum at the bottom edge. ⇩ Draw vertical lines (branches) upward. ⇩	Inoculation Loop

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		Cross-streak diagonally across the vertical lines at the end.	
3. Zig-Zag Streak	For bulk growth or propagation of an already pure culture.	Start at one end of the plate. ⇩ Drag the loop in a single, continuous "S" or "Z" shape across the entire surface without lifting or re-sterilizing.	Inoculation Loop
Pour Plate Method	To quantify (count) viable bacteria in a sample.	Add a specific volume of liquid sample to an empty sterile dish. ⇩ Pour molten agar (cooled to 45°C) over the sample. ⇩ Swirl gently to mix. ⇩ Allow to solidify (colonies will grow inside the agar).	Pipette (for sample)