Bacteria: An Overview

Section 1: General Microbiology
Section 2: Microbial Taxonomy
Section 3: Bacteria vs. Enzymes
Section 4: Aquarius Microbes
- Bacteria
  - Bacillus

Section 5: Required Techniques and Math
- Bacterial Enumeration
- Bacterial Concentration

Section 1

GENERAL MICROBIOLOGY

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What is a microbe?

- Bacteria are single celled and one of the simplest forms of life.
- Despite the sometimes negative perception, the majority of bacteria are not harmful and some can be very useful in various industry and domestic applications.
- Bacteria are the primary decomposers of organic material in our environment. They are responsible for the purification process of rivers, streams, lakes and the removal of dead biomass and waste from our soil.

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Microbes are ancient and adapt extremely well

- The single celled bacteria are the earliest forms of life on the planet.
- There are many thousands of species of microbes, and for bacteria in particular, their generation time is very fast: a couple of hours compared to our life span.
- This means there have been billions of generations of bacteria and many opportunities for them to adapt to environmental conditions. Bacteria live everywhere in almost every environment: from the surface of the human skin to our deep oceans.

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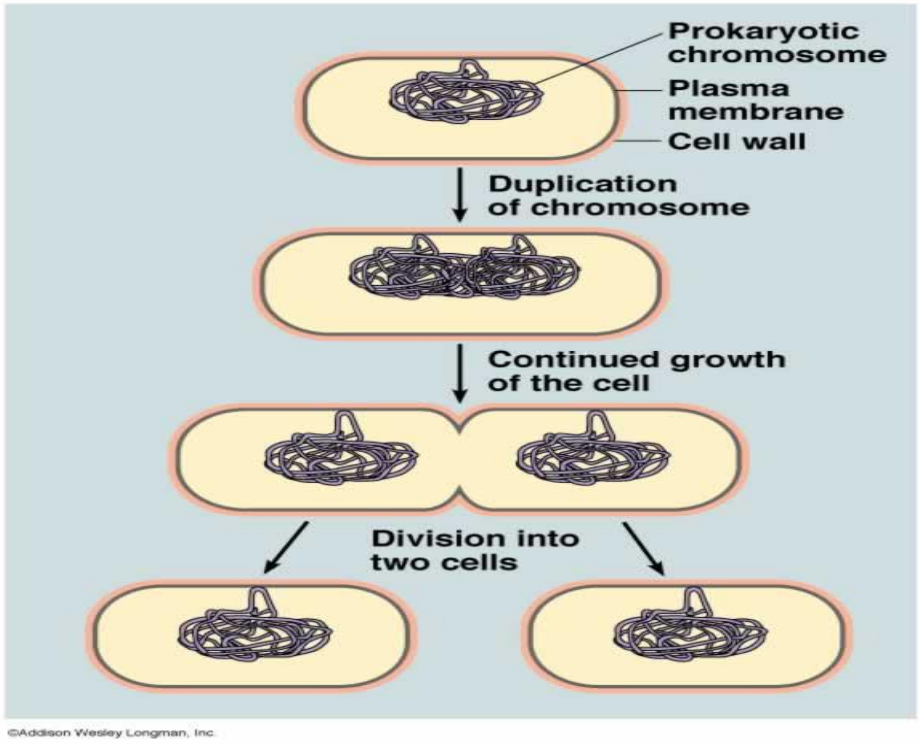
Microbes can carry out two types of processes

- **Catabolism**- these are processes where microbes break large molecules down into their constituent parts, either to use the fragments for other processes or to gain energy for growth. Bacteria can degrade many types of materials including wastes and chemical by-products, and can be used to return an environment, altered by contaminants, back to its original condition. Ex. Digestion

- **Anabolism**-these are processes where microbes convert materials into other useful materials, or build larger molecules from constituent parts. Ex. muscle growth, DNA replication

Bacteria growth

- Given optimal conditions, bacteria tend to replicate every few minutes in a logarithmic growth pattern. The time it takes to replicate varies with species and the environmental conditions they are exposed to.



Bacterial Growth

- Let's assume that it takes a certain species of bacteria 20 minutes to replicate. In 20 minutes,1 cell will divide and become two new cells, 20 minutes later those two cells can divide to become 4 cells and after 60 minutes you will have 8 cells. As this progresses, between 6 and 7 hours, the numbers pass the 1 million mark, and after 12 hours there are nearly 70 billion cells.
- Bearing in mind that our products deliver millions of bacteria to the application at the start, the numbers are even bigger: 1 million cells will divide and become 2 million in 20 minutes, etc. After 4 hours, we reach billions, between 6 and 7 hours we reach trillions, and after 12 hours in ideal conditions, we are into the quadrillions.

Microbes are involved in many beneficial applications

- Decomposition of waste in our environment
- Bioremediation of soil, effluents and water
- Bioconversion of waste to useful products such as compost or biogas
- Cleaning, soil degradation and nuisance removal
- Beneficial effects on humans and farmed animals
- Production of food, beverages, drugs & vaccines

Biosafety Levels (BSL)

The United States Center for Disease Control (CDC) issued criteria to categorize each strain of microorganism into a specific level of containment:

- BSL-1: Safest. Not known to cause disease in plants, animals or other organisms.
- BSL-2: Moderate risk of infections.
- BSL-3: Agents are known to cause disease
- BSL-4: Cause severe and fatal disease; epidemics

All of our strains at Aquarius are considered BSL 1 and are eco friendly.

Section 2

MICROBIAL TAXONOMY

Naming microorganisms

- Typical Naming of microbes consists of 2 names
  - **Genus** - noun, always capitalized
  - **species** - adjective, lowercase

- Both italicized or underlined
  - *Bacillus subtilis (B. subtiis)*
  - *Bacillus licheniformis (B. lichenformis)*
  - *Pseudomonas putida (P. putida)*

Strains

We commonly talk about "strains" of bacteria. This helps to identify different classifications among a species:

Not all bacteria are the same even though they have the same Genus and species name.

Common Name	**Dog**	**Human**	**Bacillus subitilis**
Genus	Canis	Homo	Bacillus
Species	*Canis familiaris*	*Homo sapeins*	*Bacillus subtilis*
"Sub-species" Type	Breed	Race	Strain
Common varieties	Border collie, Boxer, Great dane, etc.	Caucasian, African, Chinese	100, HP13

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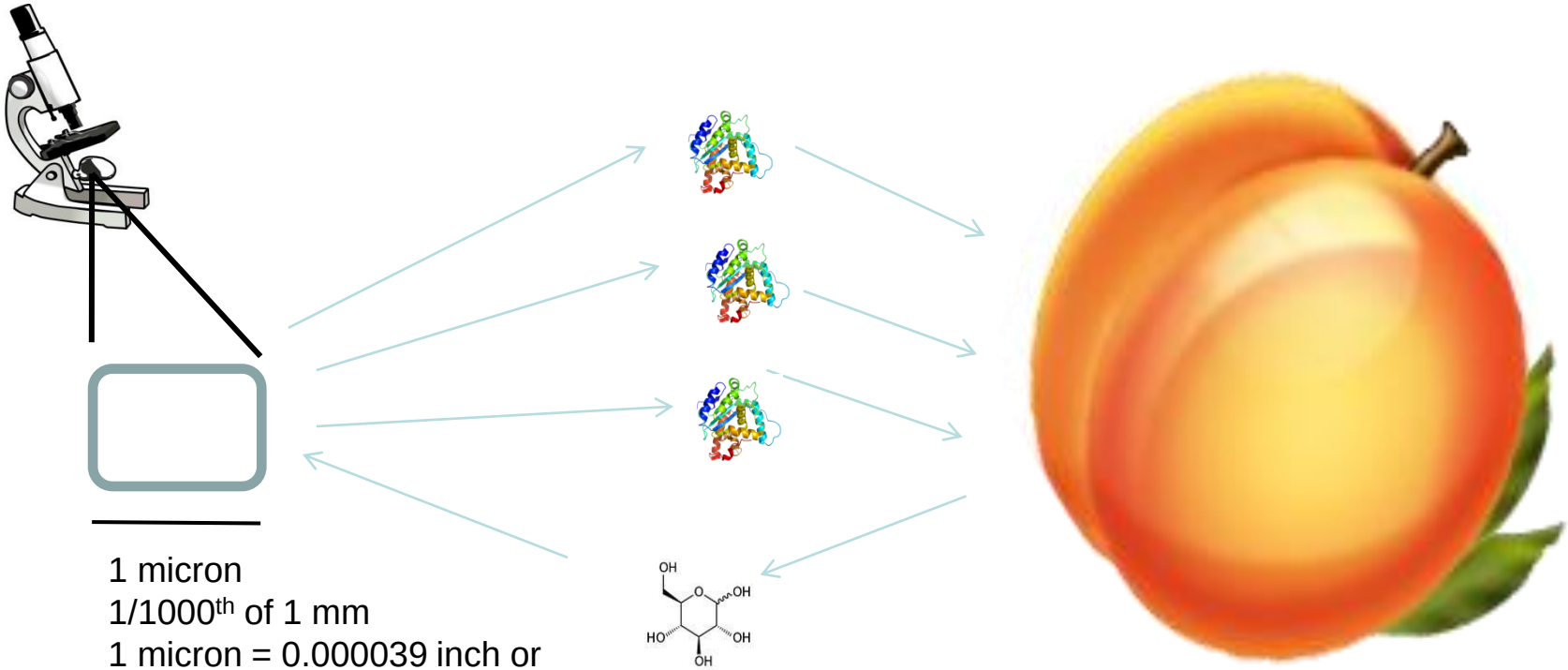
Section 3

BACTERIA VS. ENZYME

How Biotechnology Works

- Our selection process insures choosing strains that have the highest capability to carry out the chosen process.
  - Selecting the bacteria strains with the best capabilities to degrade the targeted wastes.
  - The parallel is selecting the most capable individual from the species to do the job: Usain Bolt for the 100 meters, Michael Phelps for swimming, Stephen Hawking for quantum physics.
- Synergistic blending provides improved performance for a wide range of degradation capabilities.
  - A team of the most capable individuals will always work better together to provide overall superior results: many business or sports analogies here!

How does a bacterium break down a large mass?



1 micron
1/1000th of 1 mm
1 micron = 0.000039 inch or
about 4/100,000ths of an inch

- The microscopic bacterium cannot ingest large particles to use as food.
- The bacterium produces enzymes to break down the food into smaller pieces that the bacteria can absorb and use.
- The food is used as an energy source so the bacteria can continue to produce enzymes, grow and undergo cell division producing new cells.
- Note that this is all happening at the microscopic level!

Types of Bacteria

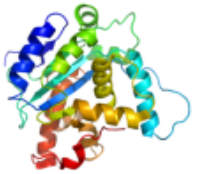
Vegetative and Spores

- Vegetative State
  - Active state
  - Undergoing respiration
  - Needs to find food

Types of Bacteria

- Spore Formers
  - Able to go dormant while conditions are unfavorable
  - Survival technique to protect itself
  - In favorable conditions the spore germinates
  - When the spore germinates it become vegetative
  - Able to go back into a spore state if conditions become un-favorable
  - Facultative capability, perform in aerobic & anaerobic conditions

What is an enzyme?



- A protein molecule produced by living organisms is able to catalyze, or facilitate, a specific chemical reaction. They are specific in the substrates it couples with, leading to the degradation of specific compounds.
- Enzymes are produced by microorganisms to accomplish the degradation process.
- Enzymes are affected by several factors: environmental conditions such as pH and temperature.; concentration of substrate (no substrates present=no reaction) and can be destroyed (denatured) by excessive heat, chemicals, etc.

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- Enzymes are a tool for microorganisms in their degradation process. Microorganisms are enzymes factories and produce a multitude of different enzymes.

➢ *Lipase*	*Lipid hydrolysis*	*cleave the ester bond*
➢ *Amylase*	*Starch hydrolysis*	*glucose formation*
➢ *Protease*	*Protein hydrolysis*	*cleave the peptide bond*
➢ *Cellulase*	*Cellulose hydrolysis*	*glucose formation*

Advantages of using bacteria vs. purified enzymes

- Our bacteria naturally produce a range of enzymes such as lipase, cellulase, amylase, and protease.

- Purified enzymes are obtained as a single enzyme type. In order to get the same range of enzymes; they would have to be purchased individually.

- Since our enzymes come from the product bacteria, as long as the organisms have nutrients, and proper environmental conditions they will continue to provide fresh enzyme activity.

Differences between bacteria & enzymes

Bacteria

- Living organisms
- Able to multiply
- Are microscopic enzyme factories
- Able to produce a continuous supply of enzymes
- Are able to react to changes in the nature of a waste stream
- Traditionally seen as a slower process compared to enzymes
- Can provide a complete degradation process

Differences between bacteria & enzymes

Enzymes

- Provide biochemical catalysis
- Cannot multiply
- Have relatively narrow optimal conditions
- Cannot react to changes in the nature of the waste stream
- Traditionally seen as faster acting
- Only catalyse a single reaction and so,
- Do not provide a complete degradation process

Section 4

AQUARIUS MICROBES

Aquarius Microbes

- All microbes supplied by Aquarius are living organisms which are safe for humans and animals (produce no toxins) and are classified as BSL 1 Strains.

- The “parents” of Aquarius microbes were isolated from the natural environment (i.e. soil, ponds, remediation systems, etc.) or purchased from Global culture banks.

- Aquarius does not supply microbes that are genetically modified (mutated).

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‘The power of microbes’

- Bacteria are very small microscopic organisms.
- Aquarius biological formulas contain hundreds of billions of microbes per gallon in our liquid formulas and billions of bacteria, per gram, in powdered formulas.
- These bacteria grow exponentially in the correct environmental conditions: this means that each bacterium doubles every 30 minutes under ideal conditions: and thus the hundreds of billions of bacteria become thousands of billions in a few hours to solve the toughest problems.
- Each individual “Selected” bacterium, within these billions, is carrying out reactions continuously, and as a “Blend” all of these activities are synergistic. As a result significant amounts of waste material can be degraded, as is done everyday in our waste water treatment plants.
- A good analogy is perhaps; a single termite can only eat a toothpick, and billions of termites can level a building.

Microbes from Aquarius

- Bacteria:
  - *Bacillus*:
    - Have the ability to form spores (a dormant stage in the life cycle).
    - This is the main type of microbe that Aquarius supplies.
    - Gram positive (a simple classification based on a staining method developed in the 1800s!).

Bacillus Life Cycle

- The *Bacillus* bacteria in all Aquarius products is packaged and shipped in the **spore** form**.**
- Once the product is applied in the application it is used for, the spores rapidly **germinate** into a **vegetative cell.**
- After germination, the bacteria start secreting their enzymes, breaking down the food for an energy source. The bacteria grow and undergo cell division and reproduce two new identical daughter cells.
- When the bacteria reach a **limiting factor,** they undergo **sporulation** (the creation of **endospores** and then **spores**).

Advantages of Spores

- Long term, shelf life stable.
- More tolerant to harsh environmental conditions.
- Competitive with other types of microbes.
- Work synergistically with other *Bacillus,* as well as other select microbes.
- Able to be formulated with many other ingredients allowing superior and unique formulas to be made.
- Are good waste degraders.

Bacillus species

- *Bacillus licheniformas*
- *Bacillus subitilis*
- *Bacillus pumilus*
- *Bacillus megaterium*
- *Bacillus amyloliquefaciens*

Bacteria Applications

- Odor eliminating
- Waste degradation
- Stain removal by degradation of organic elements
- Can degrade a vast range of organic compounds including simple hydrocarbons
- Enhances BOD/COD removal
  - Biological Oxygen Demand / Chemical Oxygen Demand

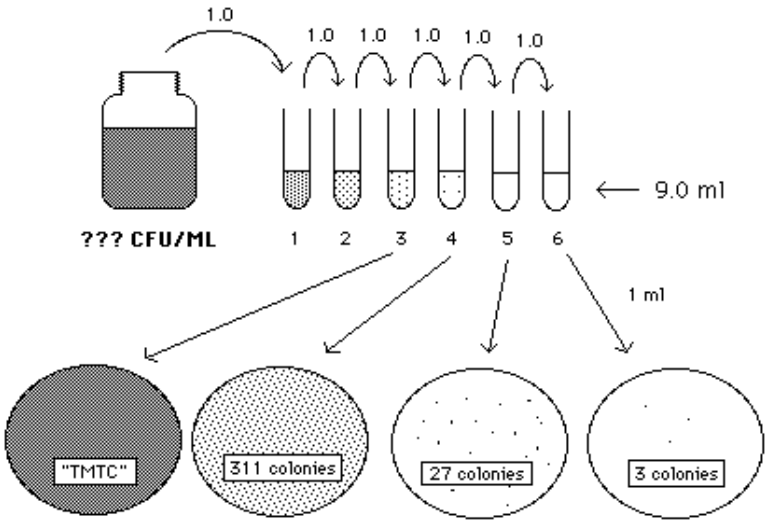
Section 5

BACTERIAL ENUMERATION

Bacterial Enumeration (plate counting)

Cell counts are primarily obtained via the plate count method.

- A sample is serially diluted to several dilute values.
- A ml of this diluted sample is set into agar plate. The plate is then incubated for 24-48 hours.
- Once colonies have formed, they are counted. The count is multiplied by the dilution factor to figure the colony forming units (CFUs) per gram.



Bacterial Concentration

EXAMPLE:

BEC 100 is a 1 billion count product…

THEREFORE

- It has 1 billion CFUs of bacteria per gram,
- Or 1,000,000,000 **C**olony **F**orming **U**nits per gram,
- OR 1 E 9 CFU/g (aka, 1 x 10^9/g)

Bacterial Concentration Calculations

The task of calculating/converting volumes, can be confusing and tedious

The following values are approximate (they imply a specific gravity of 1.00, the s.g. of water)

- 1 Gallon = 3.78 L
- 1 Pound (lb) = 16 ounces (oz) = 454 g
- 1 Gallon = 3785 g = 8.34 lbs.

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Issues with Plate Counting

- Time consuming
- Clumping
- Not feasible for fungal species
- Contamination
- Variability

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Plate Counting Solutions

- Aseptic technique
- Homogenization
- Rigorous training
- Standardized methods