Microbiology

Unit 6 · Medical, Environmental and Industrial Microbiology

Microbes make people ill, keep people well, clean rivers, feed crops and run whole industries. After this unit you can explain how a pathogen causes disease and how TB, typhoid and malaria are diagnosed and treated, describe how microbes treat sewage and clean up pollution, choose a biofertilizer or microbial insecticide for a crop, and work out yields for an industrial fermentation.

Taught at 8 of 10 | Usually Sem 3 | 8 chapters

About this unit

This unit takes the microbiology of the earlier units out of the laboratory and into hospitals, fields, rivers and factories. It starts with how microbes cause disease and three infections every Indian student should know well (tuberculosis, typhoid and malaria), moves to the gut community that keeps us healthy, then to soil, water and sewage, to microbes that clean up pollution and feed or protect crops, to the biofilms that make microbes hard to kill, and ends with the fermentations that make alcohol, enzymes, curd, idli and plastics. The early chapters use the cell biology of Units 2 to 4 and the drug ideas of Unit 5; the later chapters reuse the metabolism of Unit 4.

Chapters

1. Microbial pathogenicity; nosocomial and opportunistic infections
2. Common infections: tuberculosis, typhoid and malaria
3. Gut microbiome and dysbiosis ADVANCED
4. Soil and aquatic microbiology; wastewater treatment ADVANCED
5. Microbes in pollution control and bioremediation
6. Biofertilizers, biopesticides and microbial insecticides
7. Biofilms
8. Industrial and food fermentations: alcohol, enzymes, bioplastics

How to use this book

- Read the lead paragraph and the figure of a chapter first; they hold the one idea the rest explains.
- Cover the solution of each worked example and do it yourself on paper before you read it.

- Answer every "Check yourself" question aloud or on paper before you open it; the ones you get wrong are your revision list.

6.1 Microbial pathogenicity; nosocomial and opportunistic infections

Most microbes never harm us. A pathogen is one that can cause disease, and pathogenicity is that ability. To cause disease a microbe must enter the body, stick, multiply, dodge the immune system and damage tissue, and then leave to reach the next host. Each step needs a molecule, called a virulence factor. Hospitals add a special twist: sick patients with tubes and wounds, plus heavy antibiotic use, let even weak microbes cause disease. Those are nosocomial and opportunistic infections, and they are why hospital hygiene is a science.

You need: bacterial cell structure, capsule and the Gram-negative outer membrane (Unit 2, Bacterial cell wall: Gram-positive and Gram-negative); antimicrobial resistance (Unit 5, Antimicrobial resistance).

Pathogenicity and virulence

Pathogenicity is a yes-or-no quality: can this species cause disease? **Virulence** is a degree: how much disease does this strain cause? Two strains of *Escherichia coli* are the same species, yet one lives quietly in your colon and another (an enterohaemorrhagic strain) causes bloody diarrhoea. They differ in virulence.

Virulence is measured in animals or cell culture with two numbers. The **ID50** (infectious dose 50) is the number of microbes that infects half of the test hosts. The **LD50** (lethal dose 50) is the dose that kills half of them. A smaller ID50 or LD50 means a more virulent microbe. *Shigella* can infect with very few cells, which is why it spreads so easily through contaminated food.

A **primary (true) pathogen** causes disease in a healthy person. An **opportunistic pathogen** causes disease only when defences are weakened. The same microbe can be both: *Staphylococcus aureus* lives harmlessly in the nose of many people but causes wound and bloodstream infections in surgical patients.

The steps of infection and their virulence factors

1. **Entry.** Through a portal: the respiratory tract (tuberculosis), the gut (typhoid, cholera), the skin through a cut or an insect bite (malaria), or the genital tract.

2. **Adhesion.** Pili (fimbriae) and surface proteins called **adhesins** bind host receptors, so the microbe is not washed away by urine, mucus or gut movement. Uropathogenic *E. coli* use P-fimbriae to hold on in the urinary tract.
3. **Invasion and spread.** Some bacteria make the host cell swallow them (*Salmonella*, *Shigella*). Enzymes such as hyaluronidase and collagenase loosen tissue; coagulase and streptokinase build or dissolve fibrin clots.
4. **Evasion of defences.** A **capsule** stops phagocytes from gripping the cell (*Streptococcus pneumoniae*). IgA protease cuts the antibody of mucous surfaces. Antigenic variation changes surface proteins faster than antibodies can follow. Siderophores steal iron, which the host keeps locked away.
5. **Damage.** Mostly by toxins, compared below, and partly by the host's own inflammation.
6. **Exit and transmission.** Coughing, faeces, blood, or a vector such as a mosquito carries the microbe to the next host.



Virulence = how much damage a strain causes; measured as ID50 or LD50 (smaller = more virulent).

Opportunist: steps 2 to 5 succeed only when skin, flora or immunity is damaged (catheter, burn, surgery, HIV, cancer drugs, long antibiotic use).

Figure 6.1.1 The six steps a pathogen must complete, with the virulence factors that serve each step. Notice that damage (orange box) is only one step; blocking any earlier step, for example adhesion, also prevents disease.

Exotoxins and endotoxins

FEATURE	EXOTOXIN	ENDOTOXIN
Chemistry	Protein	Lipid A part of lipopolysaccharide (LPS)
Source	Secreted by living cells; Gram-positive and some Gram-negative bacteria	Outer membrane of Gram-negative bacteria; released mainly when cells lyse
Heat	Mostly destroyed by heat	Stable to boiling
Potency and action	Very potent, specific target (nerve, gut, heart)	Less potent; general fever, low blood pressure, septic shock

FEATURE	EXOTOXIN	ENDOTOXIN
Toxoid vaccine	Possible (tetanus, diphtheria)	Not possible

Many exotoxins are **A-B toxins**: the B part binds the host cell and the A part enters and acts as an enzyme. Diphtheria toxin stops protein synthesis; cholera toxin keeps the gut cell pumping out salt and water, which causes watery diarrhoea.

Nosocomial and opportunistic infections

A **nosocomial** infection, now usually called a **healthcare-associated infection (HAI)**, is one the patient did not have or was not incubating at admission; by convention it appears 48 hours or more after admission. The four commonest are urinary tract infection linked to a catheter (CAUTI), bloodstream infection linked to a central line (CLABSI), ventilator-associated pneumonia (VAP) and surgical site infection (SSI).

Why hospitals? The patients are already weak; tubes and wounds bypass skin and mucosa; staff hands and instruments carry microbes from bed to bed; and heavy antibiotic use selects resistant strains. The infection is **endogenous** when it comes from the patient's own flora and **exogenous** when it comes from outside. The resistant hospital group is remembered as **ESKAPE**: *Enterococcus faecium*, *Staphylococcus aureus* (including MRSA), *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* species. Hand hygiene is the single most effective control; Ignaz Semmelweis showed this in a Vienna maternity ward in the 1840s.

Opportunistic infections follow a fall in defence. In advanced HIV infection the classic ones are *Pneumocystis jirovecii* pneumonia, cryptococcal meningitis (*Cryptococcus neoformans*, a capsulated yeast seen with India ink) and oral and oesophageal candidiasis. Aspergillosis (*Aspergillus fumigatus*) affects patients on cancer treatment. In 2021 India saw many cases of mucormycosis ("black fungus") in COVID-19 patients with uncontrolled diabetes or heavy steroid use, a clear lesson in how host state decides disease.

WORKED EXAMPLE

Question (6 marks). The infection control team of a Chennai hospital ICU records, in one month, 5,000 patient-days, 3,600 urinary catheter-days and 9 catheter-associated urinary tract infections (CAUTI). Calculate the CAUTI rate and the catheter utilisation ratio. A second ICU had 6 CAUTIs in 1,500 catheter-days. Which ICU performed better, and why must you not simply compare 9 with 6?

1. A device-associated rate counts infections per 1,000 device-days: rate = (number of infections ÷ device-days) × 1,000.
2. ICU 1: (9 ÷ 3,600) × 1,000 = 2.5 CAUTIs per 1,000 catheter-days.
3. Catheter utilisation ratio = catheter-days ÷ patient-days = 3,600 ÷ 5,000 = 0.72. So catheters were in place on 72% of patient-days.
4. ICU 2: (6 ÷ 1,500) × 1,000 = 4.0 CAUTIs per 1,000 catheter-days.
5. ICU 1 performed better (2.5 is lower than 4.0) even though it had more infections. Raw counts ignore exposure: ICU 1 had more than twice the catheter-days, so more chances for infection.

Answer: 2.5 per 1,000 catheter-days; utilisation 0.72; ICU 1 is better. Its next target is the high utilisation ratio, by removing catheters that are no longer needed.

EXAM TRAPS

- Pathogenicity is the ability (species level); virulence is the degree (strain level). Do not use them as synonyms in a definition question.
- Endotoxin is lipid A of LPS, not the whole cell wall, and only Gram-negative bacteria have it. Exotoxins are not only from Gram-positives: cholera toxin is from a Gram-negative.
- A lower LD50 means a more dangerous microbe. Students often reverse this.
- An infection present on admission is not nosocomial, even if it is diagnosed in the hospital.
- Favourite question: "Differentiate exotoxin and endotoxin" (five points in a table) and "What are nosocomial infections? Give sources and control measures."

CHECK YOURSELF

– REMEMBER **Define ID50 and LD50.** 2 marks

ID50 is the number of microbes that infects 50% of test hosts; LD50 is the number that kills 50%. Both measure virulence.

– REMEMBER **Expand ESKAPE.** 2 marks

Enterococcus faecium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species.

– UNDERSTAND **Why does a capsule make a bacterium more virulent?** 3 marks

The slippery polysaccharide capsule stops phagocytes from binding and engulfing the cell and hides surface antigens from complement, so the bacterium survives and multiplies in tissue.

– UNDERSTAND **Why can a toxoid vaccine be made for tetanus but not for endotoxin shock?** 3 marks

Tetanus toxin is a protein; formaldehyde treatment removes its toxicity but keeps its antigenic shape, so antibodies form. Endotoxin is lipid A, a poor antigen with no such inactivated form.

– APPLY **A ward had 4 CLABSIs in 2,000 central-line days. Find the rate.** 2 marks

(4 ÷ 2,000) × 1,000 = 2.0 CLABSIs per 1,000 central-line days.

– APPLY **Classify each infection as endogenous or exogenous: (a) *E. coli* UTI from the patient's own gut flora after catheterisation; (b) MRSA spread from a nurse's hands.** 2 marks

(a) Endogenous; (b) exogenous.

– ANALYZE **A strain loses its pili but keeps all its toxin genes. Predict its virulence in the urinary tract and explain.** 4 marks

Virulence falls sharply. Without pili it cannot adhere, so urine flow washes it out before it multiplies or makes enough toxin. Toxin genes are useless if an earlier step fails.

BEYOND THE SYLLABUS

This is not for the exam. Modern pathogenicity research uses "molecular Koch's postulates": a gene is a virulence gene if knocking it out lowers virulence and putting it back restores it. Drug developers now target virulence factors (anti-adhesion and anti-toxin molecules) instead of killing the microbe, hoping to slow resistance. Hospitals track HAIs with surveillance software and genome sequencing of isolates to find outbreak chains. Search "anti-virulence therapy", "HAI surveillance India ICMR" and "One Health".

6.2 Common infections: tuberculosis, typhoid and malaria

Tuberculosis, typhoid and malaria are three of the biggest infectious diseases of India, and each is caused by a different kind of microbe: an acid-fast bacterium, a Gram-negative gut bacterium and a protozoan carried by a mosquito. Learning them side by side teaches the pattern every infection follows: agent, route, what happens in the body, how the lab confirms it, how it is treated and how it is prevented. Examiners ask exactly these headings.

You need: acid-fast staining (Unit 1, Special stains: acid-fast, endospore, capsule, flagella); protozoa (Unit 2, Protozoa and helminths); the steps of infection (Chapter 6.1).

HEADING	TUBERCULOSIS	TYPHOID (ENTERIC FEVER)	MALARIA
Agent	*Mycobacterium tuberculosis*, acid-fast rod	*Salmonella enterica* serovar Typhi, Gram-negative motile rod	*Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale* (and *P. knowlesi*)
Spread	Airborne droplet nuclei from coughing patients	Faecal-oral: contaminated water and food	Bite of an infected female *Anopheles* mosquito
Main site	Lungs; any organ	Gut lymphoid tissue, then blood, liver, spleen, gall bladder	Liver, then red blood cells
Key lab test	Sputum smear (Ziehl–Neelsen), NAAT (CBNAAT, Truenat), culture	Blood culture (first week); Widal test	Thick and thin blood smear; rapid antigen test
Treatment	Isoniazid, rifampicin, pyrazinamide, ethambutol for 6 months	Ceftriaxone or azithromycin (guided by sensitivity)	ACT for falciparum; chloroquine plus primaquine for vivax
Prevention	BCG at birth, case finding, treating contacts	Safe water, sanitation, typhoid conjugate vaccine	Bed nets, spraying, larval control, vaccines in Africa

Tuberculosis

M. tuberculosis has a waxy wall rich in **mycolic acids**. That wall resists drying and many drugs, keeps the Gram stain from working (so the acid-fast stain is used), and slows growth: the cell divides only about once in 15 to 20 hours, so culture on Löwenstein–Jensen medium takes weeks.

Inhaled bacilli are swallowed by lung macrophages but survive inside them. The immune system walls them off in a **granuloma (tubercle)**: a ball of macrophages and lymphocytes with a cheese-like (caseous) centre. In most people the infection stays **latent**: no symptoms, not infectious. When immunity falls (HIV, diabetes, malnutrition, old age) it becomes **active**: cough for more than two weeks, fever, night sweats, weight loss, blood in sputum.

India has the largest number of TB patients of any country, and the National TB Elimination Programme (NTEP) gives free diagnosis and drugs. Diagnosis uses sputum microscopy and molecular tests: CBNAAT (GeneXpert) and the Indian-made Truenat detect TB DNA and rifampicin resistance in about two hours. Drug-sensitive TB is treated for six months: two months of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E), then four months of H, R and E, written 2HRZE/4HRE. Several drugs are used together so that no single-drug-resistant mutant survives. **MDR-TB** is resistant to at least isoniazid and rifampicin. The BCG vaccine (live attenuated *M. bovis*) protects young children against severe forms such as TB meningitis but protects adults poorly.

Typhoid

S. Typhi is swallowed in water or food contaminated by human faeces; humans are its only host. It crosses the small-intestine lining through M cells over the **Peyer's patches**, survives inside macrophages and spreads through lymph to the blood, liver, spleen and gall bladder. The fever rises step by step over the first week; the pulse may stay slow for the temperature; ulcers of Peyer's patches can bleed or perforate in the third week. Some people carry the bacteria in the gall bladder for years and shed them in faeces: these **chronic carriers** keep the disease going. *S.* Paratyphi A, B and C cause a milder paratyphoid fever; together they are called enteric fever.

The test depends on the week of illness: blood culture is best in the first week; antibodies (Widal) rise from the second; stool and urine cultures turn positive later. The **Widal test** detects antibodies against the O (somatic) and H (flagellar) antigens. In India many healthy people already have some antibody, so a single low titre means little; a fourfold rise between two samples is meaningful. Resistance to older drugs and to fluoroquinolones is now common, so ceftriaxone or azithromycin is used. A typhoid conjugate vaccine based on the Vi capsular antigen is made in India.

Malaria

The parasite alternates between two hosts. In the **human** it multiplies asexually by schizogony; in the **mosquito** it reproduces sexually. Each burst of red cells releases merozoites and parasite waste, which causes the fever spike. The cycle in red cells takes about 48 hours for *P. vivax*, *P. ovale* and *P. falciparum* (tertian fever) and 72 hours for *P. malariae* (quartan fever).

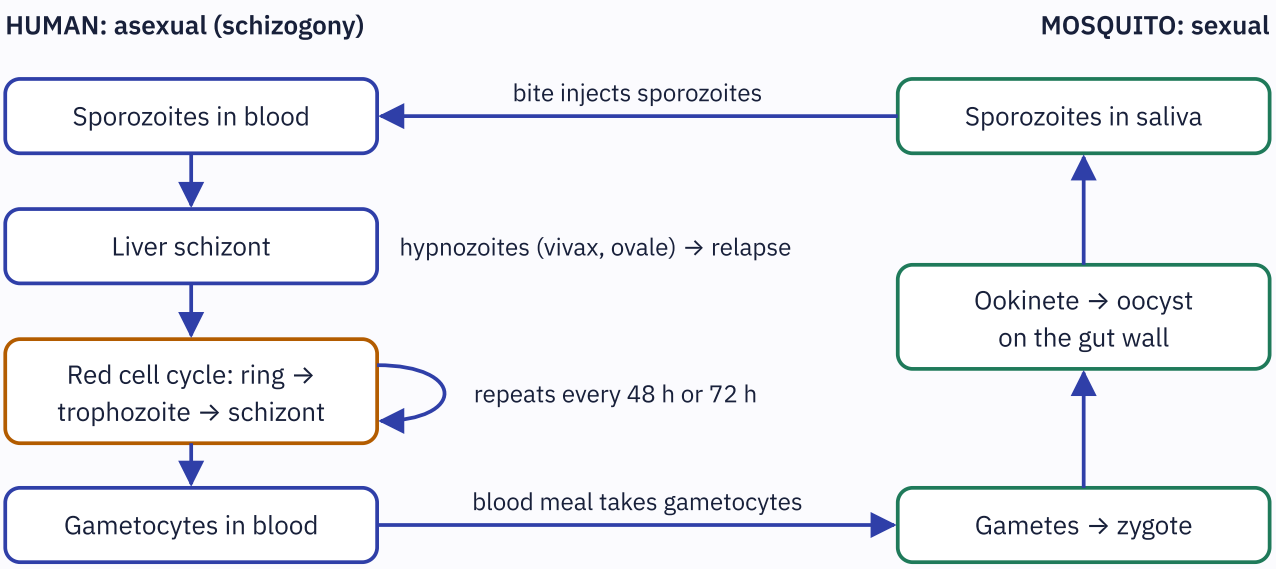


Figure 6.2.1 The malaria life cycle. Human stages are on the left (blue), mosquito stages on the right (green). Notice the loop in the red cell box: its timing sets the fever pattern, and only gametocytes can infect the mosquito.

P. falciparum is the most dangerous: it infects red cells of all ages, and infected cells stick to the walls of small blood vessels, blocking blood flow to the brain (cerebral malaria) and kidneys. *P. vivax* and *P. ovale* leave dormant liver forms called **hypnozoites**, which wake months later and cause relapse; primaquine kills them, but it can break red cells in people with G6PD deficiency, so that is checked first. Diagnosis uses Giemsa- or Leishman-stained blood smears (thick smear to find parasites, thin smear to name the species) or a rapid diagnostic test that detects parasite antigens such as HRP2 (falciparum only) or pLDH. Falciparum malaria is treated with an artemisinin-based combination therapy (ACT).

Nipah is a bat-borne virus that caused outbreaks in Kozhikode, Kerala, from 2018. The viruses themselves are in Unit 3 (Human viruses: HIV and SARS-CoV-2).

WORKED EXAMPLE

Question (5 marks). On a Giemsa-stained thick smear, a technician counts 120 asexual malaria parasites while counting 200 white blood cells. On the thin smear, 25 of 2,500 red cells are infected, and many rings are seen with banana-shaped gametocytes. Calculate the parasite density (assume 8,000 white cells per µL) and the percentage parasitaemia, and name the likely species.

1. Parasites per µL = (parasites counted ÷ white cells counted) × white cells per µL = (120 ÷ 200) × 8,000.
2. = 0.6 × 8,000 = 4,800 parasites per µL of blood.
3. Parasitaemia = (infected red cells ÷ red cells counted) × 100 = (25 ÷ 2,500) × 100 = 1%.
4. Banana- or crescent-shaped gametocytes are typical of *P. falciparum*.

Answer: 4,800 parasites/µL, 1% parasitaemia, *P. falciparum*; treat with an ACT and watch for signs of severe malaria.

EXAM TRAPS

- A Widal test is not the first test in week one of fever; blood culture is. One positive Widal in an endemic area does not prove typhoid.
- Only the female *Anopheles* bites and transmits malaria; *Aedes* carries dengue and *Culex* carries filariasis.
- "Tertian" means every 48 hours (fever on day 1 and day 3), not every three days.
- Latent TB is not infectious; active pulmonary TB is. MDR-TB needs resistance to both isoniazid and rifampicin.
- Favourite question: "Describe the life cycle of *Plasmodium* with a neat diagram" and "Laboratory diagnosis of enteric fever".

CHECK YOURSELF

– REMEMBER **Name the four first-line TB drugs and the regimen code used in India.** 2 marks

Isoniazid, rifampicin, pyrazinamide, ethambutol; 2HRZE/4HRE (six months).

– REMEMBER **Which antigens does the Widal test use?** 2 marks

The O (somatic, LPS) and H (flagellar) antigens of *S.* Typhi, plus those of Paratyphi A and B.

– UNDERSTAND **Why is TB treated with several drugs at once for months?** 3 marks

A large bacterial population contains mutants resistant to any single drug; a combination kills them all. The slow-growing, partly dormant bacilli need months of exposure to be cleared.

– UNDERSTAND **Explain why vivax malaria can return months after the blood was cleared.** 3 marks

Dormant liver hypnozoites are untouched by blood-stage drugs; when they wake they release merozoites and start a new blood cycle (relapse). Primaquine is given to kill them.

– APPLY **A thick smear shows 300 parasites per 200 WBC. Find the density at 8,000 WBC/µL.** 2 marks

(300 ÷ 200) × 8,000 = 12,000 parasites/µL.

– APPLY **A patient has fever on day 6 of illness. Which sample would you send first to confirm typhoid, and why?** 2 marks

Blood culture: the bacteria are in the blood in the first week, before antibodies rise.

– ANALYZE **Compare how the immune system "fails" in TB and in typhoid carriers.** 4 marks

In both, bacteria survive in a protected niche. TB bacilli survive inside macrophages walled in granulomas and reactivate when immunity falls. Typhoid carriers harbour *S.* Typhi in the gall bladder (often on gallstones), out of reach of immune cells and many drugs. Both create silent reservoirs that sustain spread.

BEYOND THE SYLLABUS

This is not for the exam. Whole-genome sequencing of TB isolates now predicts drug resistance and tracks transmission. New all-oral regimens for drug-resistant TB (based on bedaquiline, pretomanid and linezolid) have cut treatment from years to months. Two malaria vaccines, RTS,S and R21 (the second made by the Serum Institute of India), are being rolled out in Africa. Search "WHO Global TB Report", "Ni-kshay portal" and "malaria elimination India 2030".

6.3 Gut microbiome and dysbiosis

The human colon holds a dense community of bacteria, archaea, fungi and viruses that digests fibre we cannot, makes vitamins, trains the immune system and keeps pathogens

out. When this community loses its balance, a state called dysbiosis, diseases from *Clostridioides difficile* diarrhoea to inflammatory bowel disease become more likely.

The community and what it does

The **microbiota** is the set of microbes living in a place; the **microbiome** is that community together with its genes and activities (the two words are often used loosely). A 2016 estimate put the bacteria in an adult man at about 4×10^{13}, roughly as many as his own cells, most of them in the colon. Two phyla dominate a healthy adult gut: **Bacillota** (formerly Firmicutes, e.g. *Faecalibacterium*, *Ruminococcus*) and **Bacteroidota** (formerly Bacteroidetes, e.g. *Bacteroides*), with smaller groups such as *Bifidobacterium* and *Akkermansia*.

- **Fibre fermentation.** Gut anaerobes ferment dietary fibre into **short-chain fatty acids** (SCFAs): acetate, propionate and butyrate. Butyrate is the main fuel of colon lining cells and calms inflammation.
- **Vitamins.** Gut bacteria make vitamin K and several B vitamins.
- **Colonisation resistance.** Resident microbes use up nutrients and space and make inhibitory compounds, so newcomers such as *Salmonella* or *C. difficile* struggle to settle.
- **Immune training.** Contact with gut microbes shapes the gut immune system from birth.

Dysbiosis: causes and effects

Dysbiosis is a harmful change in the community: loss of diversity, loss of useful groups (such as butyrate makers), or overgrowth of **pathobionts**, resident microbes that cause harm when they bloom. Common causes are broad-spectrum antibiotics, a low-fibre diet, gut infections and, in babies, caesarean birth or lack of breastfeeding. The clearest disease link is *C. difficile* infection after antibiotics. Associations with inflammatory bowel disease, obesity and type 2 diabetes are well documented, but for many conditions it is still unclear whether dysbiosis is a cause or a result.

Repair options: **probiotics** (live beneficial microbes, e.g. *Lactobacillus* in curd), **prebiotics** (fibres such as inulin that feed them), **synbiotics** (both together) and **faecal microbiota transplantation** (FMT), which is very effective for recurrent *C. difficile* infection.

Diversity is measured from DNA, mostly by 16S rRNA gene sequencing (which bacteria are present) or shotgun metagenomics (all genes). A common number is the **Shannon index**, $H = -\Sigma\, p_i \ln p_i$, where p_i is the fraction of reads belonging to taxon i. H rises with more taxa and with more even shares.

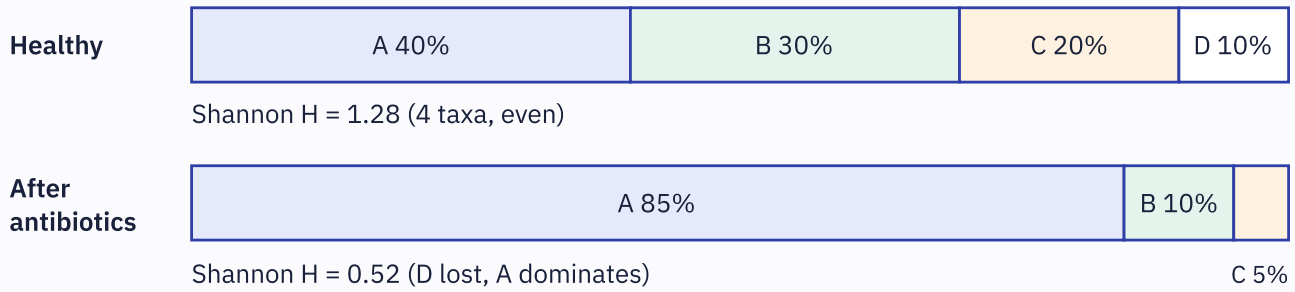


Figure 6.3.1 Relative shares of four taxa (A to D) in a stool sample before and after a course of antibiotics, drawn from the worked example below. Notice that the bar length is the same (100%); dysbiosis shows as loss of a taxon and one taxon taking over.

WORKED EXAMPLE

Question (4 marks). 16S sequencing gives the shares A 0.40, B 0.30, C 0.20, D 0.10 before antibiotics and A 0.85, B 0.10, C 0.05 after. Calculate the Shannon index for each and interpret.

1. Before: $-[0.40 \ln 0.40 + 0.30 \ln 0.30 + 0.20 \ln 0.20 + 0.10 \ln 0.10] = -[(-0.3665) + (-0.3612) + (-0.3219) + (-0.2303)] = 1.28$.
2. After: $-[0.85 \ln 0.85 + 0.10 \ln 0.10 + 0.05 \ln 0.05] = -[(-0.1381) + (-0.2303) + (-0.1498)] = 0.52$.
3. The index fell by more than half: one taxon was lost and the rest became uneven. This is dysbiosis by loss of diversity.

EXAM TRAPS

- Probiotic = live microbes; prebiotic = non-digestible food that feeds them. They are often swapped.
- Dysbiosis is not just "fewer bacteria": total numbers can stay the same while diversity and composition change.
- Use the natural log in the Shannon index unless the question says $\log_2$ or $\log_{10}$, and write H without units.

CHECK YOURSELF

– REMEMBER **Name the three main short-chain fatty acids.** 1 mark

Acetate, propionate, butyrate.

– UNDERSTAND **What is colonisation resistance?** 2 marks

The protection resident microbes give by occupying space, consuming nutrients and making inhibitors, so pathogens cannot establish.

– APPLY **Find H for two taxa at 0.5 each.** 2 marks

$-2 \times 0.5 \times \ln 0.5 = \ln 2 = 0.69$.

– ANALYZE **Why does *C. difficile* infection often follow a course of clindamycin or a cephalosporin?** 3 marks

The antibiotic kills many resident anaerobes, removing colonisation resistance; *C. difficile* (often resistant, and protected as spores) germinates in the emptied niche and makes toxins.

– UNDERSTAND **Distinguish microbiota from microbiome.** 2 marks

Microbiota: the organisms. Microbiome: the organisms plus their genes and functions in that habitat.

BEYOND THE SYLLABUS

This is not for the exam. Research now asks what microbes do (metabolomics, metatranscriptomics) rather than only who is there. Defined bacterial consortia are being developed as drugs to replace FMT. The gut–brain axis and drug metabolism by gut bacteria are active fields. Search "Human Microbiome Project" and "live biotherapeutic products".

6.4 Soil and aquatic microbiology; wastewater treatment

A gram of fertile soil holds a vast number of microbes that recycle carbon, nitrogen and phosphorus; rivers and lakes hold communities that break down what falls into them. A sewage treatment plant is these same natural processes, packed into tanks and speeded up.

Soil microbes and their interactions

The **rhizosphere** is the thin layer of soil around roots; root exudates feed it, so it is much richer in microbes than bulk soil.

- **Mutualism** (+/+): *Rhizobium* and legume roots; lichens (fungus and alga).

- **Commensalism** (+/0): one microbe uses the oxygen or waste of another.
- **Amensalism** (–/0): *Streptomyces* releases antibiotics that inhibit neighbours.
- **Competition** (–/–), **parasitism** and **predation** (+/–): phages lysing bacteria; protozoa grazing bacteria.

Soil microbes drive the **nitrogen cycle**: nitrogen fixation ($N_2 \rightarrow NH_3$), ammonification (protein → NH_4^+), nitrification ($NH_4^+ \rightarrow NO_2^-$ by *Nitrosomonas*, then $NO_2^- \rightarrow NO_3^-$ by *Nitrobacter*) and denitrification ($NO_3^- \rightarrow N_2$ under low oxygen). Fixation and its use as fertilizer are in Chapter 6.6.

Aquatic microbiology

Organic waste entering water is eaten by bacteria that use dissolved oxygen. The **biochemical oxygen demand** (BOD) is the oxygen (mg/L) microbes consume while oxidising the organic matter in a sample over a set time and temperature; India uses 3 days at 27 °C. High BOD drains oxygen and kills fish. Nitrate and phosphate run-off cause **eutrophication**: algal blooms that die, rot and drain oxygen. Faecal pollution is checked with **indicator organisms**, coliforms and especially *E. coli*, because testing for every pathogen is impractical; the most probable number (MPN) test or membrane filtration counts them.

Wastewater treatment

1. **Preliminary:** screens and grit chambers remove rags, plastic and sand.
2. **Primary:** settling tanks remove settleable solids as primary sludge; roughly a third of BOD goes with it.
3. **Secondary (biological):** aerobic microbes oxidise dissolved organics. In the **activated sludge** process, air is blown into a tank of sewage and microbial flocs; the flocs settle in a secondary clarifier and part is returned to keep the tank seeded. In a **trickling filter**, sewage trickles over stones or plastic covered with a biofilm (Chapter 6.7).
4. **Tertiary:** nitrogen and phosphorus removal, filtration and disinfection (chlorine, UV, ozone).
5. **Sludge:** digested anaerobically; hydrolytic, acid-forming and finally methanogenic archaea turn it into biogas (mostly CH_4 and CO_2).

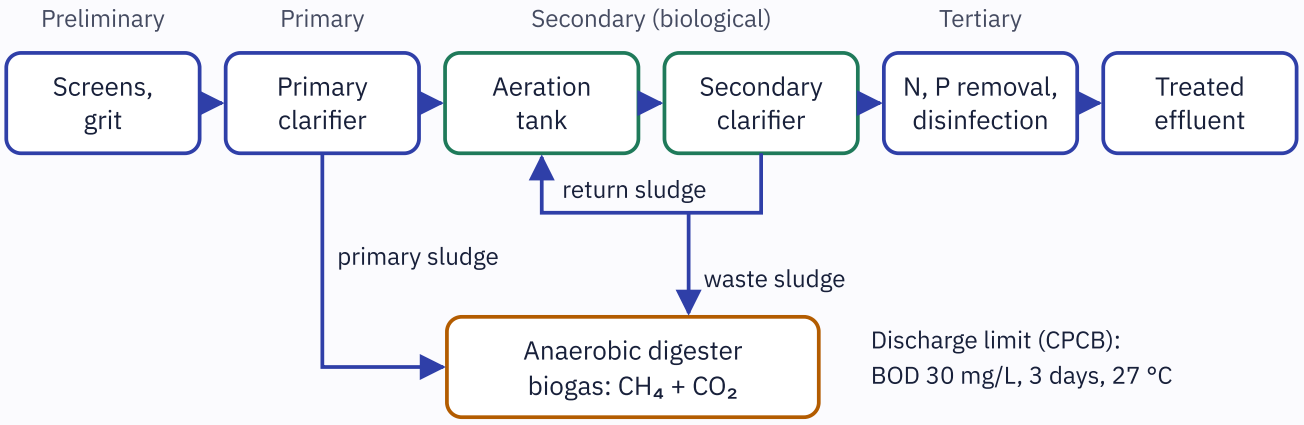


Figure 6.4.1 A conventional activated sludge plant. Notice the return-sludge loop, which keeps the aeration tank full of active microbes, and that both primary and waste sludge go to the anaerobic digester, which turns the waste into fuel.

WORKED EXAMPLE

Question (4 marks). 6 mL of sewage is diluted to 300 mL in a BOD bottle. Dissolved oxygen is 8.5 mg/L at the start and 3.5 mg/L after 3 days at 27 °C. Find the BOD and say whether it meets the CPCB limit of 30 mg/L for discharge into inland surface water.

1. Fraction of sample P = 6 ÷ 300 = 0.02.
2. BOD = $(D_1 - D_2) \div P = (8.5 - 3.5) \div 0.02 = 5.0 \div 0.02 = 250$ mg/L.
3. 250 mg/L is far above 30 mg/L: this raw sewage needs secondary treatment that removes about 88% of its BOD before discharge.

EXAM TRAPS

- Divide by the dilution fraction P; forgetting it gives 5 mg/L instead of 250 mg/L.
- *Nitrosomonas* makes nitrite, *Nitrobacter* makes nitrate. Denitrification needs low oxygen.
- Coliforms are indicators of faecal pollution, not usually the pathogens themselves.

CHECK YOURSELF

– REMEMBER **Define BOD.** 2 marks

Oxygen (mg/L) consumed by microbes oxidising the organic matter in water over a set period and temperature (3 days, 27 °C in India).

– UNDERSTAND **Why is part of the settled sludge returned to the aeration tank?** 2 marks

To keep a high concentration of active, adapted microbes in the tank so organic matter is oxidised quickly.

– APPLY **D_1 = 8.0, D_2 = 5.0 mg/L, 10 mL sample in 300 mL. Find BOD.** 2 marks

P = 0.0333; BOD = 3.0 ÷ 0.0333 = 90 mg/L.

– ANALYZE **Classify the interaction: *Streptomyces* secretes streptomycin, which inhibits nearby bacteria while it is unaffected.** 2 marks

Amensalism (–/0): one partner is harmed, the other neither gains nor loses directly.

– UNDERSTAND **How does eutrophication kill fish?** 2 marks

Nutrients cause algal blooms; dead algae are decomposed by bacteria that use up dissolved oxygen, so fish suffocate.

BEYOND THE SYLLABUS

This is not for the exam. Newer plants use membrane bioreactors, moving-bed biofilm reactors and anammox bacteria, which turn ammonium and nitrite straight into N_2 with less aeration. Sewage is now also surveyed for viruses and resistance genes (wastewater-based epidemiology, used for SARS-CoV-2 in Indian cities). Search "anammox", "MBBR" and "wastewater surveillance".

6.5 Microbes in pollution control and bioremediation

Bioremediation is the use of microbes, or plants with their root microbes, to remove or detoxify pollutants in soil, water and air. It works because microbes, as a group, can use almost any organic molecule as food and can change the chemical form of metals. Compared with digging up and burning contaminated soil, it is cheaper and gentler, but slower and harder to control. Oil spills, pesticide residues, textile dyes and chromium from tanneries are its classic targets.

You need: aerobic and anaerobic metabolism (Unit 4, Microbial metabolism: aerobic and anaerobic pathways); plasmids and gene transfer (Unit 4, Bacterial recombination).

Why microbes can clean up

A pollutant is broken down when some microbe can gain energy or carbon from it, or can transform it while growing on something else (**co-metabolism**). Complete breakdown of an organic pollutant to CO_2, water and minerals is **mineralisation**. Many breakdown genes sit on **catabolic plasmids**; the TOL plasmid of *Pseudomonas putida*, for example, carries genes to degrade toluene and xylene. In the 1970s Ananda Chakrabarty combined several such plasmids in one *Pseudomonas* strain to degrade several crude-oil components; the US Supreme Court's 1980 decision in *Diamond v. Chakrabarty* allowed it to be patented, the first patent on a living engineered organism.

A compound that resists breakdown is **recalcitrant**. Synthetic chemicals with no natural counterpart (xenobiotics), especially those with many chlorine atoms, such as DDT and PCBs, are often recalcitrant and build up in food chains.

Pollutants and the microbes that treat them

- **Petroleum hydrocarbons:** oxygenases in *Pseudomonas*, *Rhodococcus* and the marine *Alcanivorax* attack alkanes and aromatics; oxygen is usually the limiting factor.
- **Pesticides and dyes:** white-rot fungi (*Phanerochaete chrysosporium*) secrete lignin peroxidases that break many aromatic dyes and pesticides. Textile effluent with azo dyes is decolourised by bacterial azoreductases under low oxygen.
- **Heavy metals:** metals cannot be destroyed, only bound or changed. In **biosorption**, metal ions stick to dead or living cell walls; in **bioaccumulation**, living cells take them in. Some bacteria reduce toxic, soluble chromium(VI) to less toxic, less soluble chromium(III), useful for tannery waste. The *mer* operon enzyme mercuric reductase turns Hg^{2+} into volatile Hg^0.
- **Plastics:** *Ideonella sakaiensis*, found in 2016 at a bottle-recycling site in Japan, uses an enzyme called PETase to digest PET plastic.
- **Air: biofilters**, beds of compost or soil with microbes, remove odours and solvent vapours from factory exhaust.

Strategies and the factors that limit them

Techniques are grouped by whether the material stays in place (**in situ**) or is removed for treatment (**ex situ**). **Biostimulation** adds nutrients or oxygen to wake up native microbes; **bioaugmentation** adds selected microbes, which helps only when the native community lacks the needed enzymes.

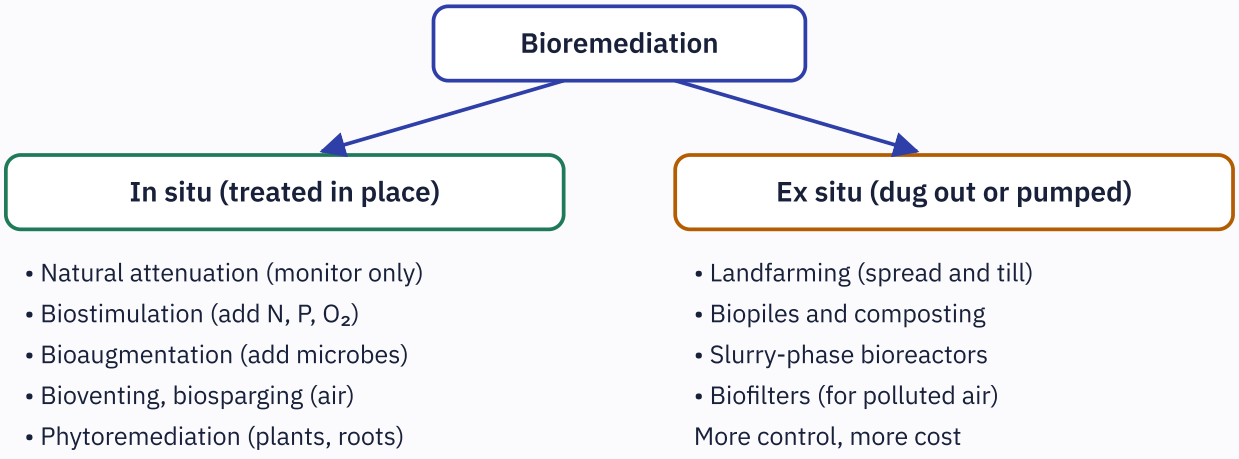


Figure 6.5.1 Bioremediation techniques grouped by where treatment happens. Notice the trade-off: in situ methods disturb less and cost less; ex situ methods give more control over oxygen, moisture and temperature, so they are faster but costlier.

Whatever the method, the rate depends on the same factors: **oxygen** or another electron acceptor (nitrate, sulphate); **nutrients** (a carbon : nitrogen : phosphorus ratio near 100 : 10 : 1 is a common rule of thumb); **moisture**; **pH** near neutral; **temperature**; and **bioavailability**, since a pollutant stuck to clay or dissolved in an oil layer is out of the microbes' reach.

In the lab and field, the fall in pollutant concentration is often close to **first-order**: $C = C_0$ e^(−kt), where C_0 is the starting concentration, C the concentration after time t, and k the rate constant (per day). The half-life is $t_{½} = \ln 2 \div k = 0.693 \div k$.

WORKED EXAMPLE 1 (NUTRIENTS)

Question (4 marks). 100 tonnes of soil near a fuel depot holds 5,000 mg of petroleum hydrocarbon per kg. Hydrocarbon is about 85% carbon by mass. Using C : N : P = 100 : 10 : 1, find the nitrogen and phosphorus to add, and the mass of urea (46% N) that supplies the nitrogen.

1. Hydrocarbon = 5,000 mg/kg × 100,000 kg = 5×10^8 mg = 500 kg.
2. Carbon = 0.85 × 500 = 425 kg.
3. N = 425 × 10/100 = 42.5 kg; P = 425 × 1/100 = 4.25 kg.
4. Urea = 42.5 ÷ 0.46 = 92.4 kg.

WORKED EXAMPLE 2 (RATE)

Question (3 marks). In a landfarming trial the hydrocarbon falls with k = 0.023 per day. Find the half-life and the time to bring 5,000 mg/kg down to 1,000 mg/kg.

1. $t_{½} = 0.693 \div 0.023 = 30.1$ days.
2. From $C = C_0 e^{(-kt)}$: $t = \ln(C_0/C) \div k = \ln(5{,}000/1{,}000) \div 0.023 = 1.609 \div 0.023 = 70.0$ days.
3. Check: 70 days is about 2.3 half-lives; 5,000 → 2,500 → 1,250 → just under 1,000. Consistent.

EXAM TRAPS

- Biostimulation feeds native microbes; bioaugmentation adds new ones. Do not mix them up.
- Heavy metals are not "degraded"; they are immobilised, accumulated or changed in oxidation state.
- Bioventing pushes air into the unsaturated zone above the water table; biosparging injects air below it.
- In rate problems, use ln (natural log) and keep k and t in the same time unit.
- Favourite question: "Explain in situ and ex situ bioremediation with examples" and "Write a note on superbug".

CHECK YOURSELF

– REMEMBER **What is mineralisation?** 1 mark

Complete breakdown of an organic compound to CO_2, water and inorganic ions.

– REMEMBER **Who made the "superbug" and what did it degrade?** 2 marks

Ananda Mohan Chakrabarty; a *Pseudomonas* strain with several catabolic plasmids that degraded several hydrocarbons of crude oil.

– UNDERSTAND **Why is DDT recalcitrant?** 2 marks

It is a chlorinated xenobiotic with no close natural counterpart, so few microbes have enzymes for it; it is also poorly soluble and binds to soil, lowering bioavailability.

– APPLY **k = 0.0139 per day. Find t½.** 1 mark

0.693 ÷ 0.0139 = 49.9, about 50 days.

– APPLY **Suggest a microbial treatment for tannery effluent rich in chromium(VI).** 3 marks

Use chromate-reducing bacteria in a bioreactor to reduce Cr(VI) to Cr(III); Cr(III) precipitates as hydroxide and is settled out; biosorbent biomass can polish what remains.

– ANALYZE **Adding a commercial oil-degrading culture to a long-polluted site gave no improvement, but adding urea and phosphate did. Explain.** 4 marks

Years of exposure had already selected native degraders, so the community had the enzymes; growth was limited by N and P, not by missing species. Added strains also struggle to compete with adapted natives.

BEYOND THE SYLLABUS

This is not for the exam. Engineered PETase variants now depolymerise PET in hours for enzymatic recycling, and companies are building plants on this idea. Microbial fuel cells pair wastewater treatment with electricity generation. Molecular monitoring (qPCR for catabolic genes, metagenomics) shows whether degraders are active at a site. Search "enzymatic PET recycling", "microbial fuel cell" and "PFAS biodegradation".

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.

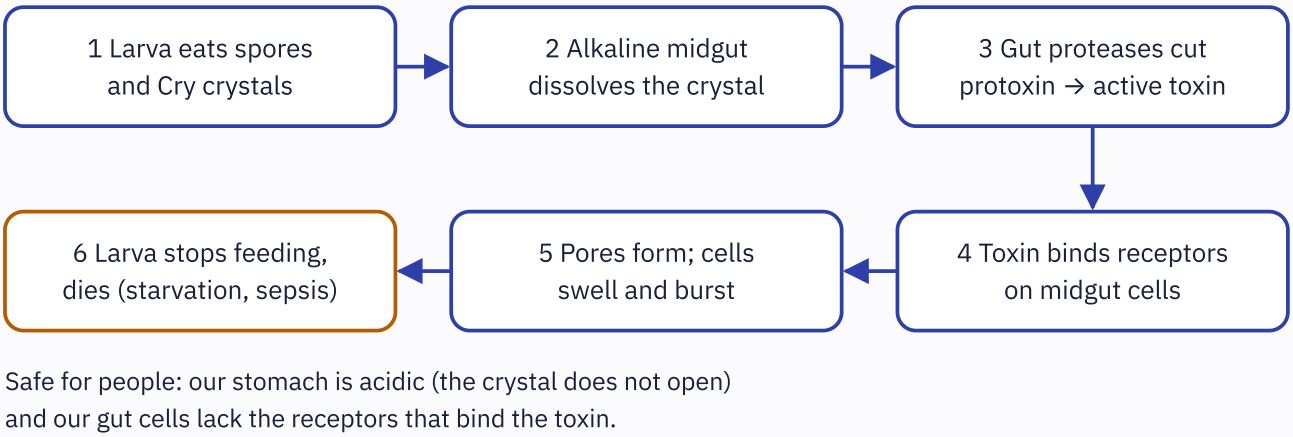


Figure 6.6.1 How Bt kills a caterpillar. Notice that steps 2 to 4 each need a feature of the target insect's gut (alkaline pH, the right proteases, the right receptor); this is why Bt is so specific.

- ***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.

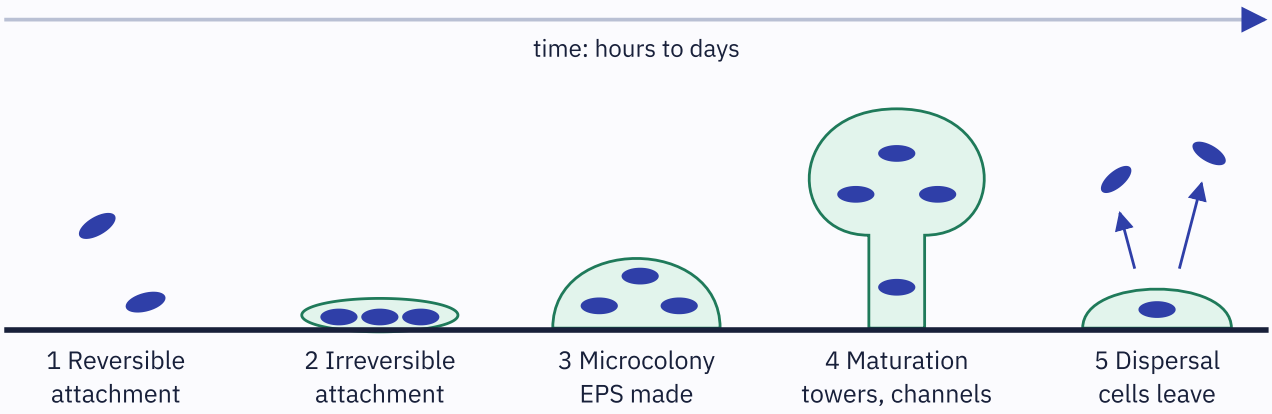


Figure 6.7.1 The five stages of biofilm development. Cells are blue, the EPS matrix green, and the thick line is the surface. Notice that matrix appears at stage 2 and grows with the community, and that dispersal (stage 5) returns cells to the free-swimming state.

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.

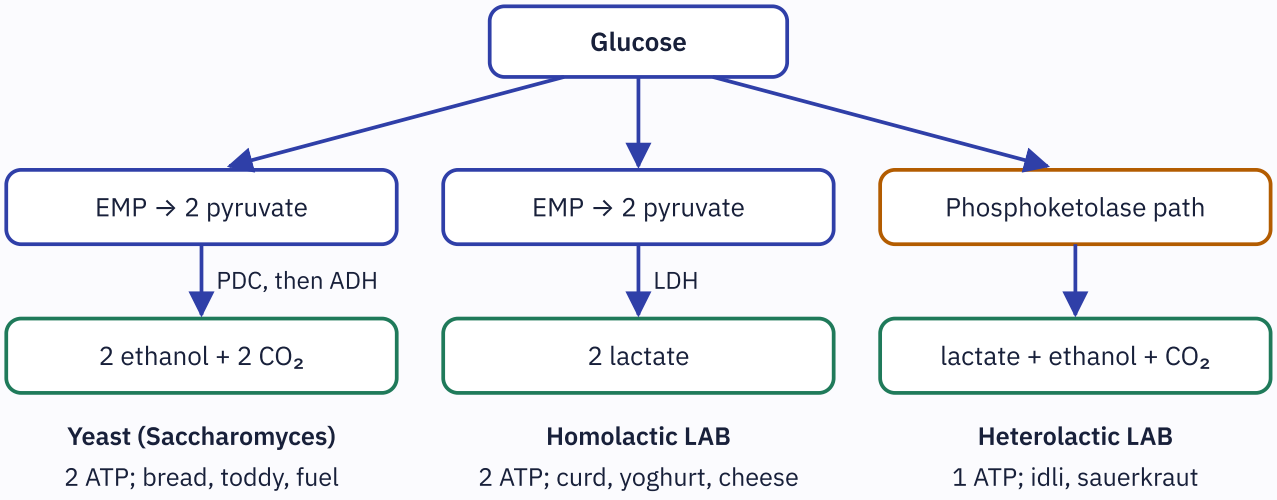


Figure 6.8.1 Three fermentations of one glucose molecule. PDC is pyruvate decarboxylase, ADH alcohol dehydrogenase, LDH lactate dehydrogenase. Notice that yeast and homolactic bacteria share glycolysis and differ only in the last step, while heterolactic bacteria take a different route and gain half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

```
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
```

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".

Unit summary

TERM	MEANING OR FORMULA	CHAPTER
Virulence (ID50, LD50)	Degree of disease; dose infecting or killing 50% of hosts (smaller = more virulent)	6.1
Device-associated infection rate	(infections ÷ device-days) × 1,000	6.1
Exotoxin vs endotoxin	Secreted protein, heat-labile, toxoid possible vs lipid A of LPS, heat-stable, no toxoid	6.1
TB regimen	2HRZE/4HRE; MDR-TB = resistant to isoniazid and rifampicin	6.2
Typhoid tests by week	Blood culture first; Widal (O, H) from week 2; stool and urine later	6.2
Parasite density	(parasites ÷ WBC counted) × 8,000 per µL	6.2
Shannon index	$H = -\Sigma\, p_i \ln p_i$	6.3
BOD (dilution method)	$(D_1 - D_2) \div P$; CPCB limit 30 mg/L (3 days, 27 °C)	6.4
First-order decay	$C = C_0\, e^{(-kt)}$; $t_{½} = 0.693 \div k$	6.5
Nutrient rule of thumb	C : N : P ≈ 100 : 10 : 1 for bioremediation	6.5
Nitrogenase	$N_2 + 8H^+ + 8e^- + 16\ ATP \rightarrow 2NH_3 + H_2 + 16\ ADP + 16\ P_i$	6.6
Biofertilizer standard (FCO)	≥ 5×10^7 CFU/g (carrier), ≥ 1×10^8 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

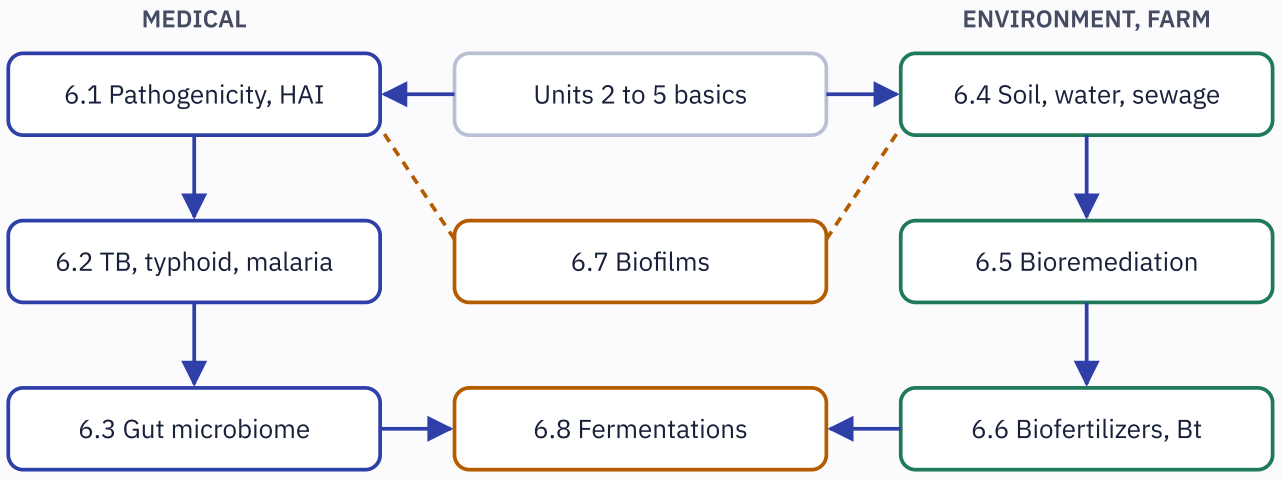


Figure 6.S.1 How the chapters connect. Solid arrows: "builds on". The basics of Units 2 to 5 feed both columns; biofilms (centre) link device infections (6.1) with trickling filters (6.4); fermentation (6.8) is the mass culture behind probiotics (6.3) and biofertilizer and Bt production (6.6).

1. Pathogenicity is the ability to cause disease; virulence is its degree, measured by ID50 and LD50, and built from adhesion, invasion, evasion and toxin factors.
2. Healthcare-associated infections come from weak hosts, devices, hands and antibiotic pressure; rates are counted per 1,000 device-days, and hand hygiene is the best control.
3. TB, typhoid and malaria follow one exam pattern: agent, spread, disease, lab diagnosis, treatment, prevention. Know the test that fits each stage of illness.
4. A healthy gut community makes short-chain fatty acids and resists invaders; dysbiosis is a loss of diversity or balance, often after antibiotics.
5. Sewage treatment is microbial oxidation in tanks: primary settling, aerobic secondary treatment (activated sludge or biofilm), tertiary polishing and anaerobic digestion to biogas; BOD measures the load.
6. Bioremediation feeds native microbes (biostimulation) or adds new ones (bioaugmentation), in situ or ex situ; metals are transformed, not destroyed.
7. Biofertilizers fix nitrogen or mobilise phosphorus; Bt, *L. sphaericus*, *P. popilliae*, baculoviruses and insect-killing fungi are specific, safe insecticides.
8. Biofilms are matrix-wrapped communities, coordinated by quorum sensing and tolerant of drugs; they matter in implants, teeth, pipes and treatment plants.
9. Fermentation turns sugar into ethanol (0.511 g/g), lactic acid foods, enzymes and bioplastics such as PHB; the right strain and conditions decide the yield.

Exam question bank

Remember and understand

- REMEMBER **Define nosocomial and opportunistic infections. List the common types of healthcare-associated infection, the ESKAPE pathogens and the control measures.** 12 marks

  Outline: definitions (48 h rule; weakened host); CAUTI, CLABSI, VAP, SSI; endogenous and exogenous sources; expand ESKAPE; reasons hospitals favour them (devices, antibiotics, weak patients); controls: hand hygiene, aseptic device care, early device removal, antibiotic stewardship, surveillance, sterilisation (Unit 5).

- UNDERSTAND **Describe the life cycle of *Plasmodium* with a labelled diagram and relate it to the symptoms, relapse and diagnosis of malaria.** 16 marks

  Outline: sporozoite inoculation; liver schizogony; hypnozoites in vivax and ovale; erythrocytic cycle (ring, trophozoite, schizont, merozoites) and 48 h or 72 h periodicity; gametocytes; mosquito stages (gametes, zygote, ookinete, oocyst, sporozoites); fever with each burst; falciparum sequestration; smear and RDT; ACT, chloroquine plus primaquine.

- UNDERSTAND **Explain the stages of biofilm formation, the role of quorum sensing and why biofilms tolerate antibiotics.** 12 marks

  Outline: conditioning film; five stages with diagram; EPS composition; AHL (LuxI/LuxR) and peptide systems; tolerance mechanisms (penetration, slow growth, persisters, gene exchange); tolerance versus resistance; two medical and two industrial examples.

- UNDERSTAND **Compare homofermentative and heterofermentative lactic acid bacteria and describe the microbiology of yoghurt, cheese and idli.** 12 marks

  Outline: EMP versus phosphoketolase; products and ATP; examples of each; starter cultures and why used; yoghurt pair and their roles; cheese starter, rennet and ripening moulds; idli LAB and CO_2 leavening.

Apply

– APPLY **A 6 mL sample in a 300 mL BOD bottle shows a fall in dissolved oxygen from 8.2 to 4.6 mg/L in 3 days at 27 °C. Find the BOD, compare it with the CPCB limit, and design a treatment scheme to meet the limit.** 10 marks

Outline: P = 0.02; BOD = 3.6 ÷ 0.02 = 180 mg/L; above 30 mg/L, needs at least 83% removal; scheme: screens, grit, primary clarifier, activated sludge with sludge return, secondary clarifier, disinfection; sludge to anaerobic digester; diagram.

– APPLY **2,000 kg of molasses with fermentable sugar equal to 45% glucose is fermented at 88% efficiency. Calculate ethanol in kg and litres and the CO_2 formed, then draw a flow sheet of the ethanol plant.** 12 marks

Outline: sugar 900 kg; theoretical ethanol 900 × 0.511 = 460 kg; actual 0.88 × 460 = 404.7 kg; ÷ 0.789 = 513 L; theoretical CO_2 900 × 0.489 = 440 kg; flow sheet: molasses dilution, sterilisation, yeast propagation, fermenter, distillation, dehydration; strain traits.

– APPLY **Plan a bioremediation of 50 tonnes of diesel-soaked soil at 8,000 mg/kg. Choose a method, compute the N and P needed (85% C; C:N:P 100:10:1) and the time to reach 1,000 mg/kg if k = 0.03 per day.** 12 marks

Outline: ex situ landfarming or biopile with aeration; hydrocarbon 400 kg; C 340 kg; N 34 kg, P 3.4 kg (urea 73.9 kg); t = ln 8 ÷ 0.03 = 69.3 days; monitoring of moisture, pH, oxygen; reasons for biostimulation over bioaugmentation.

– APPLY **Recommend biofertilizers and microbial insecticides for a farmer growing groundnut, rice and cotton, with the mechanism of each and a quality test for the products.** 12 marks

Outline: *Rhizobium* plus PSB for groundnut; *Azolla*/BGA and *Azospirillum* for rice; *Azotobacter* or *Azospirillum* plus PSB for cotton; nitrogenase and its O_2 protection; Bt for bollworms (mode of action), NPV for *Helicoverpa*, *Beauveria* for sucking pests; plate count against FCO standards.

Analyze and evaluate

– ANALYZE **Biofilms are harmful in hospitals but useful in sewage plants. Analyse this statement with examples from both settings and suggest how the same biology is controlled in one and encouraged in the other.** 12 marks

Outline (spans 6.1, 6.4, 6.7): catheter and implant biofilms, tolerance, dispersal to blood; trickling filter and MBBR biofilms retain slow-growing degraders and nitrifiers; control by coated devices, removal, stewardship; encourage by high surface area media, steady loading, aeration.

– ANALYZE **Antibiotic use links several chapters of this unit. Discuss its effects on hospital infections, the gut microbiome and the treatment of TB and typhoid.** 14 marks

Outline (spans 6.1, 6.2, 6.3): selection of ESKAPE and MRSA in hospitals; loss of colonisation resistance and *C. difficile*; MDR-TB from incomplete regimens and combination therapy logic; fluoroquinolone-resistant typhoid and current drugs; stewardship and diagnostics (CBNAAT, culture and sensitivity) as answers; link to Unit 5, Antimicrobial resistance.

– ANALYZE **Evaluate Bt as a biopesticide: its specificity, safety, limits and the problem of resistance in Indian cotton.** 10 marks

Outline: crystal, activation and receptor steps explain specificity; safety for mammals; limits (must be eaten, UV breakdown, narrow host range, no effect on sucking pests); Bt cotton and pink bollworm resistance; refuges, stacked toxins, integrated pest management.

– ANALYZE **Compare TB and typhoid as intracellular infections: how each survives the host, how chronic carriage or latency arises, and what this means for elimination programmes.** 12 marks

Outline: macrophage survival in both; granuloma and latency versus gall bladder carriage; reactivation triggers; diagnosis limits (latent TB, carrier detection); NTEP case finding and contact treatment; sanitation and conjugate vaccine for typhoid.

– ANALYZE **Should India replace petroleum plastics with PHB and PLA? Evaluate using the microbiology and economics of their production.** 10 marks

Outline: PHB under nutrient limitation in *Cupriavidus necator*; PLA from LAB lactic acid; substrate cost, yield and downstream extraction as the main costs; biodegradability conditions; use of cheap substrates (molasses, waste oils); suitable niches (packaging, medical) rather than total replacement.

Glossary

Activated sludge	Aerated tank of sewage and microbial flocs that oxidise dissolved organics; the flocs are settled and partly returned.
Adhesin	A microbial surface molecule that binds a host receptor.
Autoinducer	Signal molecule whose concentration tells bacteria their population density (quorum sensing).
Bioaugmentation	Adding selected microbes to a polluted site.
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).
Biostimulation	Adding nutrients or oxygen to activate native degrading microbes.
BOD	Biochemical oxygen demand: oxygen used by microbes oxidising organic matter in water, mg/L.
Chronic carrier	A person who sheds a pathogen for long periods without illness, e.g. *S.* Typhi in the gall bladder.
Cry protein	Crystal (δ-endotoxin) protein of *Bacillus thuringiensis* toxic to specific insect larvae.
Dysbiosis	Harmful change in the composition or diversity of a microbial community.
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.
Granuloma	Organised ball of immune cells walling off *M. tuberculosis* (tubercle).
Healthcare-associated infection	Infection acquired in a healthcare setting, appearing 48 h or more after admission; also called nosocomial.
Heterocyst	Thick-walled cyanobacterial cell where nitrogen is fixed away from oxygen.
Hypnozoite	Dormant liver stage of *P. vivax* and *P. ovale* that causes relapse.
Lactic acid bacteria	Gram-positive, acid-tolerant fermenters producing lactic acid; homo- or heterofermentative.

Leghaemoglobin	Oxygen-binding protein of legume nodules that protects nitrogenase.
MDR-TB	TB resistant to at least isoniazid and rifampicin.
Nitrogenase	Oxygen-sensitive enzyme that reduces N_2 to NH_3 using 16 ATP per N_2.
Opportunistic pathogen	Microbe that causes disease mainly when host defences are weakened.
PHB	Poly-3-hydroxybutyrate, a biodegradable bacterial storage polyester.
Probiotic	Live microbes that give a health benefit when taken in adequate amounts.
Quorum sensing	Density-dependent gene control through autoinducer signals.
Recalcitrant	Resistant to microbial breakdown.
Starter culture	Known active culture added to start a food fermentation reliably.
Virulence	Degree of disease a strain causes; measured by ID50 or LD50.
Widal test	Agglutination test for antibodies to *Salmonella* O and H antigens.

Sources and further reading

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- 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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