Microbiology

Unit 6 · Lesson 3 · Biofertilizers, biofilms and fermentation

The notes for Lesson 3 of Unit 6: chapters 6.6 to 6.8 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 3 | 3 chapters | Chapters 6.6 to 6.8

About these notes

These are the notes for **Lesson 3 · Biofertilizers, biofilms and fermentation** of the University free n easy video lessons on Microbiology Unit 6, Medical, Environmental and Industrial Microbiology. They are chapters 6.6 to 6.8 of the unit book, unchanged, so a page here is the same page in the full book.

6.6 Biofertilizers, biopesticides and microbial insecticides

Farmers can buy microbes in a packet. Biofertilizers are live microbes that add nitrogen or make phosphorus and other nutrients available to the crop. Biopesticides are microbes, or products from them, that kill or suppress pests and plant pathogens. Both cut the use of chemical urea and pesticides, which cost money, pollute water and harm useful insects. The star of the second group is *Bacillus thuringiensis*, whose crystal toxin kills caterpillars but not people.

You need: the nitrogen cycle (Chapter 6.4); endospores (Unit 2, Bacterial morphology and cell structure); viruses (Unit 3, General properties, structure and classification of viruses).

Biofertilizers

A **biofertilizer** is a preparation of live cells of a chosen strain, in a carrier (lignite, charcoal) or as a liquid, applied to seed, seedling roots or soil. The main groups:

GROUP	EXAMPLES	CROP OR HABIT
Symbiotic N-fixers	*Rhizobium*, *Bradyrhizobium*	Root nodules of legumes (pulses, groundnut, soybean)
Associative N-fixer	*Azospirillum*	Roots of cereals and millets (rice, maize, sorghum)
Free-living N-fixer	*Azotobacter* (aerobic)	Many non-legume crops
Cyanobacteria	*Anabaena*, *Nostoc*; *Azolla* fern with its *Anabaena* partner	Flooded rice fields
Phosphate solubilisers (PSB)	*Bacillus megaterium*, *Pseudomonas*, *Aspergillus*	Release P from insoluble phosphate
Phosphate mobilisers	Arbuscular mycorrhizal fungi (AMF)	Hyphae extend the root's reach for P and water

All nitrogen fixers use the enzyme **nitrogenase**:

$$N_2 + 8\,H^+ + 8\,e^- + 16\,ATP \rightarrow 2\,NH_3 + H_2 + 16\,ADP + 16\,P_i$$

Breaking the triple bond of N_2 costs 16 ATP per N_2, and nitrogenase is destroyed by oxygen. Fixers protect it in different ways: legume nodules contain **leghaemoglobin**, which binds O_2 and keeps it low around the bacteroids; cyanobacteria fix N_2 in thick-walled **heterocysts** that do not make O_2; *Azotobacter* respires so fast that it uses up oxygen at the cell surface.

Phosphate solubilisers release organic acids (such as gluconic acid) that dissolve calcium phosphate. India's Fertiliser (Control) Order sets the quality standard: carrier-based *Rhizobium*, *Azotobacter*, *Azospirillum* and PSB must have at least 5×10^7 colony-forming units (CFU) per gram, and liquid forms at least 1×10^8 CFU per mL.

Biopesticides and microbial insecticides

***Bacillus thuringiensis* (Bt)** is a Gram-positive, spore-forming soil bacterium. During sporulation it makes a **parasporal crystal** of Cry proteins (δ-endotoxins). Different subspecies kill different insects: *kurstaki* kills caterpillars (Lepidoptera), *israelensis* kills mosquito and blackfly larvae (Diptera), *tenebrionis* kills some beetles (Coleoptera). Bt cotton, grown in India since 2002, carries a *cry* gene so the plant itself kills bollworms.

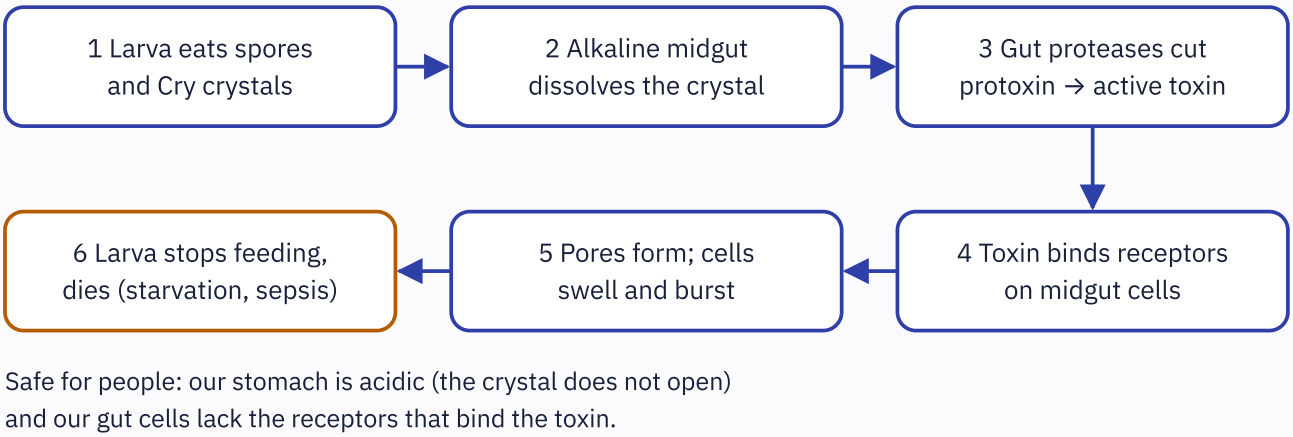


Figure 6.6.1 How Bt kills a caterpillar. Notice that steps 2 to 4 each need a feature of the target insect's gut (alkaline pH, the right proteases, the right receptor); this is why Bt is so specific.

- ***Lysinibacillus sphaericus*** (formerly *Bacillus sphaericus*): makes a binary toxin that kills mosquito larvae, especially *Culex*, and survives better than Bt in polluted water. Bti and *L. sphaericus* are used against mosquito larvae in malaria and filaria control.
- ***Paenibacillus popilliae*** (formerly *Bacillus popilliae*): causes milky disease of Japanese beetle grubs; the grub's blood turns milky with spores. It grows poorly in culture, so spores are produced in live grubs.
- **Baculoviruses**: rod-shaped DNA viruses of insects. In nucleopolyhedroviruses (NPVs) the virions are packed in protein crystals (polyhedra) that dissolve in the alkaline midgut. HaNPV against *Helicoverpa armigera* (gram pod borer) and SlNPV against *Spodoptera litura* are used in India. They are highly host-specific but kill slowly.

Fungal biopesticides, *Beauveria bassiana* and *Metarhizium anisopliae*, enter through the insect cuticle, so they also work on sucking pests that never eat a sprayed surface. *Trichoderma* species attack fungal plant pathogens in soil (mycoparasitism) and are applied as seed treatment.

WORKED EXAMPLE 1 (QUALITY CHECK)

Question (4 marks). 10 g of a carrier-based *Azospirillum* biofertilizer is mixed in 90 mL sterile water (10^{-1} dilution) and diluted tenfold in series. 0.1 mL of the 10^{-6} dilution, spread on three plates, gives 46, 50 and 48 colonies. Does the batch meet the Fertiliser (Control) Order standard?

1. Mean colonies = (46 + 50 + 48) ÷ 3 = 48.
2. CFU per g = colonies ÷ (volume plated × dilution) = 48 ÷ (0.1×10^{-6}) = 4.8×10^{8} CFU/g.
3. Standard: at least 5×10^{7} CFU/g. 4.8×10^{8} is about ten times that, so the batch passes.

WORKED EXAMPLE 2 (ENERGY COST)

Question (3 marks). How many moles of ATP does nitrogenase spend to fix 1 kg of nitrogen?

1. 1 kg N = 1,000 g ÷ 14 g/mol = 71.4 mol N atoms = 35.7 mol N_2.
2. 16 ATP per N_2: 35.7 × 16 = 571 mol ATP.
3. Interpretation: fixation is costly, so the legume pays its rhizobia with sugars, and fixers switch nitrogenase off when ammonium is plentiful.

EXAM TRAPS

- *Rhizobium* is for legumes only; *Azospirillum* and *Azotobacter* are for non-legumes. Using *Rhizobium* on rice is a common wrong answer.
- The Bt toxin is a protein crystal made at sporulation, not an exotoxin secreted during growth, and it needs an alkaline gut to dissolve.
- Phosphate *solubilisers* dissolve insoluble P; mycorrhizae *mobilise* (carry) P to the root. Different words, different mechanisms.
- Write the new names with the old ones in brackets: *Lysinibacillus* (*Bacillus*) *sphaericus*, *Paenibacillus* (*Bacillus*) *popilliae*.

CHECK YOURSELF

– REMEMBER **Name one biofertilizer each for groundnut, rice and maize.** 3 marks

Groundnut: *Rhizobium*; rice: *Azolla–Anabaena* or blue-green algae (also *Azospirillum*); maize: *Azospirillum* or *Azotobacter*.

– REMEMBER **Which Bt subspecies is used against mosquito larvae?** 1 mark

B. thuringiensis subsp. *israelensis* (Bti).

– UNDERSTAND **How do legume nodules protect nitrogenase from oxygen?** 2 marks

Leghaemoglobin binds O_2, keeping free oxygen very low around the bacteroids while still supplying enough for their respiration.

– UNDERSTAND **Why are baculoviruses considered safe for farm workers?** 2 marks

They infect only a narrow range of insect hosts and cannot replicate in vertebrate cells.

– APPLY **0.1 mL of a 10^{-5} dilution of a liquid biofertilizer gives 75 colonies. Find CFU/mL and judge it.** 2 marks

$75 \div (0.1 \times 10^{-5}) = 7.5 \times 10^{7}$ CFU/mL; below the 1×10^{8} CFU/mL standard for liquids, so it fails.

– ANALYZE **A farmer sprays Bt on cotton against whiteflies (sucking insects) and sees no effect. Explain and suggest an alternative.** 4 marks

Bt must be eaten by chewing larvae and activated in their gut; sucking insects take sap and do not ingest the sprayed crystals. A fungus that enters through the cuticle, such as *Beauveria bassiana* or *Lecanicillium*, is a better choice.

BEYOND THE SYLLABUS

This is not for the exam. Pink bollworm in India has evolved resistance to Bt cotton, which has led to refuge planting and stacked toxins. RNA-interference sprays and gene-edited microbes are newer pest-control ideas. Nitrogen-fixing microbes engineered to work on cereal roots are being sold in some countries. Search "Bt resistance pink bollworm", "PGPR consortia" and "nano-urea versus biofertilizers".

6.7 Biofilms

In nature most bacteria do not float alone; they live in **biofilms**, communities stuck to a surface and wrapped in a slime they make themselves. Dental plaque, the slippery coat on a river stone and the film inside a urinary catheter are all biofilms. Living this way protects microbes from drying, antibiotics, disinfectants and the immune system, which is why

biofilm infections are stubborn and why industry spends heavily fighting biofilms in pipes and on ship hulls.

You need: pili, flagella and capsule (Unit 2, Bacterial morphology and cell structure); how antibiotics act (Unit 5, Antibiotics and how they act).

What a biofilm is made of

A biofilm has two parts: the cells, often of several species, and the **extracellular polymeric substance** (EPS), also called the matrix. The EPS is mostly water held in a gel of polysaccharides, proteins, extracellular DNA (eDNA) and some lipids. Most of a biofilm's volume is matrix, not cells. The matrix glues cells to the surface and to each other, holds water and nutrients, and slows the entry of many chemicals. Inside it, gradients form: oxygen and food are plentiful at the surface and scarce at the bottom, so cells in different layers grow at different rates and switch on different genes.

How a biofilm forms

Any wet surface soon gets a thin **conditioning film** of proteins and other molecules from the liquid (for example saliva proteins on teeth, blood proteins on a catheter). Bacteria arrive by swimming or flow and then go through five stages:

1. **Reversible attachment.** Weak forces hold cells near the surface; they can still swim away.
2. **Irreversible attachment.** Pili and adhesins grip the surface, cells lie flat and start making EPS.
3. **Microcolony formation.** Attached cells divide and recruit others; EPS production rises.
4. **Maturation.** The film grows into a three-dimensional structure, often with tower- or mushroom-shaped clusters separated by water channels that carry nutrients in and waste out.
5. **Dispersal.** Cells are released, by enzymes that loosen the matrix or by pieces breaking off, and start new biofilms elsewhere. In an infected catheter this step seeds the bloodstream.

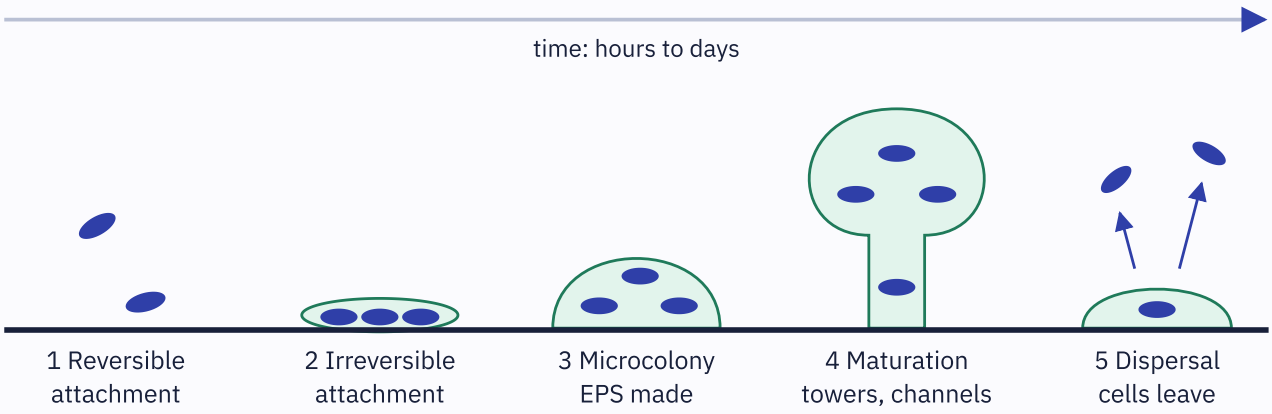


Figure 6.7.1 The five stages of biofilm development. Cells are blue, the EPS matrix green, and the thick line is the surface. Notice that matrix appears at stage 2 and grows with the community, and that dispersal (stage 5) returns cells to the free-swimming state.

Quorum sensing

Bacteria in a biofilm coordinate genes by **quorum sensing**: each cell releases a small signal molecule called an **autoinducer**. When the population is dense, the signal concentration crosses a threshold and switches on shared genes, for EPS, toxins or light. Gram-negative bacteria use **acyl-homoserine lactones** (AHLs), made by a LuxI-type enzyme and sensed by a LuxR-type receptor; the system was found in the glowing marine bacterium *Vibrio fischeri*. Gram-positive bacteria use short **autoinducing peptides** sensed by two-component systems. A third signal, AI-2, is shared across many species.

Why biofilms are hard to kill, and where they matter

- **Slow penetration:** the matrix binds or slows some drugs (positively charged aminoglycosides stick to negatively charged eDNA), and enzymes such as β-lactamase in the matrix destroy others on the way in.
- **Slow growth:** starved cells deep in the film barely grow, and most antibiotics act on growing cells.
- **Persister cells:** a few dormant cells survive almost any dose and regrow afterwards.
- **Gene exchange:** close packing speeds plasmid transfer, spreading resistance genes.

This is **tolerance**, not genetic resistance: cells shaken out of the biofilm are often killed by normal doses again. In medicine, biofilms cause infections of catheters, prosthetic joints, heart valves and contact lenses (often *Staphylococcus epidermidis* and *S. aureus*), dental caries (*Streptococcus mutans* in plaque), and chronic lung infection by *Pseudomonas aeruginosa* in cystic fibrosis. Often the only cure is removing the device. In industry, biofilms foul ship hulls, heat exchangers and water-purifier membranes, and sulphate-reducing bacteria such as *Desulfovibrio* cause microbially influenced corrosion of steel pipelines. Useful biofilms run

trickling filters and biofilm reactors in sewage plants (Chapter 6.4). Control combines prevention (coated or silver-impregnated catheters, smooth surfaces), mechanical removal (brushing teeth, scraping pipes) and newer tools such as matrix-degrading enzymes and quorum-sensing inhibitors.

WORKED EXAMPLE

Question (5 marks). Four catheter isolates are tested by the crystal violet microtitre assay. The negative control wells (broth only) give a mean OD_{570} of 0.080 with standard deviation 0.010. The isolates give mean ODs of K1 = 0.095, K2 = 0.180, K3 = 0.350, K4 = 0.620. Classify each using the cut-off method (Stepanović criteria).

1. Cut-off ODc = mean of negative control + 3 × SD = 0.080 + 3 × 0.010 = 0.110.
2. Bands: non-producer if OD ≤ ODc (0.110); weak if ≤ 2 × ODc (0.220); moderate if ≤ 4 × ODc (0.440); strong if above 0.440.
3. K1: 0.095 ≤ 0.110 → non-producer. K2: 0.180 is between 0.110 and 0.220 → weak.
4. K3: 0.350 is between 0.220 and 0.440 → moderate. K4: 0.620 > 0.440 → strong.

Answer: K1 non, K2 weak, K3 moderate, K4 strong biofilm producer. K4 is the isolate most likely to persist on the catheter.

EXAM TRAPS

- Biofilm tolerance is reversible and not caused by a resistance gene; do not write "biofilm bacteria are mutants".
- EPS is made by the microbes themselves; the conditioning film comes from the surrounding liquid.
- AHLs are Gram-negative signals; peptides are Gram-positive signals.
- Crystal violet stains the whole biomass (cells and matrix), not only live cells.
- Favourite question: "Describe the stages of biofilm formation with a diagram and explain its clinical significance."

CHECK YOURSELF

– REMEMBER **List four components of the biofilm matrix.** 2 marks

Polysaccharides, proteins, extracellular DNA, lipids (all in a mostly water gel).

– REMEMBER **What is an autoinducer?** 1 mark

A small signal molecule released by bacteria whose concentration reports population density in quorum sensing.

– UNDERSTAND **Why do cells at the base of a thick biofilm survive penicillin?** 3 marks

Little oxygen and food reach them, so they grow very slowly; penicillin acts on cell-wall synthesis in growing cells, so non-growing cells are spared. The matrix may also slow the drug or hold β-lactamase.

– APPLY **Negative control 0.070 ± 0.010 SD; an isolate reads 0.300. Classify it.** 2 marks

ODc = 0.100; 2ODc = 0.200; 4ODc = 0.400. 0.300 lies between 0.200 and 0.400 → moderate producer.

– APPLY **Suggest two ways to lower catheter biofilm infections in a ward.** 2 marks

Remove catheters as soon as they are not needed; use antimicrobial (silver or antibiotic) coated catheters with aseptic insertion.

– ANALYZE **A patient's joint-implant infection relapses after each antibiotic course, yet the isolate tests sensitive in the lab. Explain.** 4 marks

Lab tests use free-floating cells (MIC), but on the implant the bacteria live in a biofilm: the matrix, slow growth and persisters let them survive doses that kill planktonic cells. Dispersed cells cause each relapse. The implant usually has to be removed or replaced.

BEYOND THE SYLLABUS

This is not for the exam. Labs now report the minimum biofilm eradication concentration (MBEC) alongside the MIC for device infections. The signal c-di-GMP inside cells works like a switch between swimming and biofilm life and is a drug target. Phage therapy and enzyme cocktails that dissolve the matrix are being tested on chronic wounds. Search "MBEC assay", "quorum quenching" and "c-di-GMP".

6.8 Industrial and food fermentations: alcohol, enzymes, bioplastics

Humans used microbes to make curd, bread, toddy and idli long before anyone knew microbes existed. Industrial microbiology does the same thing on purpose and at scale: a chosen strain is grown in a large vessel (a fermenter or bioreactor) on a cheap raw material and makes a product worth more than the raw material, such as ethanol, an enzyme or a biodegradable plastic.

You need: glycolysis and fermentation pathways (Unit 4, Microbial metabolism: aerobic and anaerobic pathways); secondary metabolism (Unit 4, Photophosphorylation and secondary metabolism in microbes); the growth curve (Unit 4, Bacterial growth curve and growth kinetics); food spoilage (Unit 5, Microbial spoilage of food and its control).

Two meanings of fermentation

In **biochemistry**, fermentation is energy metabolism without an external electron acceptor: an organic molecule made from the sugar (pyruvate or acetaldehyde) accepts the electrons, and ATP comes only from substrate-level phosphorylation. In **industry**, "fermentation" means any large-scale culture of microbes, even a fully aerobic one such as penicillin production. A process has three parts: **upstream** (strain, medium, sterilisation, inoculum), the **fermentation** itself (batch, fed-batch or continuous) and **downstream processing** (separating and purifying the product).

A production strain is chosen for high yield, fast growth on cheap media, tolerance of its own product (ethanol, acid), genetic stability, easy separation from the product and safety (GRAS, "generally recognised as safe"). Dairy starters must also resist bacteriophages, because a phage attack can stop a cheese vat.

Food fermentations

Lactic acid bacteria (LAB) are Gram-positive, non-spore-forming, acid-tolerant bacteria that ferment sugar to lactic acid. **Homofermentative** LAB (such as *Lactococcus lactis*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii*) use glycolysis (the EMP pathway) and make almost only lactate: 2 lactate and 2 ATP per glucose. **Heterofermentative** LAB (such as *Leuconostoc mesenteroides*) use the phosphoketolase pathway and make lactate, ethanol (or acetate) and CO_2, with 1 ATP per glucose. The acid preserves the food and gives sour taste; the CO_2 of heterofermenters puffs up batters. A **starter culture** is a known, active culture added in large numbers so that the right microbes dominate quickly and every batch tastes the same.

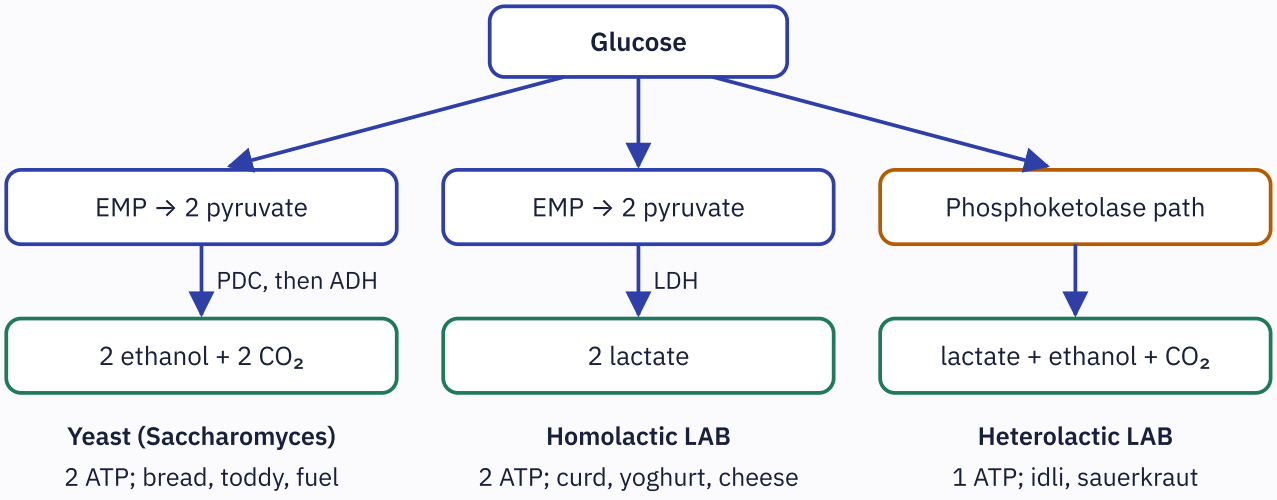


Figure 6.8.1 Three fermentations of one glucose molecule. PDC is pyruvate decarboxylase, ADH alcohol dehydrogenase, LDH lactate dehydrogenase. Notice that yeast and homolactic bacteria share glycolysis and differ only in the last step, while heterolactic bacteria take a different route and gain half the ATP.

FOOD	MAIN MICROBES	WHAT THEY DO
Yoghurt	*Streptococcus thermophilus* + *Lactobacillus delbrueckii* subsp. *bulgaricus*	Acid sets the milk protein; the two help each other grow; flavour (acetaldehyde)
Cheese	*Lactococcus lactis* starter; *Penicillium roqueforti* (blue), *P. camemberti* (white rind)	Acid and rennet (chymosin) form the curd; moulds ripen it
Bread	*Saccharomyces cerevisiae*	CO_2 raises the dough; ethanol bakes off
Idli, dosa	*Leuconostoc mesenteroides*, other LAB, wild yeasts	CO_2 leavens the rice–black gram batter overnight; acid gives sourness
Soy sauce, tempeh	*Aspergillus oryzae* (koji), then LAB and yeasts; *Rhizopus* for tempeh	Mould enzymes break soy protein and starch
Sauerkraut, pickles	*Leuconostoc* first, then *Lactobacillus plantarum*	Succession of LAB as acid rises
Fermented sausages	*Pediococcus*, *Lactobacillus*	Acid preserves and binds the meat

Alcohol

Yeast (*Saccharomyces cerevisiae*) converts glucose to pyruvate by glycolysis; **pyruvate decarboxylase** removes CO_2 to give acetaldehyde, and **alcohol dehydrogenase** reduces acetaldehyde to ethanol, regenerating the NAD^+ that glycolysis needs.

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C6H12O6  →  2 C2H5OH  +  2 CO2
180.16 g     2 × 46.07 g    2 × 44.01 g
Theoretical yield = 92.14 ÷ 180.16 = 0.511 g ethanol per g glucose
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In India most fuel ethanol is made from sugarcane molasses (and increasingly from grain and cane juice) and blended into petrol. Yeast is grown aerobically first to build cell mass, then kept anaerobic to make ethanol, which is distilled. Real plants get about 90% of the theoretical yield because some sugar becomes yeast cells and glycerol. The bacterium *Zymomonas mobilis* also makes ethanol, through the Entner–Doudoroff pathway.

Enzymes

Microbial enzymes are made by submerged or solid-state fermentation and are cheaper and more stable than plant or animal enzymes. Key examples: thermostable **α-amylase** from *Bacillus licheniformis* and **glucoamylase** from *Aspergillus niger* turn starch into glucose; alkaline **proteases** from *Bacillus* go into detergents; **glucose isomerase** (from *Streptomyces*) makes high-fructose syrup; **pectinases** from *Aspergillus* clear fruit juice; **cellulases** from *Trichoderma reesei* help make ethanol from crop waste; recombinant **chymosin** from microbes replaces calf rennet in cheese.

Bioplastics and biopolymers

Polyhydroxyalkanoates (PHAs), chiefly **poly-3-hydroxybutyrate** (PHB), are polyesters that bacteria store as granules when carbon is plentiful but nitrogen or phosphorus is limiting. *Cupriavidus necator* (formerly *Ralstonia eutropha*) is the classic producer; it is grown first with all nutrients to make cells, then starved of nitrogen with excess sugar so the cells fill with PHB. PHB is fully biodegradable but costs more than petroleum plastic. **Polylactic acid** (PLA) is made by chemically polymerising lactic acid produced by LAB fermentation.

WORKED EXAMPLE

Question (6 marks). A distillery ferments 1,000 kg of molasses containing fermentable sugars equal to 50% glucose by mass. Fermentation efficiency is 90%. Calculate (a) the theoretical ethanol, (b) the actual ethanol in kg and litres (density 0.789 kg/L) and (c) the theoretical CO_2 released.

1. Fermentable sugar = 0.50 × 1,000 = 500 kg glucose.
2. (a) Theoretical ethanol = 500 × 0.511 = 255.7 kg.
3. (b) Actual ethanol = 0.90 × 255.7 = 230.1 kg; volume = 230.1 ÷ 0.789 = 291.7 L, about 292 L.
4. (c) CO_2 per g glucose = 88.02 ÷ 180.16 = 0.489; theoretical CO_2 = 500 × 0.489 = 244.3 kg.
5. Check: 255.7 + 244.3 = 500 kg, so mass balances.

Answer: 255.7 kg theoretical; about 230 kg or 292 L actual; 244 kg CO_2 (which breweries and distilleries can capture and sell).

EXAM TRAPS

- The theoretical ethanol yield is 0.511 g/g, not 1 mol per mol: one glucose gives two ethanol.
- Heterofermentative LAB make 1 ATP per glucose, homofermentative make 2. Idli rises because of heterofermentative CO_2, not mainly yeast.
- PHB is made under nutrient limitation with excess carbon, not during balanced growth.
- Industrial "fermentation" need not be anaerobic; say which meaning you use.
- Favourite question: "Compare homo- and heterolactic fermentation" and "Describe the industrial production of ethanol with a flow sheet".

CHECK YOURSELF

– REMEMBER **Name the two bacteria of yoghurt.** 2 marks

Streptococcus thermophilus and *Lactobacillus delbrueckii* subsp. *bulgaricus*.

– REMEMBER **Name a microbe and a use for amylase, protease and pectinase.** 3 marks

α-Amylase, *Bacillus licheniformis*, starch processing; protease, *Bacillus*, detergents; pectinase, *Aspergillus*, juice clarification.

– UNDERSTAND **Why are starter cultures used instead of natural fermentation in dairies?** 3 marks

A large dose of known, active strains acidifies quickly, outgrows spoilage and pathogenic microbes and gives the same flavour and texture in every batch.

– UNDERSTAND **Why does a PHB process have two phases?** 2 marks

Cells are first grown with all nutrients to make biomass; then nitrogen or phosphorus is limited with excess carbon so the cells store carbon as PHB granules.

– APPLY **How much glucose is needed for 100 kg ethanol at 85% efficiency?** 3 marks

Theoretical needed: 100 ÷ 0.511 = 195.7 kg; at 85%: 195.7 ÷ 0.85 = 230.2 kg glucose.

– ANALYZE **Idli batter left in a cold Delhi winter night does not rise, while in Chennai it does. Explain.** 3 marks

The LAB and yeasts of the batter grow slowly at low temperature, so little CO_2 and acid form overnight; Chennai's warmth gives faster fermentation. Keeping the batter warm fixes it.

BEYOND THE SYLLABUS

This is not for the exam. Precision fermentation uses engineered yeasts and fungi to make milk proteins, sweeteners and fragrances without animals or plants. Second-generation ethanol plants in India use cellulases to ferment rice straw, which could also reduce stubble burning. Metabolic engineering and genome-scale models are used to raise PHA and lactic acid yields. Search "precision fermentation", "2G ethanol Panipat" and "PHA cost reduction".

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
Nitrogenase	$N_2 + 8H^+ + 8e^- + 16\ ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16\ P_i$	6.6
Biofertilizer standard (FCO)	$\geq 5 \times 10^7$ CFU/g (carrier), $\geq 1 \times 10^8$ CFU/mL (liquid)	6.6
CFU per g or mL	colonies ÷ (volume plated × dilution)	6.6
Biofilm cut-off (crystal violet)	ODc = mean negative + 3 SD; bands at ODc, 2ODc, 4ODc	6.7
Ethanol yield	$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$; 0.511 g/g theoretical	6.8
Homo vs heterolactic	2 lactate, 2 ATP vs lactate + ethanol + CO_2, 1 ATP	6.8

Glossary (terms used in this lesson)

Adhesin A microbial surface molecule that binds a host receptor.

Autoinducer Signal molecule whose concentration tells bacteria their population density (quorum sensing).

Biofertilizer Live microbes applied to seed, roots or soil to supply or mobilise nutrients.

Biofilm Surface-attached microbial community embedded in a self-made matrix (EPS).

Cry protein Crystal (δ-endotoxin) protein of *Bacillus thuringiensis* toxic to specific insect larvae.

Endotoxin Lipid A of Gram-negative lipopolysaccharide; causes fever and shock.

EPS Extracellular polymeric substance: polysaccharides, proteins and DNA forming the biofilm matrix.

Exotoxin Protein toxin secreted by a bacterium.

Heterocyst Thick-walled cyanobacterial cell where nitrogen is fixed away from oxygen.

Lactic acid bacteria Gram-positive, acid-tolerant fermenters producing lactic acid; homo- or heterofermentative.

Leghaemoglobin Oxygen-binding protein of legume nodules that protects nitrogenase.

Nitrogenase Oxygen-sensitive enzyme that reduces N_2 to NH_3 using 16 ATP per N_2.

PHB Poly-3-hydroxybutyrate, a biodegradable bacterial storage polyester.

Quorum sensing Density-dependent gene control through autoinducer signals.

Starter culture	Known active culture added to start a food fermentation reliably.

The full unit book

Every chapter of Unit 6, the unit summary, the exam question bank with model answers and the full glossary: Microbiology · Unit 6 · Medical, Environmental and Industrial Microbiology (one file, works offline).

Sources and further reading

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- Fertiliser (Control) Order, Schedule III (biofertilizer specifications), Department of Agriculture, Goa: agri.goa.gov.in, Schedule III of FCO. Source of the CFU standards in Chapter 6.6.
- General Standards for Discharge of Environmental Pollutants (Environment (Protection) Rules, 1986), copy at foundryinfo-india.org. Source of the BOD limit in Chapter 6.4.

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