Unit 29 · Applications of Microbiology

STUDY NOTES

WHAT NEB'S GRADE 12 EXAM ASKS

- **CDC's grid (2078):** Applied Biology (Units 27–29 together): 9 of 75 marks; 1 MCQ (knowledge) and 1 long question (8 marks: knowledge 1, understanding 3, application 2, higher ability 2)
- **The paper:** Botany 37 + Zoology 38; each part: MCQs × 1 (5 Botany, 6 Zoology) · 4 short × 4 · 2 long × 8; one OR in each short and long group; 3 hours; 1 mark per expected point
- **Our model papers:** Paper 2: MCQ (yogurt's two bacteria); Paper 3: long question on a town's sewage plant in four parts (what BOD is; the activated sludge process; BOD left after each step; why a sewage-fed stream turns black)
- **MCQ (1 mark), from the exercise**
  - *L. bulgaricus*: yogurt
  - soy sauce: *Aspergillus sojae*
  - distilled wine: brandy
  - water contaminants: rotavirus, *E. coli*, *Giardia*
  - last step: disinfection
  - tricho-cards: parasitoid
  - *Azolla–Anabaena*: mutualism
  - newest: probiotics
  - green manure: dhaincha
- **Short and long (in parts)**
  - yogurt and cheese in industry
  - alcoholic beverages; beer
  - water contamination: sources, causes, effects, detection, prevention
  - sewage and its treatment
  - drinking water treatment
  - biocontrol agents
  - biofertilizers used in Nepal
  - *Azolla–Anabaena* mutualism

Key terms

Applied microbiology: use of microbes in industry and daily life for people's benefit; a part of **biotechnology**.

Fermentation: the breakdown of sugars (and proteins, fats) by microbes into acids, alcohol, gases and flavors; shown to be microbes' work by **Pasteur** (1850s–60s).

Lactic acid bacteria (LAB): turn **lactose** into **lactic acid**.

Starter culture: a measured mix of chosen microbes added to milk.

Pasteurization: heating to kill harmful bacteria (e.g. 72 °C for 15 s).

Homogenization: milk forced through tiny holes so the fat stays spread.

Contamination of water: unwanted physical, chemical or biological matter making water unfit for use; by microbes: **microbial contamination**.

Indicator organisms: gut bacteria (total and fecal coliforms, *E. coli*) whose presence shows fecal contamination.

Sewage: a town's wastewater (households, industry, surface runoff); **effluent**: water leaving the plant.

BOD (biochemical oxygen demand): the oxygen microbes would use to break down the water's organic matter.

Potable water: safe to drink and cook with.

Biocontrol agent (BCA): a living thing used to control pests, weeds, plant diseases.

Biofertilizer: living microbes that, applied to seeds, plants or soil, live in the **rhizosphere** or the plant and raise the supply of nutrients.

1.1 Dairy industry

- dairy: milk and its foods; LAB's lactic acid **sours** milk, **stops harmful bacteria**, **coagulates** casein into a gel (pH ~4.6)
- industry: **standardize** (fat, solids) → **pasteurize** → **homogenize** → cool → **starter**

i. Curd (dahi)

- milk pasteurized, homogenized, cooled to **30–40 °C**, inoculated (starter or last batch's curd)
- *Lactococcus lactis* subsp. *lactis* and *cremoris*, often *Lactobacillus acidophilus*
- lactose → lactic acid → casein coagulates; sets in **6–12 h**; then cooled
- only bacteria make dahi (lemon, vinegar → paneer; rennet → cheese curd)

ii. Yogurt

- cooled to **42–45 °C**; *Streptococcus thermophilus* + *Lactobacillus delbrueckii* subsp. *bulgaricus*
- help each other: *S. thermophilus* makes acid and formic acid for *Lactobacillus*; *Lactobacillus* breaks proteins into pieces for *S. thermophilus*
- sets in **4–6 h**; **acetaldehyde** (from *Lactobacillus*): sharp taste; flavors, fruit, sugar; cooled

iii. Butter (नौनी)

- milk standardized, pasteurized, cooled → **cream separator** (spins: skimmed milk out, cream in the middle)

- cultured butter: cream pasteurized + *Lactococcus lactis*; biovar *diacetylactis* makes **diacetyl** (buttery smell)
- cream **ripened**, cooled, **aged** → **churned** → butter granules; **buttermilk** (मही) drained
- granules washed, salted, worked, packed cold
- hills: **theki** and **madani**

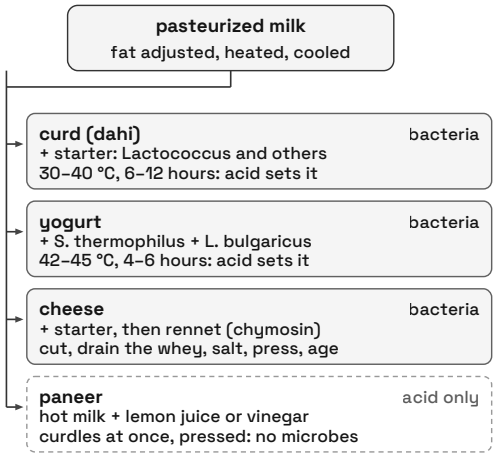
iv. Cheese

1. milk standardized, pasteurized, cooled
2. starter *Lactococcus lactis* (+ non-starter bacteria) **ripens** it
3. **rennet** (**chymosin**) cuts casein → firm **curd** in ~30 min
4. curd **cut** and **heated**; **whey** drained
5. curd knits into a **mat**; cheddar: blocks piled and turned (**cheddaring**)
6. **salted** (dry or **brine**), pressed into molds
7. **aged** (weeks to years): bacteria and enzymes make flavors; *Propionibacterium* CO_2 makes Swiss cheese's holes; molds in blue cheese

- microbial chymosin since **1990** (calf gene in bacteria, yeasts, molds)
- yak cheese: first factory **Langtang** (1950s, Swiss help); nak and chauri milk

v. Paneer: **no microbes**, no ripening

- milk heated nearly to boiling + **acid** (lemon, vinegar, citric acid, sour whey) → casein and fat clump at once
- separated from whey → **hooping** → **pressed** → chilled water
- unpressed: **chhena** (rasbari)



Curd, yogurt, cheese and paneer from the same pasteurized milk.

1.II Beverage industry

- **beverage**: any drink but plain water; alcoholic drinks for 9,000 years (China, ~7000 BCE)
- **yeast**, without oxygen (anaerobic respiration):

$$C_6H_{12}O_6 \longrightarrow 2\,C_2H_5OH + 2\,CO_2 + \text{energy}$$

- fermentation stops at ~15% alcohol (it kills the yeast); stronger = **spirits**, by **distilling** (alcohol boils at 78 °C)

i. Soy sauce

- **soybeans** soaked, steamed + **wheat** roasted, ground (~6 : 4) + mold *Aspergillus oryzae* or *A. sojae* → **koji** (2–3 days; starch → sugar, protein → amino acids)
- koji + **brine** → **moromi**; months of salt-loving *Tetragenococcus halophilus* (LAB) and yeast *Zygosaccharomyces rouxii*
- pressed → heated (~80 °C) → clarified → preservative (benzoic acid) → bottled

ii. Vinegar (4–8% **acetic acid**): two fermentations

- yeast (*Saccharomyces cerevisiae*): sugar → alcohol
- **acetic acid bacteria** (*Acetobacter aceti*, *A. pasteurianus*, *A. pomorum*, *Gluconacetobacter europaeus*, *Komagataeibacter hansenii*), with air:

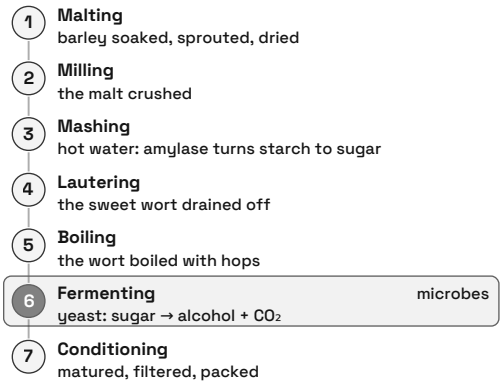
$$C_2H_5OH + O_2 \longrightarrow CH_3COOH + H_2O$$

- fruit cut, soaked in hot water, **pressed**; **sulfite** (~50 ppm) stops wild microbes; sugar set in °**Brix**
- bacteria grow as a film, **mother of vinegar**; uses: flavor, **preserving** (achar), cleaning

iii. Beer (barley; yeast can't use starch)

1. **Malting**: **steeping** (~2 days), **germination** (~5 days, makes **amylase**), **kilning** (dried, browned) → malt
2. **Milling**: malt crushed
3. **Mashing**: hot water (+ maize, sorghum, rye, wheat, rice); amylase: starch → sugar
4. **Lautering**: sweet **wort** separated
5. **Boiling** with **hops** (bitter, aroma, keeping)
6. **Fermenting**: *S. cerevisiae* at the top, 15–24 °C (**ale**) or *S. pastorianus* (*S. carlsbergensis*) at the bottom, 7–13 °C (**lager**); clarified, fined with **isinglass**
7. **Conditioning**: matured weeks, **filtered**, packaged

- only step 6 uses microbes; *Acetobacter* in a brewery = **spoilage**



The seven steps of making beer.

iv. Wine (grapes)

- harvest → crush and press → **must** (red: skins left in)
- fermentation: *S. cerevisiae*, *S. bayanus*, wild yeasts (*Pichia*, *Cyberlindnera*)
- **malolactic fermentation**: *Oenococcus oeni* (some *Lactobacillus*, *Leuconostoc*): malic → lactic acid
- clarification → aging (tanks, oak) → bottling

Others

- **cider**: apple juice + yeast; *Lactobacillus collinoides*, *Oenococcus* soften the acid; Marpha, Mustang
- **rum**: **molasses** + yeast, 1–2 days → distilled → aged in oak
- **brandy**: **distilled wine**; 35–60% (70–120 US proof)
- **gin**: neutral grain spirit redistilled with **juniper**; 35–50%
- **vodka**: grain or potato mash distilled many times, charcoal-filtered
- **whisky**: grain mash distilled, aged in **oak casks**

- Nepal (J. P. Tamang): **marcha** (*Rhizopus*, *Mucor*, *Saccharomycopsis fibuligera*) → jaand, tongba, raksi; **kinema** (*Bacillus subtilis*); **gundruk**, **sinki** (LAB)

2. Microbial contamination of water

- contaminants: **physical** (silt, plastic), **chemical** (ammonia, nitrates, arsenic, pesticides), **biological** (microbes)
- matters most for drinking, bathing, fish; cause of **water-borne diseases** (diarrhea, gastroenteritis, cholera, typhoid, dysentery, hepatitis A, E)
- **turbidity**: microbes hide on particles from disinfectants

Sources and causes

- **point sources**: outflows of sewage and treatment plants, hospitals, slaughterhouses, factories; leaking septic tanks, latrines; **sediments** and **water plants**
- **diffuse sources**, washed in by rain: human and animal **feces**, **slurry**, **manure**, **sludge**; floods, broken pipes

Microbes

- viruses: rotavirus, norovirus, hepatitis A and E, polio
- bacteria: *E. coli* (O157:H7), fecal coliforms, *Shigella*, *Vibrio cholerae*, *Salmonella*, *Campylobacter*
- fungi: yeasts, molds (taste, spoilage)
- protozoa: *Entamoeba histolytica*, *Giardia*, *Cryptosporidium* (cysts resist chlorine)
- cyanobacterial **toxins**

Effects

- human health (children, elderly worst)
- animal health
- food and drink factories, dairies, hospitals: spoiled products, costly treatment

Detection: **indicator** bacteria; WHO and Nepal: **no** *E. coli* **in 100 mL**

- **membrane filtration**: 100 mL filtered, filter incubated, colonies counted
- **MPN** (most probable number): broth tubes that turn cloudy or make gas
- **H_2S strip**: turns black in 1–2 days (cheap; villages, schools; ENPHO)
- **molecular**: **PCR**, gene probes, RFLP, sequencing

Prevention

- toilets, no open defecation (Nepal 2019); pits 10–15 m from wells, downhill
- sewage treatment; safe hospital and factory waste
- protected sources: covered wells, spring boxes, fences, pipes repaired
- hygiene: hand washing, clean containers
- treating drinking water: boiling, filters, chlorine (Piyush), SODIS
- regular **monitoring**

3.I Sewage treatment

- raw sewage > 99.9% water; high **BOD** uses a river's oxygen → fish die, black and smelly (Bagmati, dry season)
- **low-tech** (extensive, nature-based): **stabilization ponds**, **constructed wetlands** (reed beds; Dhulikhel Hospital 1997), soak pits; cheap, much land
- **high-tech** (intensive, mechanized): treatment plants (Guheshwori, activated sludge); costly, need electricity
- land disposal: sludge as fertilizer, **irrigation**, **leach fields**, soak pits, **fish ponds**, **floating plant ponds**

Preliminary

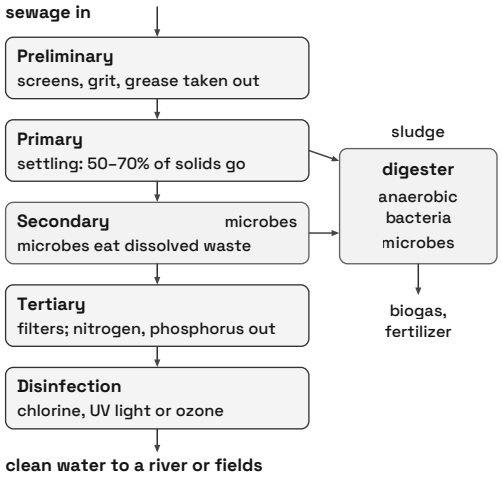
- **A. screening**: bar screens (cans, rags, plastic)
- **B. grit removal**: grit chamber (sand, gravel)
- **C. flow equalization**: even flow
- **D. fat and grease** skimmed

Main steps

- **A. Primary** (physical): **settling tanks**; **primary sludge**; removes **50–70% of suspended solids**, **25–40% of BOD**
- **B. Secondary** (biological): aerobic bacteria + protozoa break down dissolved organic matter → CO_2, water, microbes; removes **85–95%** of BOD and solids
  - **activated sludge** (suspended growth): air blown through an **aeration tank**; **flocs** settle in a **secondary clarifier**; some sludge **returned**
  - **trickling filter** (biofilm): sewage sprinkled over stones covered with microbes
- **C. Tertiary** (advanced): **polishing** by sand filters, membranes, wetlands

Then

- **disinfection**: **chlorine**, **UV**, **ozone**
- **nutrient removal**: N by **nitrification** (ammonia → nitrate, with O_2) and **denitrification** (nitrate → N_2, no O_2); P by bacteria or chemicals; else **eutrophication**
- **sludge**: thickened, **digested anaerobically** → **biogas** (methane), dried fertilizer; water to rivers or **irrigation**



A sewage treatment plant, step by step.

3.II Drinking water treatment

- sources may carry *E. coli*, *Giardia intestinalis*, *Cryptosporidium*, hepatitis A virus
- **mineral water** (protected spring; still or sparkling); **purified water** (microfiltration, **reverse osmosis**, advanced oxidation; jar water); **unsafe water**

1. **Coagulation and flocculation**: particles **negative**; coagulant **positive** (**alum**, ferric chloride), mixed fast → charges cancelled; stirred slowly → **floc**
2. **Sedimentation**: floc settles in a large tank, with many microbes

3. **Filtration**: **sand**, **gravel**, charcoal (activated carbon): floc, dust, *Giardia*, *Cryptosporidium*, most bacteria; charcoal: taste, smell; **slow sand filter**: living film eats bacteria
4. **Disinfection** (last): **chlorine**, chloramine (UV, ozone); **residual chlorine** for the pipes

- filters don't remove dissolved salts, arsenic, nitrate or, reliably, viruses → disinfection

4.I Biocontrol agents

- **biocontrol**: pests (insects, ticks, mites), weeds, plant diseases controlled by **predators**, **parasitoids**, **pathogens**, **competitors**, **plants**; sustainable, environment-friendly
- part of **IPM** (resistant varieties, rotation, traps, enemies; chemicals last)

Strategies

- **importation** (classical): a natural enemy from the pest's home (**vedalia beetle**, California, 1888; cane toad, Australia, 1935, a failure)
- **augmentation**: more enemies released: **inundative** (many, quick: tricho-cards, Bt) or **inoculative** (a few early)
- **conservation**: protect those already there (less spraying, flowers, hedges)

Kinds

- **predators**: **ladybird beetles** (aphids, mites, scale insects), lacewing larvae, spiders, predatory mites
- **parasitoids**: lay eggs **in or on** an insect, larvae kill it; *Trichogramma* wasps in moth eggs (sugarcane borer, rice stem borer, tomato fruit borer); **tricho-cards**
- **pathogens** (**biopesticides**): *Bacillus thuringiensis* (Bt; protein crystal poisons caterpillars; Bt cotton, maize); **NPV** viruses; fungi *Beauveria*, *Metarhizium*; *Trichoderma* (attacks root-rot and wilt fungi; made in Nepal)
- **plants**: **neem** (*Azadirachta indica*, **azadirachtin**); **tobacco** (**nicotine**); **lemon** extract (fungi); titepati, bakaino

4.II Biofertilizers

- add nutrients by **fixing nitrogen**, **solubilizing phosphorus**, **making growth hormones**
- groups: bacteria, cyanobacteria, fungi; green manure alongside

i. Bacteria

- *Rhizobium*: **root nodules** of legumes; N_2 → ammonia for sugar (mutualism); seeds coated (**inoculation**); *Sesbania rostrata* (dhaincha) has **stem nodules** by *Azorhizobium caulinodans* (fixes when flooded)
- *Azotobacter*: **free-living** in soil; fixes N, makes hormones; cereals, vegetables
- *Streptomyces*: threads; earthy smell; **does not fix N**; **antibiotics** (*S. griseus*: streptomycin; *S. griseoflavus*: bicozamycin); biocontrol, growth

ii. Cyanobacteria (blue-green algae, BGA)

- photosynthetic; *Anabaena*, *Nostoc* fix N in **heterocysts** (keep out O_2); flooded paddies
- *Azolla–Anabaena*: fern (eukaryote) + *Anabaena azollae* (*Trichormus*; prokaryote) in leaf cavities; *Anabaena* fixes N, fern gives shelter and food; **mutualism** (positive symbiosis); doubles in days; China, Vietnam > 1,000 years
- *Nostoc*: filaments in jelly; with liverworts, hornworts, cycad roots, *Gunnera*, lichens
- *Spirulina* (*Arthrospira*): protein **food supplement**; **no heterocysts, no N fixing**

iii. Mycorrhiza (fungus + root; mutualism): **hyphae** bring **water**, **phosphorus**; plant gives sugar

- **ectomycorrhiza**: sheath **outside**, hyphae **between** cells; **trees** (pine, oak, sal, birch, eucalyptus); mushroom fungi, *Pisolithus*
- **endomycorrhiza**: **inside** cortex cells; **arbuscular (AM)**, **Glomeromycota**, **arbuscules**; most crops, peach, apple; **orchid mycorrhiza** (seeds need it to germinate)

iv. Green manure: crop plowed in green, an **organic manure**

- **legumes** (dhaincha, cowpea, peas, beans, clover, sunn hemp), **grasses** (berseem, para, napier), cereals (oats, millet)
- adds organic matter, nutrients; legumes add *Rhizobium*'s N; dhaincha 6–8 weeks before rice

v. Commercial: powder in peat or liquid: *Rhizobium*, *Azotobacter*, *Azospirillum*, phosphate solubilizers, AM fungi; **Uni-Grow** (palm-oil by-products + microbes)

- **probiotics**: live microbes that benefit the host (*Lactobacillus*, *Bifidobacterium* in dahi); **plant probiotics** = biofertilizers, biocontrol microbes (newest idea)

COMMON MISTAKES

✗ Curd can be made with rennet, lemon or vinegar
✓ those give cheese curd or **paneer**; dahi only by **bacteria**.

✗ Bacteria make the alcohol for vinegar
✓ **yeast** makes it; **Acetobacter** turns it into acetic acid.

✗ *Acetobacter cerevisiae* brews beer
✓ beer is brewed by **yeast** alone; *Acetobacter* spoils it.

✗ Filtration removes dissolved chemicals and viruses
✓ not salts, arsenic, nitrate, nor reliably viruses; hence **disinfection**.

✗ Inoculative release is conservation
✓ it is **augmentation**; conservation adds nothing.

✗ *Streptomyces* fixes nitrogen; "Azobacter"
✓ *Streptomyces* makes **antibiotics**; spell *Azotobacter*.

✗ Peach has ectomycorrhiza; orchids have AM
✓ peach has **arbuscular**; orchids **orchid mycorrhiza**.

✗ Green manure is a biofertilizer
✓ it is an **organic manure**; a biofertilizer is **living microbes**.

✗ *Spirulina* is a nitrogen biofertilizer
✓ **no heterocysts**; it is a protein food.