Microbiology

Unit 4 · Lesson 3 · Microbial metabolism

The notes for Lesson 3 of Unit 4: chapters 4.5 to 4.6 of the unit book, with their figures, worked examples, exam traps and quizzes, in one file. The full unit book, with every chapter, the exam question bank and the glossary, is linked at the end.

Lesson 3 of 4 | 2 chapters | Chapters 4.5 to 4.6

About these notes

These are the notes for **Lesson 3 · Microbial metabolism** of the University free n easy video lessons on Microbiology Unit 4, Microbial Nutrition, Growth and Genetics. They are chapters 4.5 to 4.6 of the unit book, unchanged, so a page here is the same page in the full book.

4.5 Microbial metabolism: aerobic and anaerobic pathways

Metabolism is the sum of a cell's chemical reactions. **Catabolism** breaks food down to release energy, trapped as ATP, and electrons, carried as NADH. **Anabolism** (biosynthesis) spends that ATP and those electrons to build cell parts. A chemoheterotroph such as E. coli breaks glucose to pyruvate, and then what happens depends on whether oxygen, another electron acceptor, or none at all is available. The three answers are aerobic respiration, anaerobic respiration and fermentation.

You need: glycolysis and the TCA cycle from Biochemistry; redox potential means how strongly a substance pulls electrons.

Glucose to pyruvate: three routes

- **Embden–Meyerhof–Parnas (EMP) pathway**, ordinary glycolysis: glucose → 2 pyruvate, net 2 ATP and 2 NADH. ATP here is made by **substrate-level phosphorylation**: a phosphate is passed directly from a high-energy intermediate to ADP.
- **Entner–Doudoroff (ED) pathway**, used by Pseudomonas and Zymomonas among others: glucose is oxidised to 6-phosphogluconate, then split by KDPG aldolase. It gives 1 ATP, 1 NADH and 1 NADPH per glucose.

- **Pentose phosphate pathway**: runs alongside EMP. Its main job is not ATP but NADPH (for biosynthesis) and sugar building blocks, ribose-5-phosphate for nucleotides and erythrose-4-phosphate for aromatic amino acids.

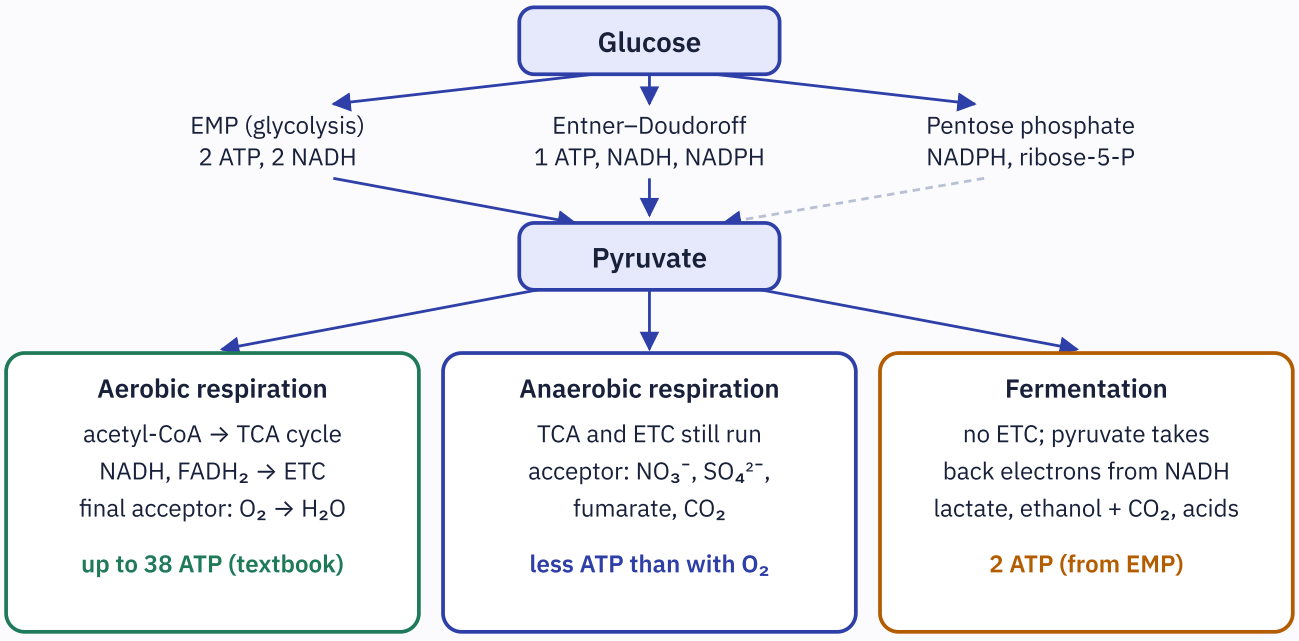


Figure 4.5.1 The main routes of glucose catabolism in bacteria. The three routes meet at pyruvate. What happens next is set by the electron acceptor: oxygen, another inorganic or organic acceptor, or an internal organic molecule (fermentation). ATP yield falls from left to right.

Aerobic respiration

Pyruvate is oxidised to acetyl-CoA (releasing CO_2 and NADH), and acetyl-CoA enters the **TCA cycle**, which oxidises it fully to CO_2 while filling NADH and $FADH_2$. These carriers hand electrons to the **electron transport chain** (ETC). In bacteria the ETC sits in the plasma membrane, since there are no mitochondria. As electrons pass to O_2, protons are pumped out of the cell, and the proton gradient (the **proton motive force**) drives ATP synthase. This is **oxidative phosphorylation**.

The classic textbook count for one glucose in a prokaryote is 38 ATP: 4 by substrate-level phosphorylation, and 34 from 10 NADH (× 3) and 2 $FADH_2$ (× 2). With the modern values of about 2.5 ATP per NADH and 1.5 per $FADH_2$ the total is about 32. Real bacteria often get less because their chains pump fewer protons. Exams usually accept 38 for prokaryotes; say which convention you use.

Anaerobic respiration

Some bacteria run the ETC with a final electron acceptor other than O_2. Because these acceptors pull electrons less strongly, less energy is released and less ATP made. Examples:

- Nitrate (NO_3^-) → nitrite → N_2: **denitrification** by Pseudomonas and Paracoccus; E. coli stops at nitrite.
- Sulfate (SO_4^{2-}) → H_2S: **sulfate reducers** such as Desulfovibrio, which blacken mud and corrode iron pipes.
- CO_2 → CH_4: **methanogens**, which are archaea, in biogas plants and the rumen.
- Fumarate → succinate: E. coli without oxygen.

Organisms are grouped by their oxygen use: **obligate aerobes** need O_2; **facultative anaerobes** (E. coli, yeast) respire with O_2 and ferment or respire anaerobically without it; **obligate anaerobes** (Clostridium, methanogens) are harmed by O_2; **microaerophiles** (Campylobacter) need a little.

Fermentation

In **fermentation** there is no ETC. Its only purpose is to reoxidise NADH to NAD^+ so that glycolysis can keep going; an organic molecule made from pyruvate takes the electrons. ATP comes only from glycolysis: 2 ATP per glucose by EMP. Fermentations are named after their products:

- **Homolactic**: pyruvate → 2 lactate (Lactobacillus, Lactococcus: curd, yogurt).
- **Heterolactic**: lactate + ethanol + CO_2 through the phosphoketolase pathway, 1 ATP (Leuconostoc: idli and dosa batter rise partly from this CO_2).
- **Alcoholic**: pyruvate → acetaldehyde + CO_2 → ethanol (Saccharomyces, Zymomonas: toddy, beer, bread).
- **Mixed acid**: lactate, acetate, succinate, formate, ethanol, H_2 and CO_2 (E. coli; methyl red test positive). **Butanediol** fermentation (Enterobacter; Voges–Proskauer test positive) gives neutral products.
- **Butyric and acetone–butanol**: Clostridium species; the Weizmann process made acetone this way.

Biosynthesis draws its precursors from the same pathways. Pyruvate, acetyl-CoA, oxaloacetate and α-ketoglutarate feed amino acid, lipid and nucleotide synthesis, which makes the TCA cycle **amphibolic** (both catabolic and anabolic). Reactions that refill drained cycle intermediates, such as pyruvate carboxylation to oxaloacetate, are **anaplerotic**. Bacteria growing on acetate use the **glyoxylate cycle** to make sugars from two-carbon units.

WORKED EXAMPLE 1: ENERGY FROM DIFFERENT ACCEPTORS

Question. Standard reduction potentials (E°′) are: $NAD^+/NADH$ −0.32 V, O_2/H_2O +0.82 V, NO_3^-/NO_2^- +0.42 V. Calculate ΔG°′ for passing two electrons from NADH to O_2 and to nitrate, and explain why aerobic respiration gives more ATP. (F = 96.5 kJ per V per mol.) (5 marks)

1. ΔG°′ = −n F ΔE°′, where n = electrons moved (2) and ΔE°′ = E°′(acceptor) − E°′(donor).
2. To O_2: ΔE°′ = 0.82 − (−0.32) = 1.14 V; ΔG°′ = −2 × 96.5 × 1.14 = −220 kJ/mol.
3. To nitrate: ΔE°′ = 0.42 − (−0.32) = 0.74 V; ΔG°′ = −2 × 96.5 × 0.74 = −143 kJ/mol.
4. Oxygen releases about 1.5 times more free energy per NADH, so more protons are pumped and more ATP made. Facultative organisms use O_2 first when it is present.

WORKED EXAMPLE 2: ATP BALANCE

Question. How many glucose molecules must Lactococcus ferment to make as much ATP as E. coli makes from one glucose by aerobic respiration (textbook values)? (3 marks)

1. Aerobic respiration: 38 ATP per glucose. Homolactic fermentation: 2 ATP per glucose.
2. 38 ÷ 2 = 19 glucose molecules.
3. That is why fermenters use sugar fast and pile up products (lactic acid in curd), which industry then harvests.

EXAM TRAPS

- Fermentation is not "anaerobic respiration": fermentation has no ETC and no external acceptor.
- Substrate-level phosphorylation (in glycolysis and the TCA cycle) differs from oxidative phosphorylation (ATP synthase driven by a proton gradient).
- In prokaryotes the ETC is in the plasma membrane; writing "mitochondria" loses marks.
- Methanogens are archaea, not bacteria.

CHECK YOURSELF

– REMEMBER **Give the net yield of the EMP and ED pathways per glucose.** 2 marks

EMP: 2 ATP, 2 NADH. ED: 1 ATP, 1 NADH, 1 NADPH.

– REMEMBER **Name three final electron acceptors used in anaerobic respiration.** 2 marks

Nitrate, sulfate, CO_2 (also fumarate, Fe^{3+}).

– UNDERSTAND **Why must fermenting cells regenerate NAD^+?** 2 marks

Glycolysis needs NAD^+ to accept electrons at the glyceraldehyde-3-phosphate step; without regeneration it stops and so does ATP supply.

– UNDERSTAND **Why is the TCA cycle called amphibolic?** 2 marks

It oxidises acetyl-CoA for energy and also supplies precursors (oxaloacetate, α-ketoglutarate) for biosynthesis.

– APPLY **$\Delta G^{\circ\prime}$ for NADH (−0.32 V) to fumarate/succinate (+0.03 V)?** 2 marks

$\Delta E^{\circ\prime} = 0.35$ V; $\Delta G^{\circ\prime} = -2 \times 96.5 \times 0.35 = -68$ kJ/mol.

– APPLY **A culture is methyl red positive and VP negative. Which fermentation?** 2 marks

Mixed acid fermentation, typical of E. coli.

– ANALYZE **Yeast in a sealed sugar solution makes ethanol, but stirred with air it grows far more and makes little ethanol. Explain.** 4 marks

With O_2 yeast respires, getting much more ATP per glucose, so more biomass forms and pyruvate goes to the TCA cycle. Without O_2 it ferments: 2 ATP per glucose, and NADH is reoxidised by reducing acetaldehyde to ethanol. (At high sugar levels yeast can ferment even with air, the Crabtree effect.)

BEYOND THE SYLLABUS

Metabolic engineering redirects these pathways: E. coli strains are built to make succinate, isobutanol or 1,3-propanediol, and flux balance analysis models every reaction in a genome-scale network. Electroactive bacteria such as Geobacter respire onto electrodes, which is the basis of microbial fuel cells. Search "flux balance analysis COBRA" and "extracellular electron transfer". This is not for the exam.

4.6 Photophosphorylation and

secondary metabolism in microbes

Phototrophic microbes make ATP with light. **Photophosphorylation** uses light energy to drive electrons through a membrane chain, which pumps protons; ATP synthase then makes ATP as in respiration (see 4.5). The second half of this chapter turns to **secondary metabolism**: compounds such as antibiotics and pigments that a cell does not need to grow but makes mainly after growth slows. The other universities leave them to Biochemistry or Industrial Microbiology.

Cyclic and non-cyclic photophosphorylation

Light is captured by chlorophyll (cyanobacteria) or bacteriochlorophyll (purple and green bacteria) in a **reaction centre**. An excited electron leaves the reaction centre and passes along carriers such as quinones and cytochromes.

- **Non-cyclic** (oxygenic, in cyanobacteria): two photosystems in series. Photosystem II splits water, releasing O_2; electrons flow through the cytochrome b_6f complex to photosystem I and finally reduce $NADP^+$ to NADPH. Both ATP and NADPH are made.
- **Cyclic**: electrons from photosystem I return to the cytochrome complex and back to photosystem I. Only ATP is made, no NADPH and no O_2. Cells use it to top up ATP.
- **Anoxygenic** photosynthesis (purple and green sulfur bacteria, heliobacteria): only one photosystem, and electrons cycle, so ATP comes by cyclic photophosphorylation. Water is not split and no O_2 forms. For reducing power they take electrons from H_2S, H_2 or organic compounds, which is why purple sulfur bacteria deposit sulfur granules.

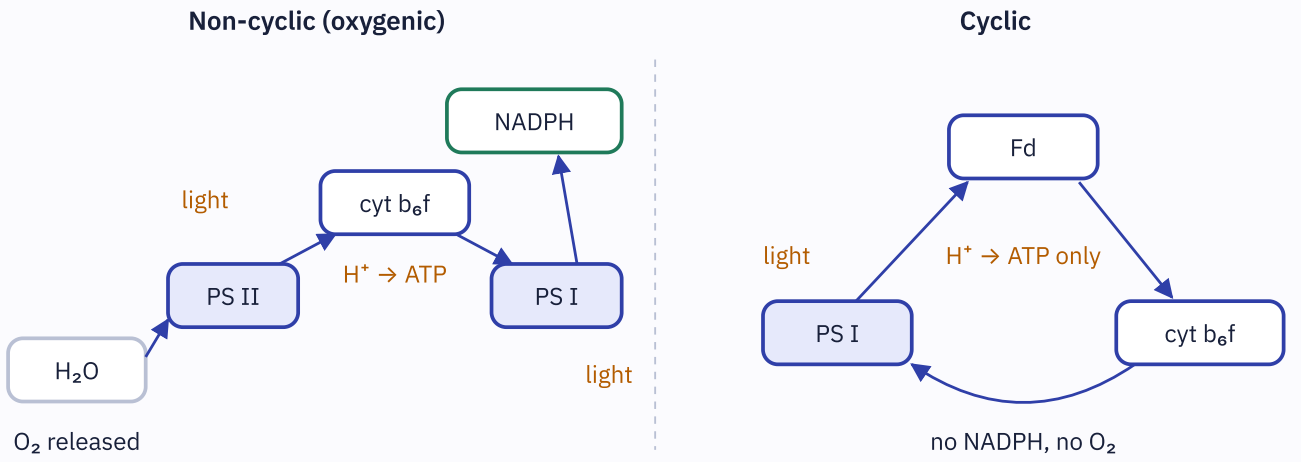


Figure 4.6.1 Electron flow in cyanobacterial photophosphorylation. Left, non-cyclic: water is the electron donor, NADPH is the end point, and O_2 is a by-product. Right, cyclic: electrons return to photosystem I, so only the proton gradient and ATP are produced. Anoxygenic bacteria work like the right panel with one photosystem (Fd = ferredoxin).

Primary and secondary metabolism

Primary metabolites are needed for growth and are made during the log phase: amino acids, nucleotides, ethanol, organic acids. **Secondary metabolites** are not needed for growth, are often species-specific, and are usually made in the late log and stationary phase. Industry calls the growth phase the **trophophase** and the production phase the **idiophase**.

Examples: penicillin (Penicillium chrysogenum), streptomycin (Streptomyces griseus), and many other antibiotics from Streptomyces; pigments such as prodigiosin (Serratia); toxins such as aflatoxin (Aspergillus flavus); and immunosuppressants such as cyclosporin (Tolypocladium). Secondary metabolites are made from primary building blocks (acetyl-CoA, amino acids) by long enzyme pathways, often encoded together in a **biosynthetic gene cluster**.

Production is switched on by signals of slowing growth. Rich, quickly used carbon such as glucose represses many antibiotic pathways, so penicillin fermentations feed lactose or sugar slowly. Phosphate and nitrogen limitation, and quorum sensing (cell density signals), also trigger production.

WORKED EXAMPLE

Question. A purple sulfur bacterium grows in light with H_2S and CO_2 but no O_2 forms. (a) Name its nutritional type. (b) How does it make ATP and reducing power? (c) Why does it not release O_2? (4 marks)

1. Energy from light, electrons from H_2S (inorganic), carbon from CO_2: photolithoautotroph (see 4.1).
2. ATP: cyclic photophosphorylation through one bacteriochlorophyll photosystem. Reducing power: electrons from H_2S, which is oxidised to sulfur.
3. It has no photosystem II and cannot split water, so no O_2 forms; this is anoxygenic photosynthesis.

EXAM TRAPS

- Cyclic flow makes only ATP; non-cyclic makes ATP and NADPH.
- Only cyanobacteria (among bacteria) make O_2; purple and green bacteria are anoxygenic.
- Secondary does not mean unimportant: antibiotics are secondary metabolites.
- Ethanol and citric acid are primary metabolites, even though they are big industrial products.

CHECK YOURSELF

- REMEMBER **Define photophosphorylation.** 1 mark

  ATP synthesis driven by light-powered electron flow and the resulting proton gradient.

- REMEMBER **Name two secondary metabolites and their producers.** 2 marks

  Penicillin from Penicillium chrysogenum; streptomycin from Streptomyces griseus.

- UNDERSTAND **Distinguish trophophase and idiophase.** 2 marks

  Trophophase: rapid growth, primary metabolites. Idiophase: slow or no growth, secondary metabolites made.

- APPLY **A cell has excess NADPH but needs ATP. Which flow should it use?** 2 marks

  Cyclic photophosphorylation, which makes ATP without more NADPH.

- ANALYZE **Why does a penicillin fermentation fed glucose all at once give low yield?** 3 marks

  Plentiful glucose keeps cells in fast growth and represses the antibiotic pathway (carbon catabolite repression). Slow feeding or lactose lets cells enter the idiophase.

BEYOND THE SYLLABUS

Genome mining with tools such as antiSMASH finds silent biosynthetic gene clusters that could encode new antibiotics; switching them on is a major research field. Cyanobacteria are being engineered to turn light and CO_2 directly into fuels and chemicals. Search "antiSMASH" and "cyanobacterial biofactories". This is not for the exam.

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Free energy from redox	$\Delta G^{\circ\prime} = -n\ F\ \Delta E^{\circ\prime}$	4.5
ATP per glucose	aerobic up to 38 (textbook); homolactic fermentation 2	4.5
Cyclic / non-cyclic	ATP only / ATP + NADPH + O_2 (oxygenic)	4.6
Idiophase	stationary-phase production of secondary metabolites	4.6

Glossary (terms used in this lesson)

Amphibolic — A pathway that serves both breakdown and synthesis, such as the TCA cycle.

Fermentation — Energy metabolism with no electron transport chain, in which an organic product accepts electrons from NADH.

Idiophase — Production phase of secondary metabolites, after rapid growth.

Oxidative phosphorylation — ATP synthesis driven by a proton gradient made by an electron transport chain.

Photophosphorylation — ATP synthesis driven by light-powered electron flow.

Substrate-level phosphorylation — Direct transfer of phosphate from an intermediate to ADP.

The full unit book

Every chapter of Unit 4, the unit summary, the exam question bank with model answers and the full glossary: Microbiology · Unit 4 · Microbial Nutrition, Growth and Genetics (one file, works offline).

Sources and further reading

Textbooks

- Willey, Sandman and Wood, *Prescott's Microbiology*, McGraw-Hill: microbial nutrition, growth, metabolism and genetics chapters (from memory; check).
- Madigan and others, *Brock Biology of Microorganisms*, Pearson: reduction potentials, anaerobic respiration and phototrophy (from memory; check).
- Pelczar, Chan and Krieg, *Microbiology*, McGraw-Hill (Indian edition widely used) (from memory; check).
- Tortora, Funke and Case, *Microbiology: An Introduction*, Pearson (from memory; check).
- Shuler and Kargi, *Bioprocess Engineering: Basic Concepts*, for Monod and chemostat kinetics (from memory; check).

Open resources

- OpenStax, *Microbiology*, section 9.1 "How Microbes Grow": growth phases, 30–300 plate rule, direct and indirect counts. openstax.org/books/microbiology/pages/9-1-how-microbes-grow
- OpenStax, *Microbiology*, section 11.6 "How Asexual Prokaryotes Achieve Genetic Diversity": Griffith and Avery, Hfr and F′ cells, 100-minute E. coli transfer. openstax.org/books/microbiology/pages/11-6-how-asexual-prokaryotes-achieve-genetic-diversity

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