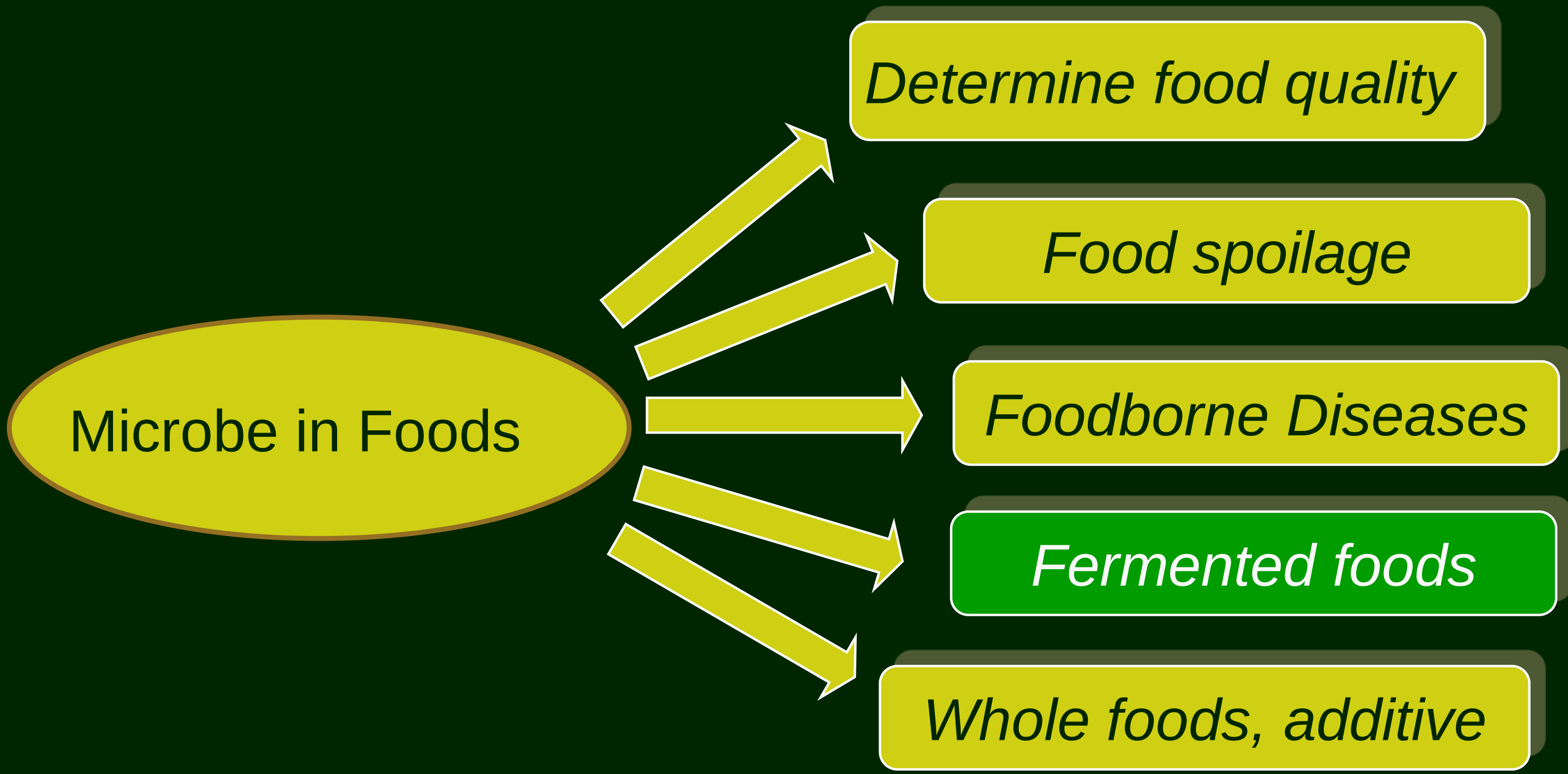
FOOD MICROBIOLOGY

FERMENTATION

By
Mochamad Nurcholis

MICROBE IN FOODS



FERMENTATION

- Definition & benefits of Fermentation
- Fermentation Medium
- Biodegradation & Fermentation Pathway
- Fermentation Types
- Microorganism in Fermented Foods
- Fermentation Products
- Condition & Resources

DEFINITION
&
BENEFITS

Definition of Fermentation

- Fermentation → *fervere* (Latin)
- To boil ???
- CO_2 production → Boiling appearance
- Different definition (Biochemist vs Microbiologist)

Definition of Fermentation

- ***Biochemist :***

  Generation of energy by the catabolism of organic compounds.

- ***Microbiologist :***

  The definition tends to be much broader.

Definition cont'd

- Gradual change process by microorganism activity or their metabolites (ex : enzyme) to produce product.
- Fermented foods → foods or food ingredients that rely on microbial growth as part of their processing or production.

Benefits of Fermentation

The growth of microorganism and their metabolic activities influence :

- Extend or prolong shelf life
- Change and Increase flavor
- Change texture
- Inhibit pathogen & spoilage microbes
- Improve nutritive value of the product

Fermented Foods

- Fermentation: *"good food microbiology"*
  - any desirable change a microorganism makes to food
  - Food Spoilage ???

    → undesirable changes

Microorganisms in Fermented Food

- Using microorganisms for food production has been done for thousands of years
  - cheese, yeast, beer
- Microorganisms used in food often produce an acidic by-product as a result of metabolism
  - can inhibit growth of many spoilage microorganisms
  - can inhibit growth of many food-borne pathogens

Yeast cells

FERMENTED FOODS

Application of biological agents (whole cells or its metabolites) on fermented foods :

- Beer
- Wine
- Yoghurt
- Cheese
- Tempe
- Bread
- Kefir
- Soy sauce, etc

1822	C.J. Person named the microscopic organism found on the surface of wine during vinegar production as *Mycoderma mesentericum*. Pasteur in 1868 proved that this organism was associated with the conversion of alcohol to acetic acid and named it *Mycoderma aceti*. In 1898, Martinus Beijerinck renamed it *Acetobacter aceti*.
1837	Theodor Schwann named the organism involved in sugar fermentation as *Saccharomyces* (sugar fungus).
1838	Charles Cogniard-Latour suggested that growth of yeasts was associated with alcohol fermentation.
1860	Louis Pasteur showed that fermentation of lactic acid and alcohol from sugar was the result of growth of specific bacteria and yeasts, respectively.
1883	Emil Christian Hansen used pure cultures of yeasts to ferment beer.

Sources : Food Microbiology (Bibek Ray)

Trends in Food Fermentation

- Development of strains with desirable metabolic activities by genetic transfer among strains
- Development of bacteriophage-resistant lactic acid bacteria
- Metabolic engineering of strains for overproduction of desirable metabolites
- Development of methods to use lactic acid bacteria to deliver immunity proteins
- Sequencing genomes of important lactic acid bacteria and bacteriophages for better understanding of their characteristics
- Food biopreservation with desirable bacteria and their antimicrobial metabolites
- Understanding of important characteristics of probiotic bacteria and development of desirable strains
- Effective methods to produce starter cultures for direct use in food processing

Sources : Food Microbiology (Bibek Ray)

FERMENTATION MEDIUM

Cell Nutrients

Nutrients required by cells can be classified in two categories:

Macronutrients are needed in larger concentrations

Ex : C, N, O, H, S, P, Mg^{2+}, and K^{+}.

Micronutrients are needed in less concentrations

Ex : Mo, Zn, Cu, Mn, Ca, Na, vitamins, growth hormones and metabolic precursors.

Carbon as the Major Sources of Cellular Carbon and Energy.

- Heterotrophs

  Use organic carbon sources, (ex : carbohydrates, lipid, proteins)

- Autotrophs

  Use carbon dioxide as a carbon source.

  They can form carbohydrate through light or chemical oxidation.

- Aerobic fermentations

  About 50% of substrate carbon is incorporated into cell mass and about 50% of it is used as energy sources.

- Anaerobic fermentation

  A large fraction of substrate carbon is converted to products and a smaller fraction is converted to cell mass (less than 30%).

Carbon Sources as Macronutrients

- In industrial fermentation

  → the most common carbon sources are :

  - Molasses (sucrose) - Corn syrup

  - Starch (dextrin) - waste sulfite liquor (glucose).

- In laboratory fermentations :

  - Glucose

  - Sucrose

  - Fructose

  - Ethanol, methanol and methane also constitute cheap carbon sources.

Nitrogens as Macronutrients

Nitrogen compounds are important sources for synthesizing protein, nucleic acid.

- Nitrogen constitutes 10 – 14 % of cell dry weight.

- The most commonly used nitrogen sources are :
  - Ammonia or ammonium salts (ex : ammonium chloride, sulfate and nitrate).
  - Protein, peptides, and amino acids.
  - Urea can be cheap source.

- In industrial fermentation used :
  - Soya meal
  - Distillers solubles
  - Dry Blood
  - Yeast extract
  - Corn steep liquor

Oxygen as Macronutrients

Oxygen constitutes about 20% of the cell dry weight.

- Molecular oxygen is required as terminal electron acceptor in the aerobic metabolism of carbon compounds.

- Gaseous oxygen is introduced into growth media by sparging air or by surface aeration.

- Improving the mass transfer of oxygen in a bioreactor is a challenge in reactor control.

H & P as Macronutrients

Hydrogen: 8% of dry cell weight

major source: carbohydrates.

Phosphorus: 3% of cell dry weight

- Present in nucleic acids and in the cell wall of some gram-positive bacteria.
- A key element in the regulation of cell metabolism.
- Sources: Inorganic phosphates.

The phosphate level should be less than 1 mM for the formation of many secondary metabolites such as antibiotics.

Other Macronutrients

- **Sulfur** :
  - 1% of cell dry weight
  - present in protein and some coenzymes.
  - source: Ammonium sulfate, Sulfur containing amino acids, cycteine some autotrophs can use S^0 and S^{2+} as energy sources.

- **Potassium** :
  - a cofactor for some enzyme and is required in carbohydrate metabolism.
  - cofactor : any of various organic or inorganic substances necessary to the function of an enzyme.
  - source: potassium phosphates.

- **Magnesium:**

  - a cofactor for some enzyme and is present in cell walls and membranes. Ribosomes specifically requires Mg^{2+} .
  - sources: Magnesium sulfate or chloride

Micronutrients

Micronutrients could be classified into the following categories (required less than 10^{-4} M):

- Most widely needed elements.
- Trace elements needed under specific growth conditions.
- Trace elements rarely require.
- Growth factor.

Micronutrients

Micronutrients could be classified into the following categories:

- Most widely needed elements

  Fe, Zn and Mn. Such elements are cofactors for some enzyme and regulate the metabolism.

- Trace elements needed under specific growth conditions

  Cu, Co, Mo, Ca, Na, Cl, Ni, and Se. For example, copper is present in certain respiratory-chain components and enzymes.

Cell Nutrients- Micronutrients

- **Trace elements rarely required**

  B, Al, Si, Cr, V, Sn, Be, F, Ti, Ga, Ge, Br, Zr, W, Li and I. These elements are required in concentrations of less than 10^{-6}M and are toxic at high concentration.

- **Growth factor is also micronutrient**.

  Growth factor stimulates the growth and synthesis of some metabolites. e.g. vitamin, hormones and amino acids. They are required less than 10^{-6}M.

Source of	Typical ingredients	Concentration (gram/liter)
Carbon	Glucose	20
	Sucrose	20
	Glycerol	20
Nitrogen	(NH)42SO4	5
	NaNO3	7
	Na4NO3	3
	Alanine or other amino acids	7
Phosphorus	KH2PO4	1
	K2HPO4	1
Sulfur	K2SO4	0.4
	MgSO4 7H2O	0.5
	Methionine	0.3

Component of chemically defines fermentation medium needed to obtain about 10 g of dry cell

Component of chemically defines fermentation medium needed to obtain about 10 g of dry cell

Source of	Typical ingredients	Concentration (gram/liter)
Mg	$MgSO_4\,7H_2O$	0.1
K	K2SO4	0.1
Ca	CaCl2	0.05
Fe	$FeSO_4\,7H_2O$	0.001
Zn	$ZnSO_4\,7H_2O$	0.001
Cu	$CuSO_4\,7H_2O$	0.004
Mn	$MnSO_4\,7H_2O$	0.004

BIODEGRADATION

&

FERMENTATION PATHWAY

How do they grow: requirements for biodegradation?

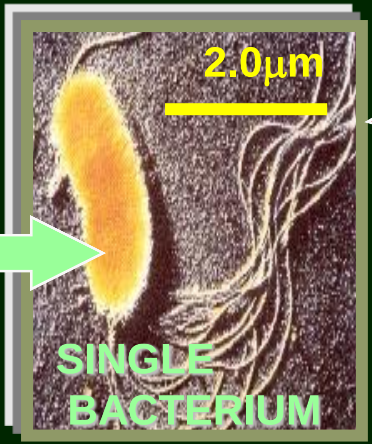
- Nutrients
- Carbon, Nitrogen, Phosphorus, Sulfur
- Many chemicals supply these
- Micronutrients/ trace metals/ vitamins
- Electron acceptors - usually O_2
- Converts / burns carbon substrate to CO_2
  Energy and biomass ie GROWTH

Biodegradation

O_2 consumption

GROWTH - CELL DIVISION
INCREASE IN BIOMASS

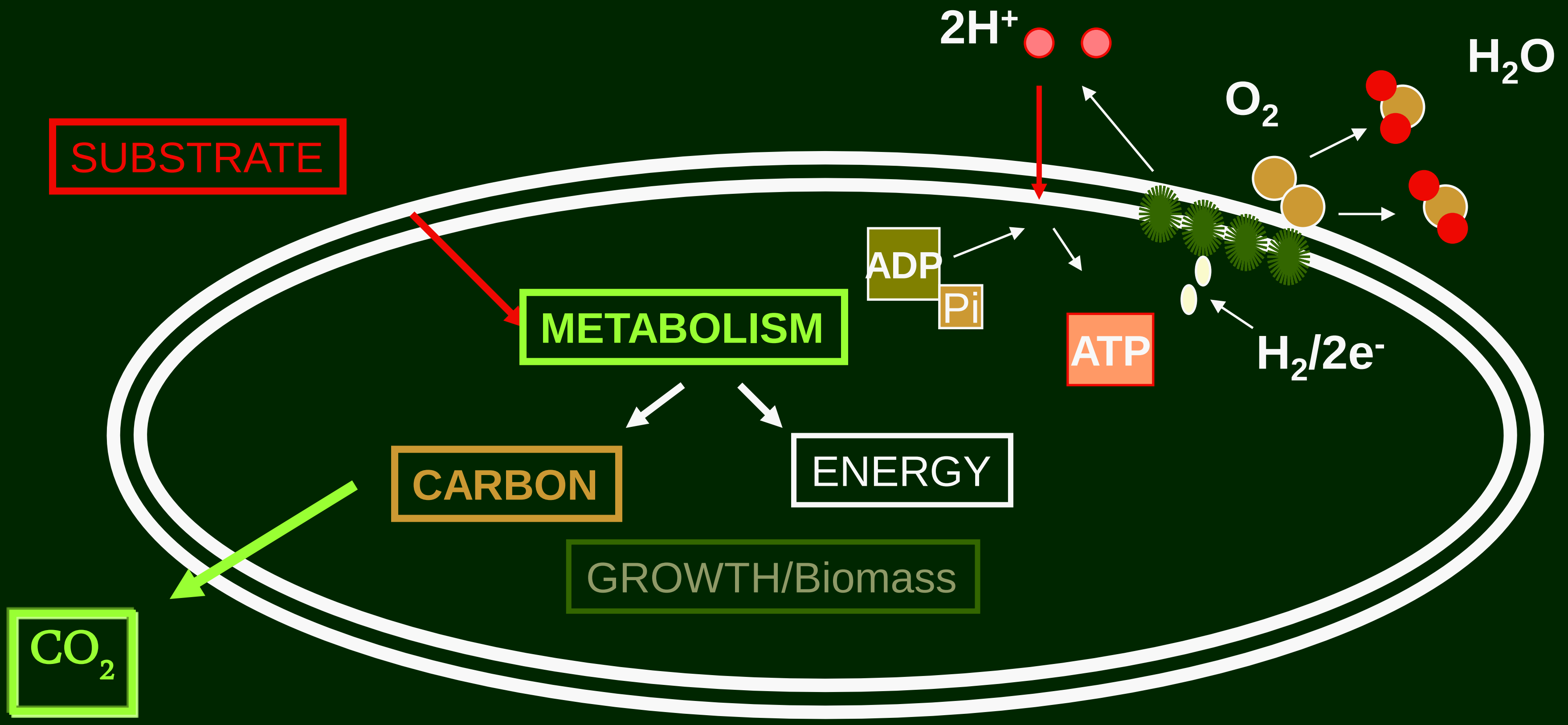
ORGANIC AND
INORGANIC NUTRIENTS
(C,H,O, N,P,Fe,S…)



CO_2 evolved

Controlled release of energy
Slow Burning!

Oxygen and Electron Acceptors: crucial for Biodegradation reactions in the environment.

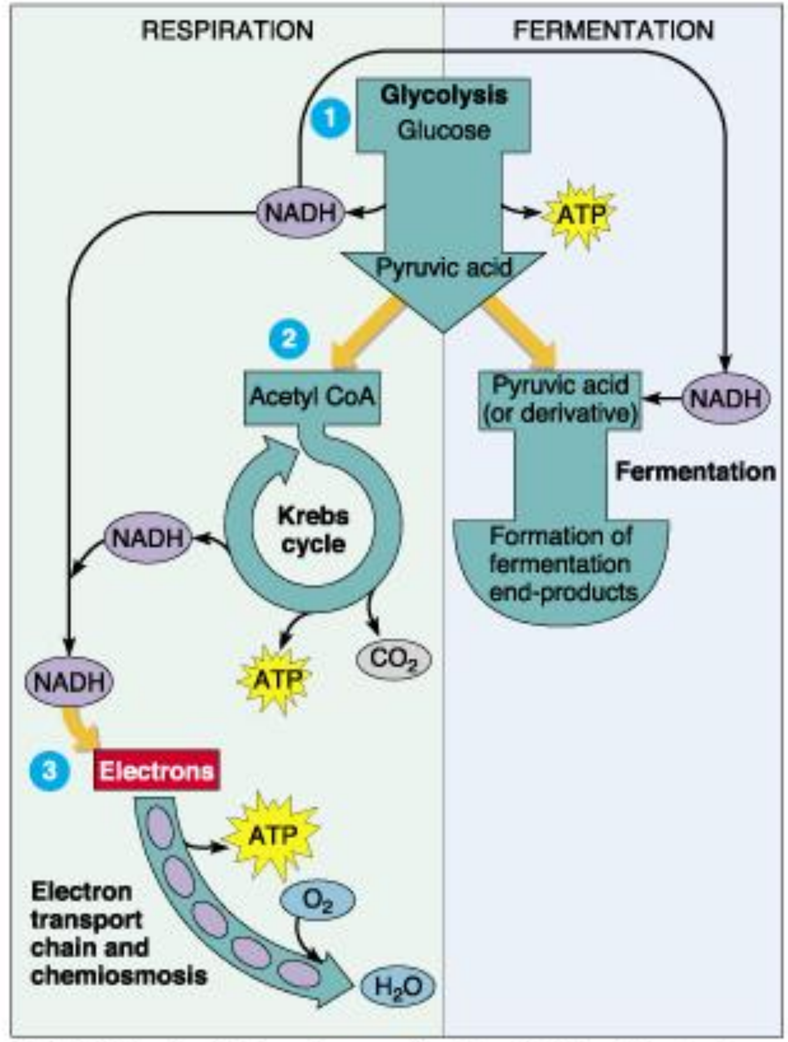


Carbohydrate Catabolism

Microbes use ***two general processes*** to generate energy from glucose

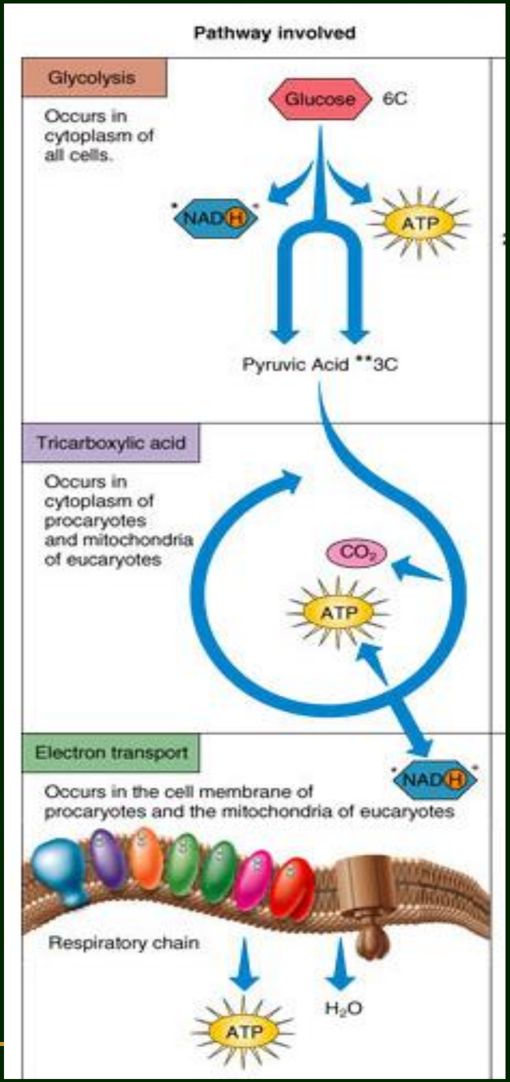
- Cellular respiration
- Fermentation

Both start with "glycolysis"



Cellular Respiration

- Glycolysis (Embden-Meyerhof-Parnas)
  - Glucose is oxidized to pyruvic acid with ATP and energy-containing NADH produced
- Pyruvic acid is converted → acetyl CoA with NADH produced
- TCA Cycle (Kreb's cycle)
  - Acetyl CoA is oxidized to CO_2 with ATP, NADH and $FADH_2$ is produced
- Electron Transport Chain
  - NADH and $FADH_2$ are oxidized through a series of redox reactions and a considerable amount of ATP is produced



Glycolysis

- Starting point for cellular respiration also fermentation.
- 10 step catabolic pathway
- Two stages
  - Preparatory stage
  - Energy conserving stage

Fermentation versus Respiration

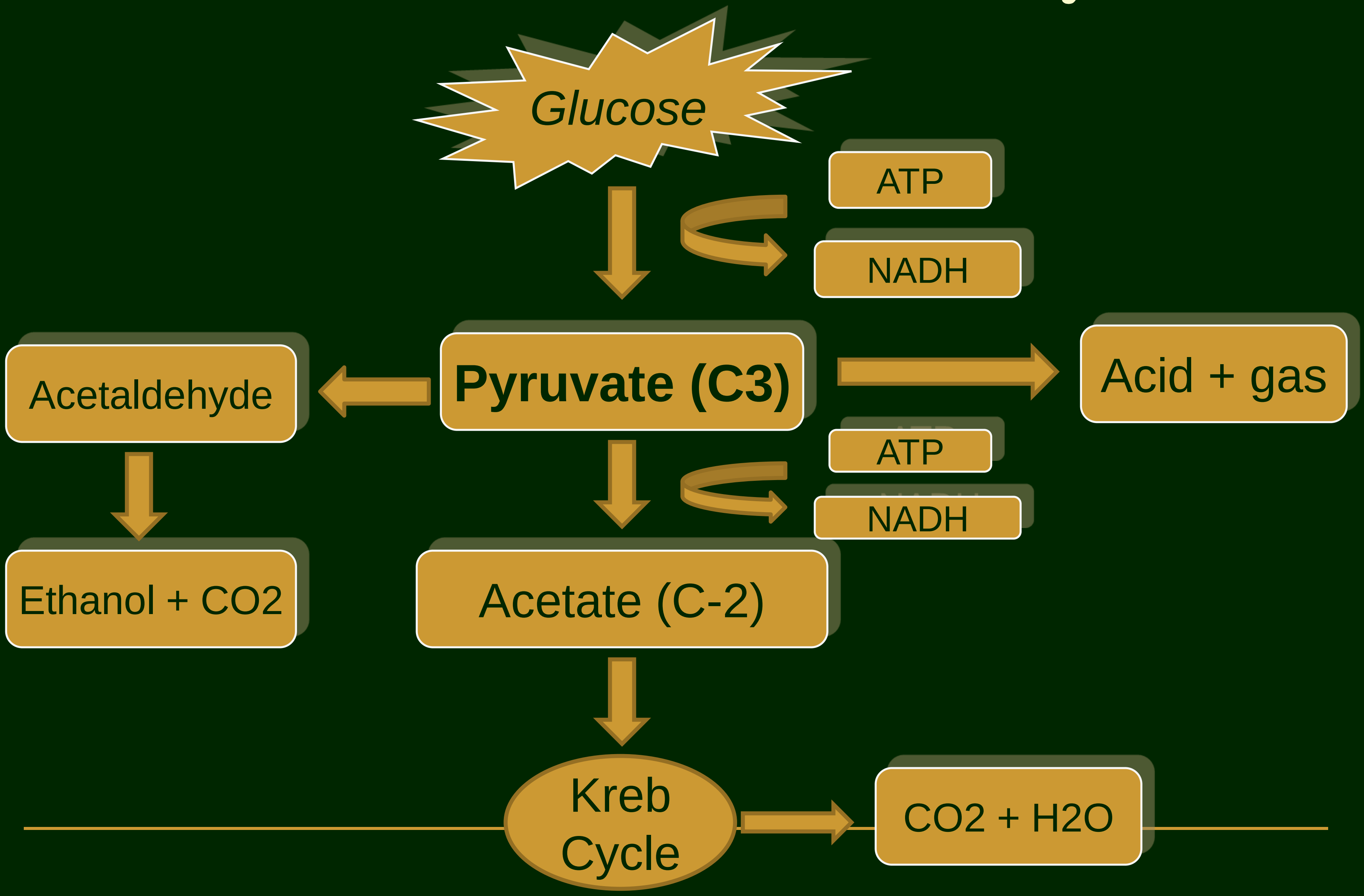
The Difference	Fermentation	Respiration
Final e- acceptor	Organic compounds	Oxygen
Electron donors	Organic compounds	
Process	Glucose is coverted to 1 or 3 Carbon compound	Glucose is oxidized to CO_2
Product	Organic acid, alcohol & 1-2 ATP	6 CO_2, 6 H_2O & energy (38 ATP)
Step	Glycolysis → acid or alcohol fermentation	Glycolysis → TCA cycle → electron transport

Fermented Foods

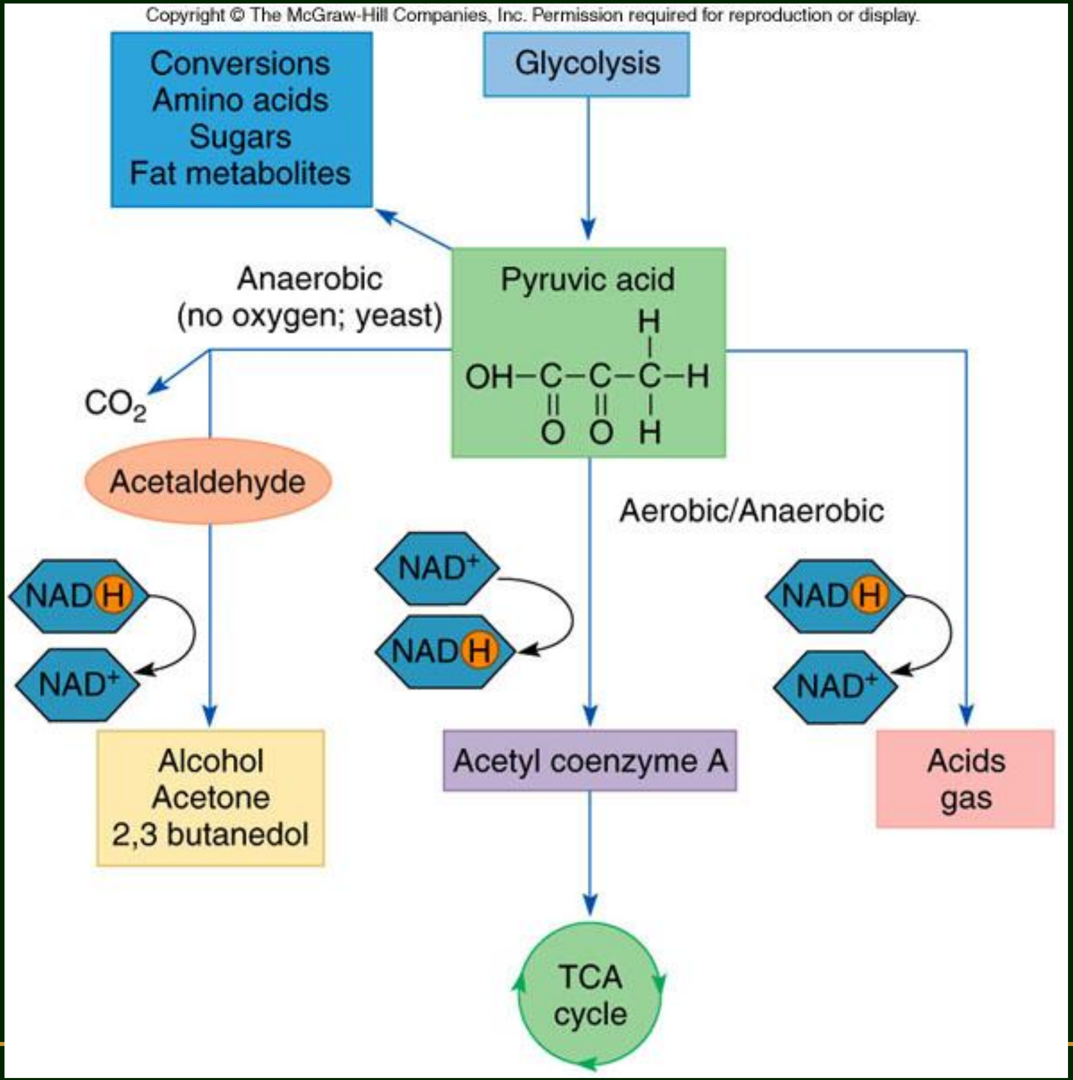
- Carbohydrates → Glucose → Acids/Alcohols
- Protein → Amino acids → Alcohol, aldehyde
- Lipid → fatty acids → FFA, ketones

Note : FFA is Free Fatty Acids

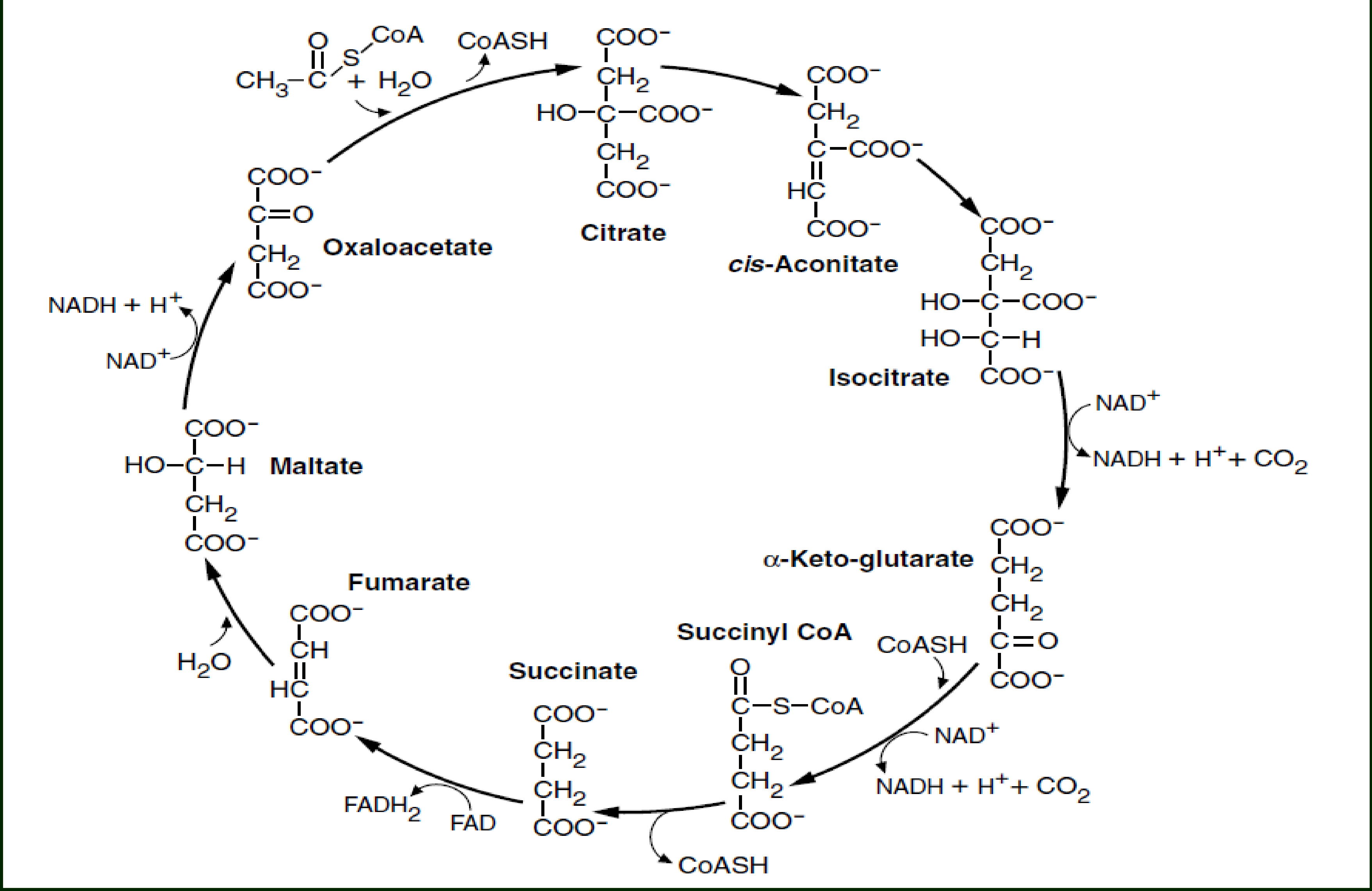
Fermentation Pathway



Pyruvic acid-a central metabolite

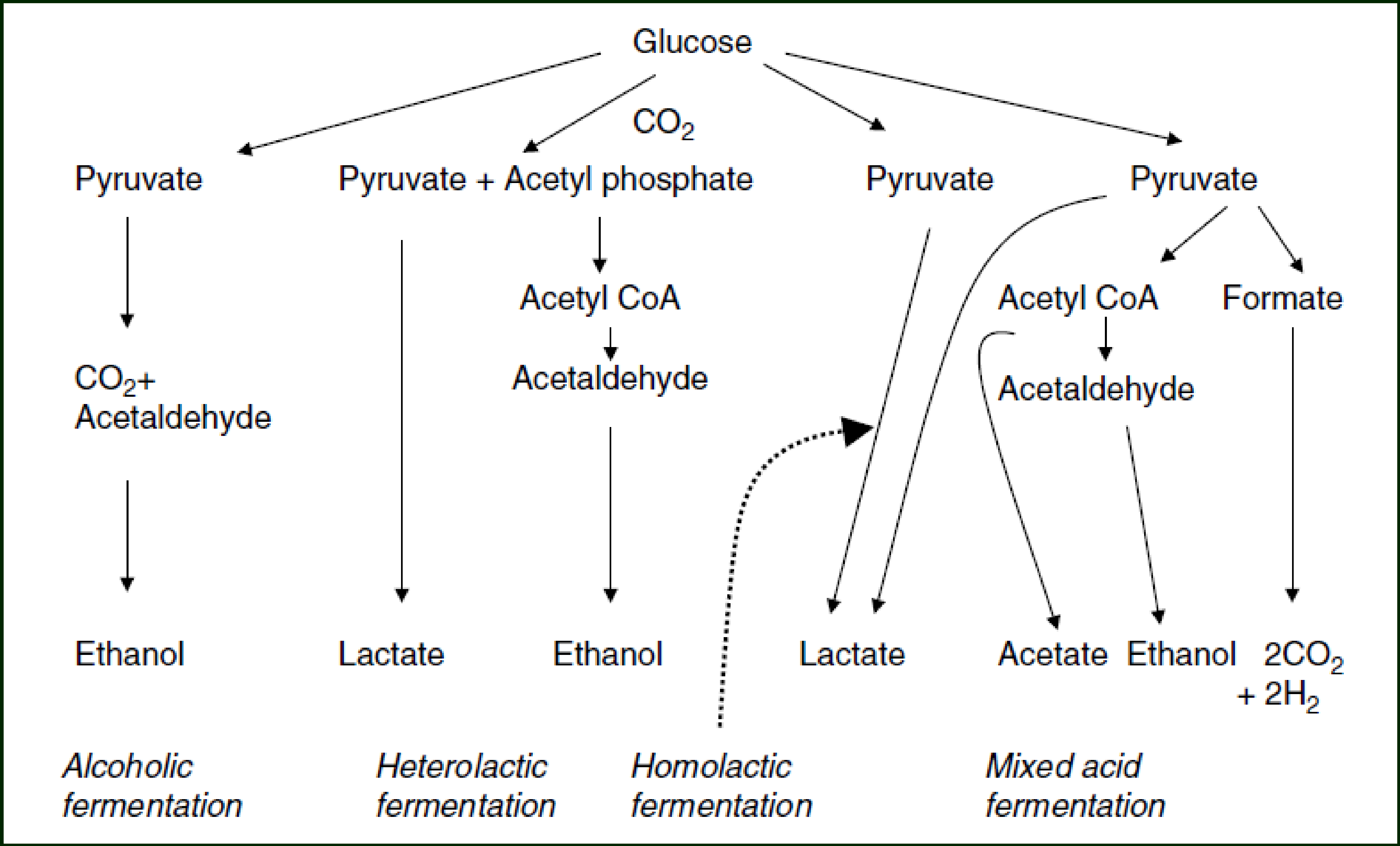


TCA Cycle

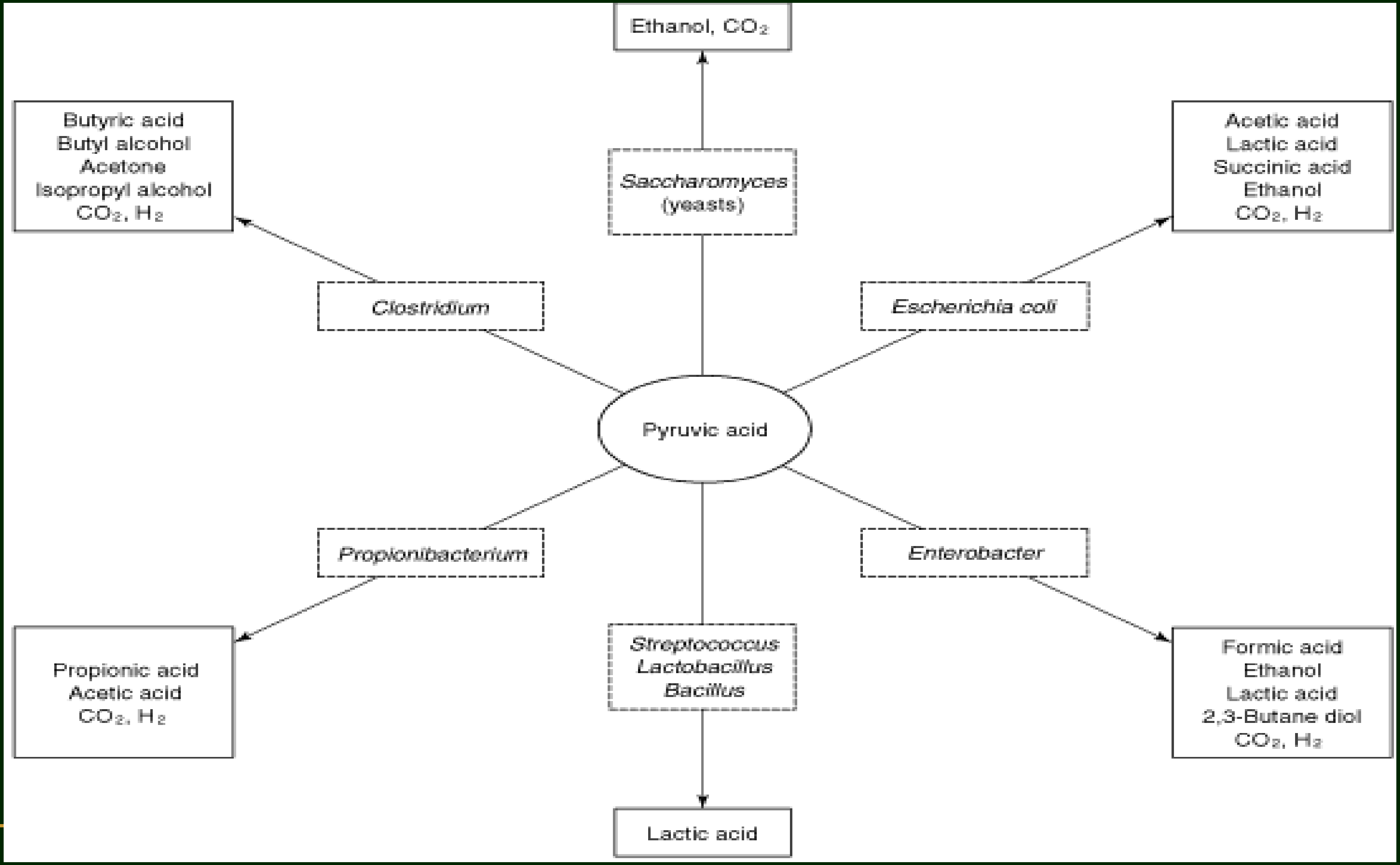


CoA
S
CH3–C + H2O
CoASH
Citrate
cis-Aconitate
Isocitrate
NAD+
NADH + H+ + CO2
α-Keto-glutarate
CoASH
NAD+
NADH + H+ + CO2
Succinyl CoA
C–S–CoA
CoASH
Succinate
FAD
FADH2
Fumarate
H2O
Maltate
NAD+
NADH + H+
Oxaloacetate

Alternative Product End in Fermentation



Overview of Fermentation Products Formed from Pyruvic Acid by Different Microorganism



FERMENTATION TYPE

Fermentation Process

- Natural Fermentation
- Back Slopping
- Controlled Fermentation

Fermentation Types

- Aerobic
- Anaerobic

Fermentation Types

- Acid fermentation
  - Hetero-lactic
  - Homo-lactic

- Alcohol fermentation

Fermentation Types

1. Solid state fermentation
2. Semi-solid state fermentation
3. Submerged fermentation

Fermentation Types

1. Batch fermentation
2. Fed-batch fermentation
3. Continuous fermentation

MICROORGANISM
IN
FERMENTATED FOOD

Microbiological Laboratory Principles

There are 3 principles when using microorganism in microbiological laboratory :

- Viability
    - Purity
        - Productivity

Microorganisms

- Isolate Culture → *pure or mix culture*
- Optimization of microbial growth
- *Product Recovery* :

  Intracellular or Extracellular

Cont'd.

- ***How to get*** ?
  Isolation or buy from *Culture collection.*
- ***How to maintain*** ?
  Deep freeze, lyophilization or adding glycerol as cryo-protectant.
- ***How to improve*** ?
  Optimization fermentation condition or Genetic Engineering techniques.

Cont'd

- *How about the metabolites ?*
  Primary or secondary

  Primary Metabolites : (Logaritmic Phase)
  - ✓ Organic acids
  - ✓ Enzymes
  - ✓ Alcohol, etc

  Secondary Metabolites : (Stationary Phase)
  - ✓ Antibiotic
  - ✓ Bacteriocin
  - ✓ Growth Hormone

Some Microorganisms Involved in Fermentation

Bacteria		Fungi	
Gram negative[a]	Gram positive[a]	Filamentous	Yeasts and non-filamentous fungi
Acetobacter	Arthrobacter	Aspergillus	Brettanomyces
Acinetobacter	Bacillus	Aureobasidium	Candida
Alcaligenes	Bifidobacterium	Fusarium	Cryptococcus
Escherichia	Cellulomonas	Mucor	Debaromyces
Flavobacterium	Corynebacter	Neurospora	Endomycopsis
	Lactobacillus	Penicillium	Geotrichum
Gluconobacter	Lactococcus	Rhizomucor	Hanseniaspora (Kloeckera)
Klebsiella	Leuconostoc	Rhizopus	Hansenula
Methylococcus	Micrococcus	Trichoderma	Kluyveromyces
Methylomonas	Mycoderma		Monascus
Propionibacter	Staphylococcus		Pichia
Pseudomonas	Streptococcus		Rhodotorula
Thermoanaerobium	Streptomyces		Saccharomyces
Xanthomonas			Saccharomycopsis
Zymomonas			Schizosaccharomyces
			Torulopsis
			Trichosporon
			Yarrowia
			Zygosaccharomyces

Microorganism in Fermented Food

- Mold

Aspergillus oryzae hydrolyze starch → sake, soy sauce

Aspergillus niger converse sucrose → citric acid

Rhizopus oryzae produce tempe

- Yeast

Saccharomyces cerevisiae → produce alcohol & CO2, commonly used in bakery product, alcoholic fermentation

- Bacteria

Lactic Acid Bacteria → produce lactic acid & SCFA

Xanthomonas → produce xanthan gum

Acetobacter oxidize ethanol → acetic acid (vinegar)

Microorganism in Fermented Food

Organism	Type of organism	Foodstuff
Aspergillus oryzae	Mould	Miso, soy sauce
Brevibacterium linens	Bacterium	Cheese pigment and surface growth
Lactobacillus casei	Bacterium	Cheese and other fermented dairy products
Lactobacillus curvatus	Bacterium	Sausage
Lactobacillus delbrueckii ssp. *bulgaricus*	Bacterium	Cheese, yoghurt
Lactobacillus helveticus	Bacterium	Cheese and other fermented dairy products
Lactobacillus lactis (various ssp.)	Bacterium	Cheese and other fermented dairy products
Lactobacillus plantarum	Bacterium	Fermented vegetables, sausage
Lactobacillus sakei	Bacterium	Sausage
Lactobacillus sanfranciscensis	Bacterium	Sourdough bread
Leuconostoc lactis	Bacterium	Cheese and other fermented dairy products
Leuconostoc mesenteroides	Bacterium	Fermented vegetables, cheese and other fermented dairy products

Microorganism in Fermented Food

Oenococcus oeni	Bacterium	Wine
Pediococcus acidilactici	Bacterium	Fermented vegetables, sausage
Pediococcus halophilus	Bacterium	Soy sauce
Pediococcus pentosaceus	Bacterium	Sausage
Penicillium camemberti	Mould	Surface ripening of cheese
Penicillium chrysogenum	Mould	sausage
Penicillium roqueforti	Mould	Blue-veined cheeses
Propionibacterium freudenreichii	Bacterium	Eyes in Swiss cheese
Rhizopus microsporus	Mould	Tempeh
Saccharomyces cerevisiae	Fungus	Bread, ale, wine
Saccharomyces pastorianus	Fungus	Lager
Staphylococcus carnosus	Fungus	Meat
Streptococcus thermophilus	Bacterium	Cheese, yoghurt

FERMENTATION PRODUCT

OVERALL PRODUCTS

Fermentation by Microorganisms

- *Amino acids*
- *Nucleic acids*
- *Organic acids*
- *Antibiotics*
- *Biofuels*
- *Enzymes*
- *Hormones*
- *Vitamins*
- *Fermented foods*

Overall Products :

1. Microbial cell (Biomass)
2. Microbial enzymes
3. Microbial metabolite
4. Recombinant products
5. Transformation process

FERMENTED FOOD

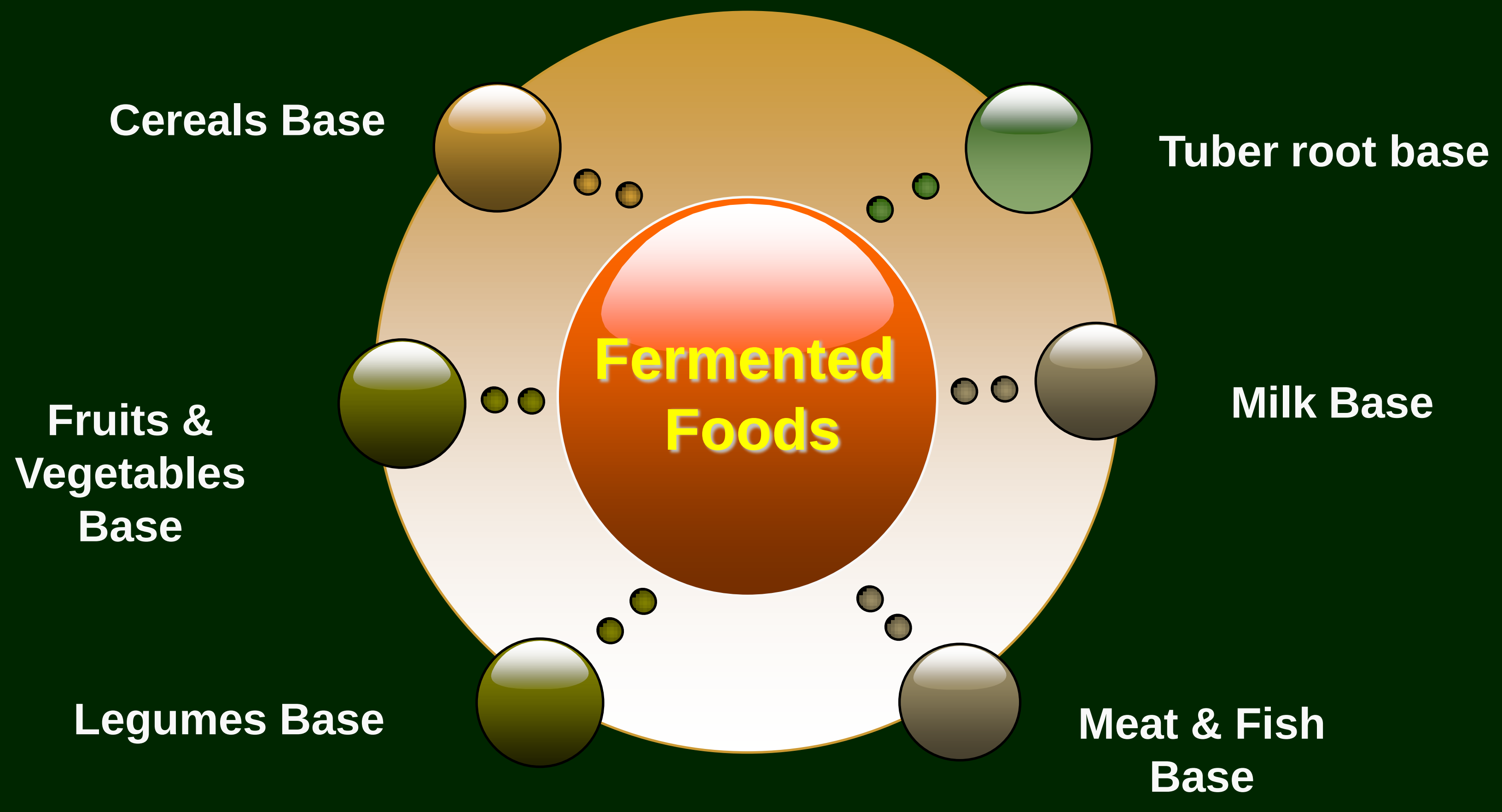


Table 14.1 Fermented Food Groups and Examples

Food Groups	Examples
Dairy products	Cheeses, yogurt, buttermilk, sour cream, dahi, kumiss, kefir, acidophilus milk
Meat products	Salami, pepperoni, chorizo, Thüringer, sausage, pickled meat, nahm
Cereal products	Breads, pancake, crackers, pizza, nun, idli, dosa, sour rice, miso
Fruits and vegetable products	Pickled fruits, pickled vegetables, olives, sauerkraut, kimchi, achar
Legume products	Tofu, fermented soymilk, tempe, soy sauce, koji, mizo, natto, papadam
Fish products	Bagoong, fish sauces, pickled fish, tarama, paak, mamoni, izushi
Beverages	Beer, wine, distilled spirits, coffee, cocoa, tea
Starch crop products	Fermented products from potato, cassava, sweet potato, bananas, plantains, etc.
Miscellaneous products	Fermented eggs, ghee (from fermented cream), vinegar, red palm oil, bongkrek, dage

Source: Adapted from Campbell-Platt, G., *Fermented Foods of the World*, Butterworths, Boston, 1987.

Traditional Products

Product	Bacteria	Yeast/Fungi
Bread, beer, wine	-	Mainly *Saccharomyces cereviciae*
Cheeses,yoghurt & other dairy product	*LAB*	-
Ripening of blue and Chamembert Cheeses	-	*Penicillium* species
Fermented meat & vegetables	Mostly *LAB*	-
Mushrooms	-	*Agaricus bisporus, Lentinula edodes*
Soy sauce	-	*Aspergillus oryzae*
Sufu (Soya bean curd)	-	*Mucor* species
Vinegar	*Acetobacter*	-
Nata	*Acetobacter*	-

Products from Microorganisms

- Various foods and drinks
- Enzymes for varied uses (GM enzymes); biocatalysts
- Engineered proteins (antibodies)
- Vaccines and antibiotics (secondary metabolites)
- Primary metabolites and bulk chemicals (amino acids (glutamic acid) and organic acids (acetic acid)
- Pharmaceuticals and novel chiral chemicals
- Recovery of metals in bioleaching
- Biosensors (use of enzymes to specifically detect chemicals in medical and)

CONDITION
VS
RESOURCES

Conditions versus Resources

- Conditions = factors that influence the growth and survival rates of organisms
- Resources = factors that influence the growth and survival rates of organisms **and can be consumed**

Conditions that Impact Microbial Growth

1. Temperature
2. pH
3. Salinity
4. Radiation
5. Pressure

1. Temperature

Groups	T min (^oC)	T opt (^oC)	T max (^oC)
Psycrofilic	-15	10	20
Psycotroph	-5	25	35
Mesofilic	5-10	30-37	45
Thermoduric/Thermotroph	15	45	50
Thermofilic	40	45-55	60-80
Hyperthermofilic	60	65-80	90

- Arrhenius plot is used to determine the minimum, maximum, and optimal temperature for growth

2. pH (hydrogen ion concentration)

- Microorganisms generally cannot tolerate extreme pH values.
- Under highly alkaline or acidic conditions, some microbial cell components may be hydrolyzed or enzymes may be denatured.
- However, there are acidophilic and alkaliphilic bacteria can tolerate or even require extreme pH conditions for growth.

Microorganism Classification (pH)

Groups	pH min	pH opt	pH max
Acidofilic	1-2	2-4	4-6
Neutrofilic	6-7	7-8,5	9-10
Basidofilic	8,5-9	9,5-11,5	12,5

Organism	Min pH	Opt pH	Max pH
Thermoplasma acidophilus	1.0	1.5	4.0
Nitrosomonas spp.	7.0 – 7.6	8.0 – 8.8	9.4
Bacillus alcalophilus	8.5	9.5	11.5

3. Salinity

- All organisms must deal with osmotic pressure, which results from differences in solute concentrations on opposite sides of a semi-permeable membrane.
- Microorganisms have evolved adaptive mechanisms to permit them to tolerate osmotic pressure within certain ranges.

Salinity cont'd

- Microorganisms that tolerate or require high salt concentrations are called **halotolerant** and **halophilic** respectively.

- These organisms tend to exclude from their cell interiors the high [Na^+] in their surroundings

4. Radiation

The spectrum of electromagnetic radiation is continuous from extremely energetic, short-wavelength gamma rays to long-wavelength low-energy radio waves.

Radiation Cont'd

- Both gamma rays and x-rays are highly penetrating, and their energy levels are destructive to microorganisms.
- Low-level irradiation may cause mutations, and high-exposure doses destroy both nucleic acids and enzymes and kill microorganisms.

Radiation Cont'd

- As is true with other environmental extremes, microorganisms tend to be more tolerant of ionizing radiation than macroorganisms.
- Bacterial endospores are highly resistant to gamma radiation. It takes 0.3 - 0.4 million rads (Mrads) to cause a 90% kill, whereas 1/10 of this dose kills most vegetative bacteria.

5. Pressure

- Atmospheric pressure reflects the weight of the air column.
- Hydrostatic pressure reflects the weight of a column of water (discuss more later)
- Microorganisms have adaptations that allow them to survive (thrive) in high pressure environments

THANK YOU

TUGAS KELOMPOK

- Buatlah makalah tentang produk pangan fermentasi (1 klp @ 4 orang).
- Format makalah :
  - Huruf Arial ukuran 11 point
  - Kertas A4
  - Margin (kiri 3 cm, kanan, atas dan bawah @ 2 cm)
  - Spasi 1,5
  - Jumlah halaman maksimal 20

TUGAS KELOMPOK

Topik makalah (Pilih salah satu)

- Pangan fermentasi berbasis sayuran : Pickle, sauerkraut)
- Pangan fermentasi berbasis buah : wine
- Pangan fermentasi berbasis susu : keju, yoghurt, sour cream, buttermilk
- Pangan fermentasi berbasis daging dan ikan : fermented sausage, hams

TUGAS KELOMPOK

- Pangan fermentasi berbasis serealia : bir, angkak, roti
- Pangan fermentasi berbasis kacang-kacangan : tempe, tofu, tauco, kecap
- Kisi-kisi makalah :
- Pendahuluan (latar belakang, definisi produk, keunggulan)
- Isi : bahan baku (komposisi fisiko kimia), mikroorganisme yang berperan

TUGAS KELOMPOK

- Proses pembuatan (penyiapan starter, *pre-treatment* bahan baku, inokulasi, tahapan fermentasi, pemanenan, pengemasan, penyimpanan)
- Kontrol kualitas dan karakteristik produk akhir
- Inovasi dan prospek produk di masa depan
- Makalah dikumpulkan hari Jumat, 7 Januari 2010 (Waktu UAS Mikrobiologi Pangan)