2. Medium Pertumbuhan

1. Prof. Dr. Ir. Wignyanto, MS
2. Dr.Ir. Nur Hidayat, MP
3. Irnia Nurika, STP, MP, PhD.
4. Sri Suhartini, STP, M.Env.Man. PhD
5. Suprayogi, STP, MP, PhD

Agroindustrial Technology – Brawijaya University

1 Pendahuluan

- Menentukan nutrisi yang dibutuhkan oleh mikroorgansime pada dasarnya identik dengan mengetahui komposisi sel. Hal ini disebabkan nutrisi dibutuhkan oleh sel mikroorganisme untuk tumbuh dan berkembang.
- Unsur yang paling banyak dibutuhkan oleh mikroorganisme secara umum disebut dengan makroelemen yang mencakup karbon, oksigen, hydrogen, nitrogen, sulfur, fosfor, kalium, kalsium, magnesium dan besi.
- Selain makronutrien dikenal pula mikronutrien yaitu nutrien yang dibutuhkan dalam jumlah kecil/sedikit. Contoh mikro nutrien adalah mangan, seng, kobalt, molybdenum, nikel dan tembaga

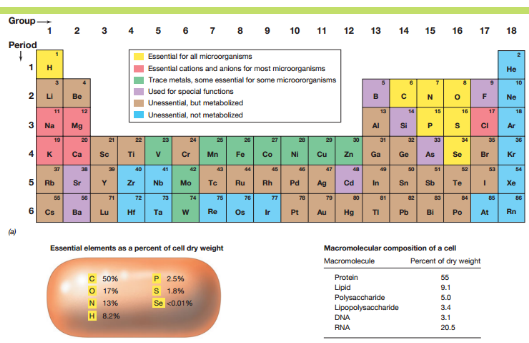

Figure 4.1 Elemental and macromolecular composition of a bacterial cell. *(a)* A microbial periodic table of the elements. With the exception of uranium, which can be metabolized by some prokaryotes, elements in period 7 or beyond in the complete periodic table of the elements are not known to be metabolized. *(b)* Contributions of the essential elements to cell dry weight. *(c)* Relative abundance of macromolecules in a bacterial cell. Data in *(b)* from *Aquat. Microb. Ecol. 10:* 15–27 (1996) and in *(c)* from Escherichia coli *and* Salmonella typhimurium: *Cellular and Molecular Biology.* ASM, Washington, DC (1996).

Tipe mikroorganisme

Table 5.1 Sources of Carbon, Energy, and Electrons

Carbon Sources	
Autotrophs	CO_2 sole or principal biosynthetic carbon source (section 10.3)
Heterotrophs	Reduced, preformed, organic molecules from other organisms (*chapters 9 and 10*)
Energy Sources	
Phototrophs	Light (section 9.12)
Chemotrophs	Oxidation of organic or inorganic compounds (*chapter 9*)
Electron Sources	
Lithotrophs	Reduced inorganic molecules (section 9.11)
Organotrophs	Organic molecules (chapter 9)

Tipe mikroorganisme berdasar nutrisi utama

Table 5.2 Major Nutritional Types of Microorganisms

Nutritional Type	Carbon Source	Energy Source	Electron Source	Representative Microorganisms
Photolithoautotrophy (photolithotrophic autotrophy)	CO_2	Light	Inorganic e^- donor	Purple and green sulfur bacteria, cyanobacteria
Photoorganoheterotrophy (photoorganotrophic heterotrophy)	Organic carbon, but CO_2 may also be used	Light	Organic e^- donor	Purple nonsulfur bacteria, green nonsulfur bacteria
Chemolithoautotrophy (chemolithotrophic autotrophy)	CO_2	Inorganic chemicals	Inorganic e^- donor	Sulfur-oxidizing bacteria, hydrogen-oxidizing bacteria, methanogens, nitrifying bacteria, iron-oxidizing bacteria
Chemolithoheterotrophy or mixotrophy (chemolithotrophic heterotrophy)	Organic carbon, but CO_2 may also be used	Inorganic chemicals	Inorganic e^- donor	Some sulfur-oxidizing bacteria (e.g., *Beggiatoa*)
Chemoorganoheterotrophy (chemoorganotrophic heterotrophy)	Organic carbon	Organic chemicals often same as C source	Organic e^- donor, often same as C source	Most nonphotosynthetic microbes, including most pathogens, fungi, many protists, and many archaea

❖ Carbon and Nitrogen

- All cells require carbon, and most prokaryotes require organic (carbon-containing) compounds as their source of carbon
- Heterotrophic bacteria assimilate organic compounds and use them to make new cell material.
- Amino acids, fatty acids, organic acids, sugars, nitrogen bases, aromatic compounds, and count-less other organic compounds can be transported and catabolized by one or another bacterium.
- Autotrophic microorganisms build their cellular structures from carbon dioxide (CO_2) with energy obtained from light or inorganic chemicals.

❖ Carbon and Nitrogen

- A bacterial cell is about 13% nitrogen, which is present in proteins, nucleic acids, and several other cell constituents.
- The bulk of nitrogen available in nature is in inorganic form as ammonia (NH^3), nitrate (NO^{3-}), or nitrogen gas (N_2).
- Nitrogen in organic compounds, for example, in amino acids, may also be available to microorganisms; if organic N is available and is taken up, the compound can immediately enter the monomer pool for biosynthesis or be catabolized as an energy source

❖ Other Macronutrients: P, S, K, Mg, Ca, Na

- Phosphorus is a key element in nucleic acids and phospholipids and is typically supplied to a cell as phosphate ($PO_4{}^{2-}$).
- Sulfur is present in the amino acids cysteine and methionine and also in several vitamins, including thiamine, biotin, and lipoic acid. Sulfur can be supplied to cells in several forms, including sulfide (HS^-) and sulfate ($SO_4{}^{2-}$).
- Potassium (K) is required for the activity of several enzymes, whereas magnesium (Mg) functions to stabilize ribosomes, membranes, and nucleic acids and is also required for the activity of many enzymes.
- Calcium (Ca) is not required by all cells but can play a role in helping to stabilize microbial cell walls, and it plays a key role in the heat stability of endospores.
- Sodium (Na) is required by some, but not all, microorganisms, and its requirement is typically a reflection of the habitat. For example, seawater contains relatively high levels of Na^+, and marine microorganisms typically require Na^+ for growth.

❖ Micronutrients: Iron and Other Trace Metals

- Iron (Fe), which plays a major role in cellular respiration. Iron is a key component of cytochromes and of iron–sulfur proteins involved in electron transport reactions
- Under anoxic conditions, iron is generally in the ferrous (Fe^{2+}) form and soluble. However, under oxic conditions, iron is typically in the ferric (Fe^{3+}) form as part of insoluble minerals.
- To obtain Fe^{3+} from such minerals, cells produce iron-binding molecules called siderophores that function to bind Fe^{3+} and transport it into the cell.
- A major group of siderophores is the hydroxamic acids, organic molecules that chelate Fe^{3+} strongly.

❖ Micronutrients: Iron and Other Trace Metals

- However, as important as iron is for most cells, some organisms can grow in the absence of iron.
- For example, many lactic acid bacteria such as species of Lactobacillus do not contain detectable iron and grow normally in its absence. In these organisms, manganese (Mn^{2+}) often plays a role similar to that just described for iron.
- Many other metals are required or otherwise metabolized by microorganisms (Figure 4.1a).
- Like iron, these micronutrients are called trace elements or trace metals.
- Micronutrients typically play a role as cofactors for enzymes.
- Table 4.1 lists the major micronutrients and examples of enzymes in which each plays a role in the cell

Table 4.1 *Micronutrients (trace elements) needed by microorganisms*[a]

Element	*Cellular function or molecule of which a part*
Boron (B)	Autoinducer for quorum sensing in bacteria; also found in some polyketide antibiotics
Chromium (Cr)	Possible but not proven component for glucose metabolism (necessary in mammals)
Cobalt (Co)	Vitamin B_{12}; transcarboxylase (only in propionic acid bacteria)
Copper (Cu)	In respiration, cytochrome *c* oxidase; in photosynthesis, plastocyanin, some superoxide dismutases
Iron (Fe)[b]	Cytochromes; catalases; peroxidases; iron–sulfur proteins; oxygenases; all nitrogenases
Manganese (Mn)	Activator of many enzymes; component of certain superoxide dismutases and of the water-splitting enzyme in oxygenic phototrophs (photosystem II)
Molybdenum (Mo)	Certain flavin-containing enzymes; some nitrogenases, nitrate reductases, sulfite oxidases, DMSO-TMAO reductases; some formate dehydrogenases
Nickel (Ni)	Most hydrogenases; coenzyme F_{430} of methanogens; carbon monoxide dehydrogenase; urease
Selenium (Se)	Formate dehydrogenase; some hydrogenases; the amino acid selenocysteine
Tungsten (W)	Some formate dehydrogenases; oxotransferases of hyperthermophiles
Vanadium (V)	Vanadium nitrogenase; bromoperoxidase
Zinc (Zn)	Carbonic anhydrase; alcohol dehydrogenase; RNA and DNA polymerases; and many DNA-binding proteins

[a]Not every micronutrient listed is required by all cells; some metals listed are found in enzymes or cofactors present in only specific microorganisms.
[b]Needed in greater amounts than other trace metals.

❖ Micronutrients: Growth Factors

- Growth factors are organic compounds that, like trace metals, are required in only very small amounts.
- Growth factors are vitamins, amino acids, purines, pyrimidines, or various other organic molecules.
- Although most microorganisms are able to biosynthesize the growth factors they need, some must obtain one or more of them from the environment and thus must be supplied with these compounds when cultured in the laboratory.
- Vitamins are the most commonly required growth factors.
- Most vitamins function as coenzymes, which are non protein components of enzymes.
- Vitamin requirements vary among microorganisms, ranging from none to several. Lactic acid bacteria, which include the genera Streptococcus, Lactobacillus, and Leuconostoc, are renowned for their many vitamin requirements, which are even more extensive than those of humans (see Table 4.2).

Kebutuhan vitamin

Table 5.3 Functions of Some Common Vitamins in Microorganisms

Vitamin	Functions	Examples of Microorganisms Requiring Vitamin[a]
Biotin	Carboxylation (CO_2 fixation) One-carbon metabolism	*Leuconostoc mesenteroides* (B) *Saccharomyces cerevisiae* (F) *Ochromonas malhamensis* (P) *Acanthamoeba castellanii* (P)
Cyanocobalamin (B_{12})	Molecular rearrangements One-carbon metabolism—carries methyl groups	*Lactobacillus* spp. (B) *Euglena gracilis* (P) Diatoms (P) *Acanthamoeba castellanii* (P)
Folic acid	One-carbon metabolism	*Enterococcus faecalis* (B) *Tetrahymena pyriformis* (P)
Lipoic acid	Transfer of acyl groups	*Lactobacillus casei* (B) *Tetrahymena* spp. (P)
Pantothenic acid	Precursor of coenzyme A—carries acyl groups (pyruvate oxidation, fatty acid metabolism)	*Proteus morganii* (B) *Hanseniaspora* spp. (F) *Paramecium* spp. (P)
Pyridoxine (B_6)	Amino acid metabolism (e.g., transamination)	*Lactobacillus* spp. (B) *Tetrahymena pyriformis* (P)
Niacin (nicotinic acid)	Precursor of NAD and NADP—carry electrons and hydrogen atoms	*Brucella abortus, Haemophilus influenzae* (B) *Blastocladia pringsheimii* (F) *Crithidia fasciculata* (P)
Riboflavin (B_2)	Precursor of FAD and FMN—carry electrons or hydrogen atoms	*Caulobacter vibrioides* (B) *Dictyostelium* spp. (P) *Tetrahymena pyriformis* (P)
Thiamine (B_1)	Aldehyde group transfer (pyruvate decarboxylation, α-keto acid oxidation)	*Bacillus anthracis* (B) *Phycomyces blakesleeanus* (F) *Ochromonas malhamensis* (P) *Colpidium campylum* (P)

[a] The representative microorganisms are members of the following groups: *Bacteria* (B), *Fungi* (F), and protists (P).

3 Culture Media

- Culture media are the nutrient solutions used to grow microorganisms in the laboratory
- Classes of Culture Media : Defined Media and Complex Media
- Defined media are prepared by adding precise amounts of highly purified inorganic or organic chemicals to distilled water
- Complex media employ digests of microbial, animal or plant products, such as casein (milk protein), beef (beef extract), soy-beans (tryptic soy broth), yeast cells (yeast extract), or any of a number of other highly nutritious yet impure substances.

Table 4.2 *Examples of culture media for microorganisms with simple and demanding nutritional requirements*[a]

Defined culture medium for Escherichia coli	*Defined culture medium for* Leuconostoc mesenteroides	*Complex culture medium for either* E. coli *or* L. mesenteroides	*Defined culture medium for* Thiobacillus thioparus
K_2HPO_4 7 g KH_2PO_4 2 g $(NH_4)_2SO_4$ 1 g $MgSO_4$ 0.1 g $CaCl_2$ 0.02 g Glucose 4–10 g Trace elements (Fe, Co, Mn, Zn, Cu, Ni, Mo) 2–10 μg each Distilled water 1000 ml pH 7	K_2HPO_4 0.6 g KH_2PO_4 0.6 g NH_4Cl 3 g $MgSO_4$ 0.1 g Glucose 25 g Sodium acetate 25 g Amino acids (alanine, arginine, asparagine, aspartate, cysteine, glutamate, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine) 100–200 μg of each Purines and pyrimidines (adenine, guanine, uracil, xanthine) 10 mg of each Vitamins (biotin, folate, nicotinic acid, pyridoxal, pyridoxamine, pyridoxine, riboflavin, thiamine, pantothenate, *p*-aminobenzoic acid) 0.01–1 mg of each Trace elements (as in first column) 2–10 μg each Distilled water 1000 ml pH 7	Glucose 15 g Yeast extract 5 g Peptone 5 g KH_2PO_4 2 g Distilled water 1000 ml pH 7	KH_2PO_4 0.5 g NH_4Cl 0.5 g $MgSO_4$ 0.1 g $CaCl_2$ 0.05 g KCl 0.5 g $Na_2S_2O_3$ 2 g Trace elements (as in first column) Distilled water 1000 ml pH 7 Carbon source: CO_2 from air


(a)


(b)

[a]The photos are tubes of *(a)* the defined medium described, and *(b)* the complex medium described. Note how the complex medium is colored from the various organic extracts and digests that it contains. Photo credits: Cheryl L. Broadie and John Vercillo, Southern Illinois University at Carbondale.

- An enriched medium, often used for the culture of otherwise difficult-to-grow nutritionally demanding (fastidious) microorganisms, starts with a complex base and is embellished with additional nutrients such as serum, blood, or other highly nutritious substances
- A selective medium contains compounds that inhibit the growth of some microorganisms but not others.
- Differential media are quite useful for distinguishing different species of bacteria and are therefore widely used in clinical diagnostics and systematic microbiology

Nutritional Requirements and Biosynthetic Capacity

- The complex medium is easiest to prepare and supports growth of both of the chemoorganotrophs, Escherichia coli and Leuconostoc mesenteroides, the examples used in the table.
- However, the simple defined medium supports growth of E. Coli but not of L. mesenteroides.
- Growth of the latter organism, a fastidious (nutritionally demanding) bacterium, in a defined medium requires the addition of several nutrients not needed by E. coli.
- By contrast, E. Coli can synthesize everything it needs from a single carbon compound, in this case, glucose.
- The nutritional needs of L. mesenteroides can be satisfied by preparing either a highly supplemented defined medium, a rather laborious under-taking because of all the individual nutrients that need to be added (Table 4.2), or by preparing a complex medium, a much less demanding operation.

Media Kultur

Table 5.4 Types of Media

Physical Nature	Chemical Composition	Functional Type
Liquid	Defined (synthetic)	Supportive (general purpose)
Semisolid	Complex	Enriched
Solid		Selective
		Differential

Table 5.5 Examples of Defined Media

BG–11 Medium for Cyanobacteria	Amount (g/liter)
$NaNO_3$	1.5
$K_2HPO_4 \cdot 3H_2O$	0.04
$MgSO_4 \cdot 7H_2O$	0.075
$CaCl_2 \cdot 2H_2O$	0.036
Citric acid	0.006
Ferric ammonium citrate	0.006
EDTA (Na_2Mg salt)	0.001
Na_2CO_3	0.02
Trace metal solution[a]	1.0 ml/liter
Final pH 7.4	
Medium for *Escherichia coli*	**Amount (g/liter)**
Glucose	1.0
Na_2HPO_4	16.4
KH_2PO_4	1.5
$(NH_4)_2SO_4$	2.0
$MgSO_4 \cdot 7H_2O$	200.0 mg
$CaCl_2$	10.0 mg
$FeSO_4 \cdot 7H_2O$	0.5 mg
Final pH 6.8–7.0	

Sources: Data from Rippka, et al. *Journal of General Microbiology,* 111:1–61, 1979; and S. S. Cohen, and R. Arbogast, *Journal of Experimental Medicine,* 91:619, 1950.

[a]The trace metal solution contains H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $Na_2Mo_4 \cdot 2H_2O$, $CuSO_4 \cdot 5H_2O$, and $Co(NO_3)_2 \cdot 6H_2O$.

Contoh medium komplek

Table 5.6 Some Common Complex Media

Nutrient Broth	Amount (g/liter)
Peptone (gelatin hydrolysate)	5
Beef extract	3
Tryptic Soy Broth	
Tryptone (pancreatic digest of casein)	17
Peptone (soybean digest)	3
Glucose	2.5
Sodium chloride	5
Dipotassium phosphate	2.5
MacConkey Agar	
Pancreatic digest of gelatin	17.0
Pancreatic digest of casein	1.5
Peptic digest of animal tissue	1.5
Lactose	10.0
Bile salts	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	13.5

Table 2
Composition of enrichment broths used for the isolation of *Acinetobacter* species.

Baumann enrichment medium[a]	Dijkshoorn enrichment medium[b]
2 g $CH_3COONa \cdot 3H_2O$	1.5 g KH_2PO_4
2 g KNO_3,	16.5 g $Na_2HPO_4 \cdot H_2O$
0.2 g $MgSO_4 \cdot 7H_2O$,	0.2 g $MgSO_4 \cdot 7H_2O$
0.04 M KH_2PO_4/NA_2HPO_4 buffer (pH 6.0)	2.0 g NH_4Cl
1 L deionised water	0.01 g $CaCl_2$
Autoclaved for 15 min at 121 °C	0.0005 g $FeSO_4 \cdot 7H_2O$
	2.0 g CH_3COONa
	1 L water deionised
	Adjust pH to 7.5 ± 0.2
	Autoclaved for 15 min at 118 °C

[a] According to Baumann (1968) without the addition of Hutner's mineral base (Cohen-Bazire et al., 1957); according to Towner (2006), this is not necessary as there are sufficient nutrient trace elements in the other medium components.
[b] Saline solution according to Monod and Wollman (1947) enriched with 0.2% (w/v) sodium acetate according to Dijkshoorn et al. (1987).

Short communication

Enrichment of *Acinetobacter* spp. from food samples

Ana Carvalheira, Vânia Ferreira, Joana Silva, Paula Teixeira*

CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Universidade Católica Portuguesa/Porto, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal

Food Microbiology 55 (2016) 123–127

Tugas individu

Tulis media pertumbuhan

1. Sintetik
2. Kompleks
3. Cair
4. Padat

(dari jurnal berbahasa Inggris – lampirkan jurnalnya).

Sebut mikroorganisme yang ditumbuhkan pada media tersebut


Microbiology
microorganisms
microbes
organisms
biodegradation

Thank You!