Microbiology

Unit 6 · Lesson 2 · Gut, soil, water and clean-up

The notes for Lesson 2 of Unit 6: chapters 6.3 to 6.5 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 2 of 3 | 3 chapters | Chapters 6.3 to 6.5

About these notes

These are the notes for **Lesson 2 · Gut, soil, water and clean-up** of the University free n easy video lessons on Microbiology Unit 6, Medical, Environmental and Industrial Microbiology. They are chapters 6.3 to 6.5 of the unit book, unchanged, so a page here is the same page in the full book.

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.

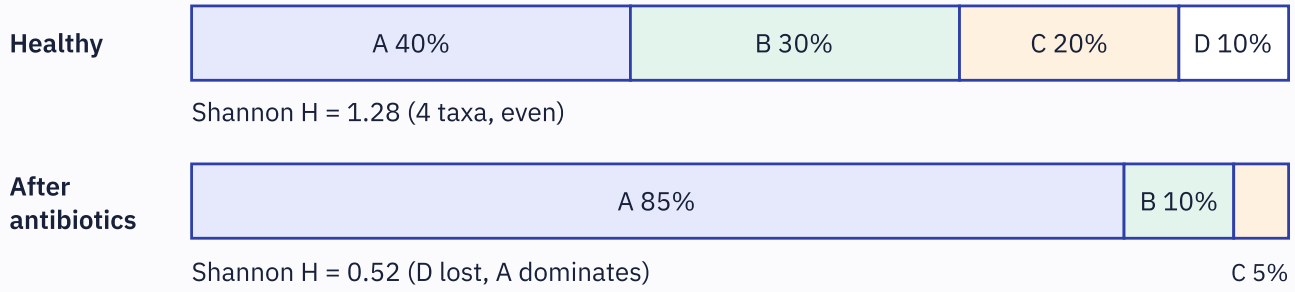


Figure 6.3.1 Relative shares of four taxa (A to D) in a stool sample before and after a course of antibiotics, drawn from the worked example below. Notice that the bar length is the same (100%); dysbiosis shows as loss of a taxon and one taxon taking over.

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).

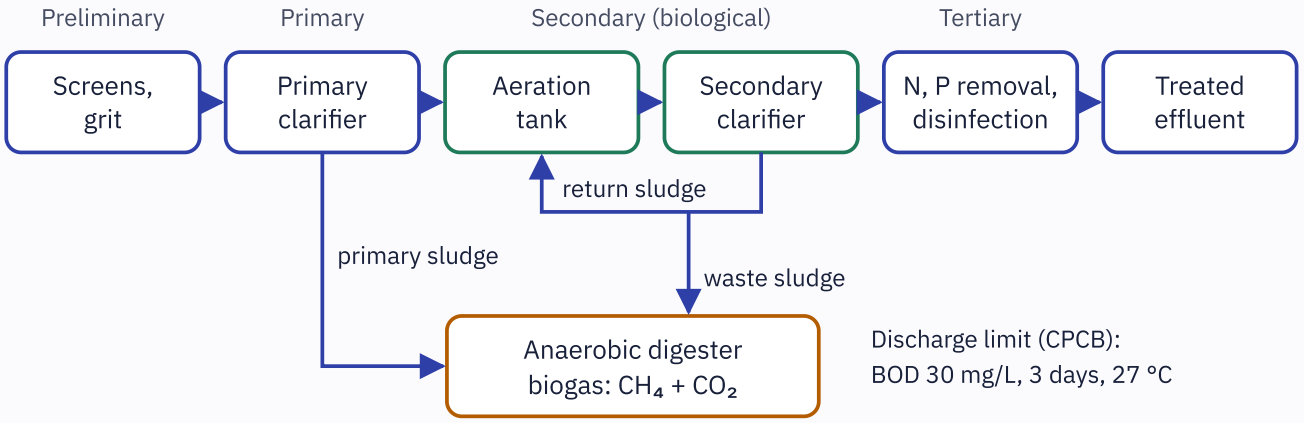


Figure 6.4.1 A conventional activated sludge plant. Notice the return-sludge loop, which keeps the aeration tank full of active microbes, and that both primary and waste sludge go to the anaerobic digester, which turns the waste into fuel.

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.

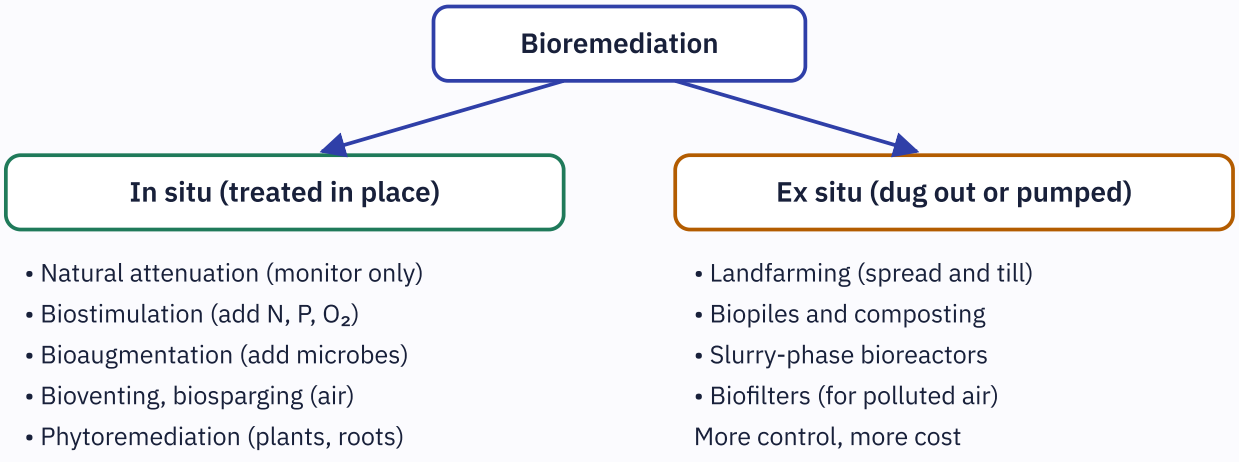


Figure 6.5.1 Bioremediation techniques grouped by where treatment happens. Notice the trade-off: in situ methods disturb less and cost less; ex situ methods give more control over oxygen, moisture and temperature, so they are faster but costlier.

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".

Key terms of this lesson

TERM	MEANING OR FORMULA	CHAPTER
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	$C = C_o\, e^{(-kt)}$; $t½ = 0.693 \div k$	6.5
Nutrient rule of thumb	C : N : P ≈ 100 : 10 : 1 for bioremediation	6.5

Glossary (terms used in this lesson)

Activated sludge Aerated tank of sewage and microbial flocs that oxidise dissolved organics; the flocs are settled and partly returned.

Bioaugmentation Adding selected microbes to a polluted site.

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.

Dysbiosis Harmful change in the composition or diversity of a microbial community.

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

Probiotic Live microbes that give a health benefit when taken in adequate amounts.

Recalcitrant Resistant to microbial breakdown.

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

- Willey, J. M., Sandman, K. M. and Wood, D. H. *Prescott's Microbiology*. McGraw-Hill. Chapters on pathogenicity, environmental and industrial microbiology (from memory; check the edition you are prescribed).
- Madigan, M. T. and others. *Brock Biology of Microorganisms*. Pearson (from memory; check).
- Pelczar, M. J., Chan, E. C. S. and Krieg, N. R. *Microbiology*. Tata McGraw-Hill (from memory; check).
- *Ananthanarayan and Paniker's Textbook of Microbiology*. Universities Press. For tuberculosis, enteric fever and laboratory diagnosis (from memory; check).
- Frazier, W. C. and Westhoff, D. C. *Food Microbiology*. McGraw-Hill. For food fermentations (from memory; check).
- Stanbury, P. F., Whitaker, A. and Hall, S. J. *Principles of Fermentation Technology*. Elsevier. For industrial fermentation (from memory; check).
- 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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