Bacterial Abundance

Objective
- Measure bacterial numbers and mass per unit volume.
- Note, we are not concerned with identification here.

Why do we want to know abundance?
- Allows determination of biomass pool size.
- Provides crude estimate of element fluxes.
- Helps to characterize dynamics of ecosystem.

Challenges with natural samples
- Low concentrations

Methods
- Dry and weigh (not with natural samples).
- Plate (or viable) count (Today).
- Direct count. (Thursday).
Why do we want to measure bacterial concentration?

**E.g., Bacterial concentration** is 100 cells ml⁻¹ or 100 fg C ml⁻¹

**Estimate bacterial pool size**

- Ocean: \(10^9\) cells l⁻¹
  - \(20\) fg C cell⁻¹ (\(20 \times 10^{-15}\) g C cell⁻¹)
  - \(1.37 \times 10^{21}\) l oceans⁻¹

**Crude estimate of element fluxes** (\(x\): bacterial biomass)

- **Growth rate**: \(G = \mu x\); \(\mu\): specific growth rate
- **Uptake rate**: \(U = \mu x / \varepsilon\); \(\varepsilon\): growth efficiency
- Typical: \(\mu = 1\) d⁻¹; \(\varepsilon = 0.2\)

**Ecosystem dynamics**

- **U**: \(U\) is the uptake rate, leading to bacterial growth (\(G\)).
- **R**: \(R\) represents respiration, converting bacterial biomass to CO₂.
How is bacterial concentration measured?

Laboratory cultures

- Measure optical density and cell dry weight

Problems

- High cell densities required.
- Must be only cells (i.e., no debris or detritus)
- High predator abundance would also skew results.

⇒ Technique does not work in the field!

Dilution Plates

- Grow single cells on Petri plate until colonies are visible, then count colonies.
- Must use serial dilution so that colonies are in countable range.
- This method has a major problem. What is it? (Akin to growing fish in chicken soup)

Direct Counts

- Use microscope to directly count bacteria.

Problem: Bacteria in natural environments are very small and difficult to see and distinguish from detritus using standard light microscopy.
Dilution Plates

1 ml 1 ml 1 ml 1 ml 1 ml 1 ml
10⁻¹ 10⁻² 10⁻³ 10⁻⁴ 10⁻⁵ 10⁻⁶

Statistically relevant colony density: 30 - 300

Technique largely used for isolation or water testing, such as coliform test.
Dilution Plate Calculations

N: Number of colonies on plate

\( V_S \): Volume pipetted onto Petri plate.

D: Dilution factor for test tube plated out.

\( \rho \): Concentration of cells in original sample (cells ml\(^{-1}\))

\[
\rho = \frac{N}{V_S D}
\]

**Example:**

N: 33

\( V_S \): 100 \( \mu l \)

D: 10\(^{-4}\)

\[
\rho = \frac{33}{100 \times 10^{-4}} = 3.3 \times 10^6 \text{ cells ml}^{-1}
\]
Fecal Coliform Counts

The abundance of fecal coliform bacteria are used as an *indicator* of fecal contamination of both drinking water and recreational water (i.e., swimming, shellfishing).

Fecal coliform bacteria inhabit the intestinal tracks of animals. While the indicator bacteria are typically not pathogens, they indicate that the water has become contaminated with fecal material, either by human or other animals.

Although it would be better to assay for pathogens directly (such as hepatitis), it is too difficult to culture these organism quickly and reliably.

**Basic method:**
- Aseptically collect and filter water onto sterile filter.
- Place filter on sterile pad that contains medium for the culturing of fecal coliform bacteria (contains eosin-methylene blue dye)
- Incubate filter at 37°C (or higher)
- Count colonies to determine colonies/100 ml water

**EPA requirements (cfu/100ml):**
- Drinking water: None
- Shell fishing: ≤ 14
- Swimming ≤ 200
Some Drinking Water Pathogens

**Viruses:**
- Hepatitis

**Bacteria:**
- Cholera (*Vibrio cholera*)
- typhoid fever (*Salmonella typhi*)
- Fecal bacteria (often *Escherichia coli*)

**Protists:**
- Cryptosporidium
- Giardia
Direct Bacterial Counts

Challenges with Direct Count Method

• Natural samples contain low concentrations of bacteria (10^6 cells ml⁻¹)
  ⇒ Must concentrate bacteria

• Bacteria are small (0.2 - 1 µm) so difficult to see and differentiate from detritus using microscope with normal or phase contrast lighting techniques.
  ⇒ Must stain with fluorescent dye and use epifluorescence microscopy.

Procedure outline

• Incubate water sample with fluorescent dye.
• Concentrate sample onto 0.2 µm filter.
• Place filter on slide, and count bacteria in grid
• Calculate bacterial numbers.
Epifluorescence Microscopy

Fluorescence

• Compound is “excited” at a particular wavelength of light (usually in the UV)
• Compound then emits light at a different, lower, wavelength.

• Advantage: contrast is extremely high, which allows detection of weak light.

Dyes used

• Acridine orange (AO)
• DAPI (4’6-diamidino-2-phenylindole)

Mechanisms

• AO fluoresces when bound to DNA or RNA. Cells appear orange.
• DAPI fluoresces when bound to DNA and is more specific. Cells appear blue.
Epifluorescence Details

UV Light source

Excitation filter

Beam splitter, Emission filter

Objective

Sample

Eyepiece
Slide Preparation for DAPI

Notes:
• Place filter so that bacteria are on the top side.
• Use small drops of immersion oil
• Cover slips stick together. If you have more than one, you will not be able to focus well.
• Label slide.
Cell Density Calculations

**Known or measured**
- Volume of sample filtered: $V_S$
- Area of filter occupied by sample: $A_F$
- Area of grid in field of view: $A_G$
- Average number of cells grid$^{-1}$: $N$

**Cell Concentration**
- Cell Conc: $\rho$

\[
\rho = \frac{A_F}{A_G} \frac{N}{V_S}
\]

What is the main assumption in this calculation?