



**Innova**<sup>TM</sup>  
CLEAN WATER SOLUTIONS

# How biological products are engineered in 2019

**Greg Page**



# Common challenges in wastewater



- 40-60% of WWTP operating costs related to sludge management
- Accumulates in lift stations, sewers, WWTP's and lagoons

SLUDGE



Odor: Complaints, tourism losses and discomfort

H<sub>2</sub>S: Permit fines and corrosion damage to equipment

ODORS &  
H<sub>2</sub>S



- Equipment malfunctions and inefficiencies
- High collection and disposal labor/costs
- Inhibits dissolved oxygen & evaporation

FAT, OIL &  
GREASE

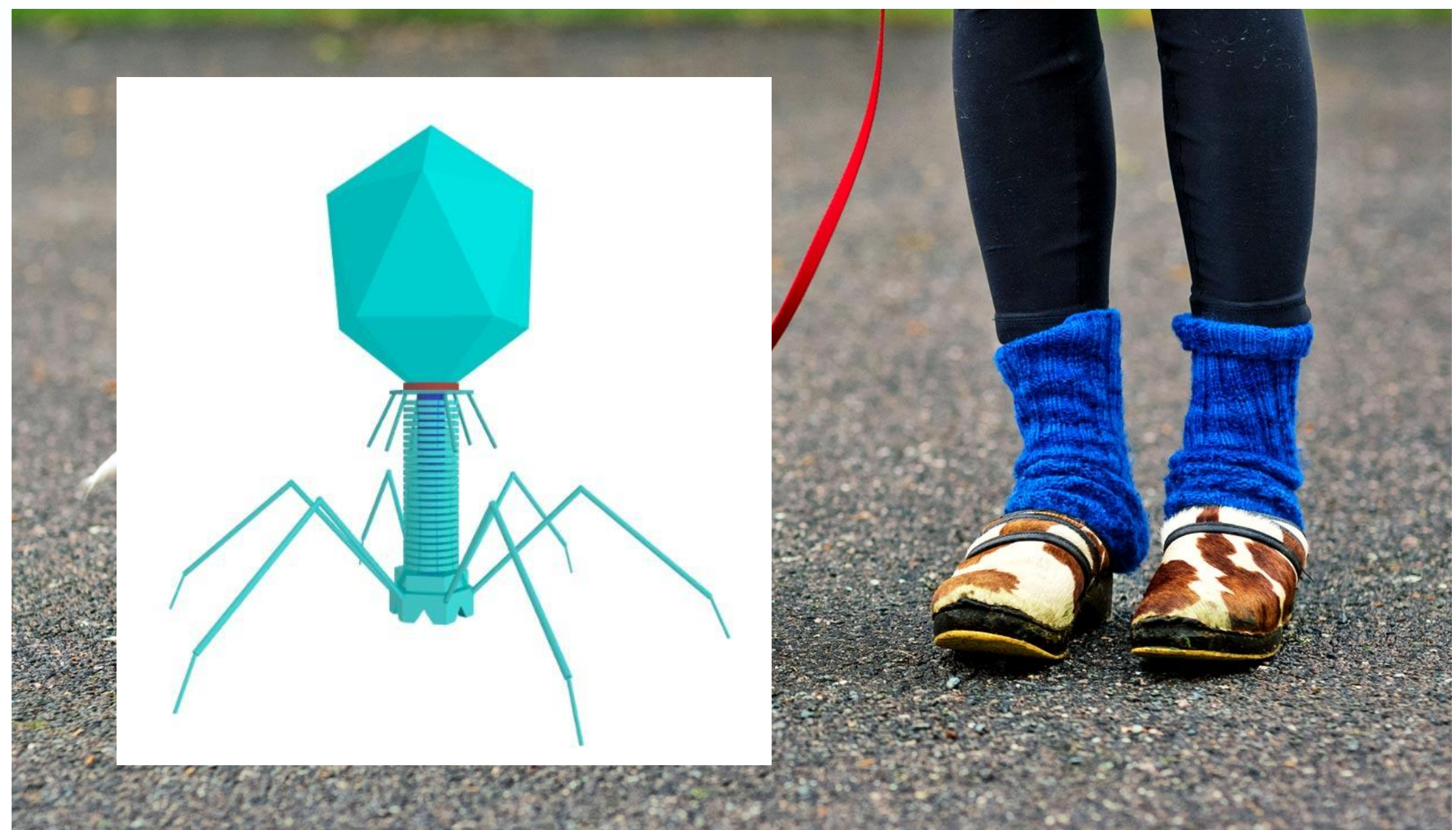


# Canis Lupus Familiaris

- \*When did we domesticate the first “dogs”?
  - ~15,000 years ago
  - \*How many breeds?
    - Over 360
- Dogs bred/trained for different purposes depending on their phenotypes/abilities
- Dogs (wolves) were once a predator to be feared...
- Now they are incredibly useful



# WELCOME TO THE FUTURE





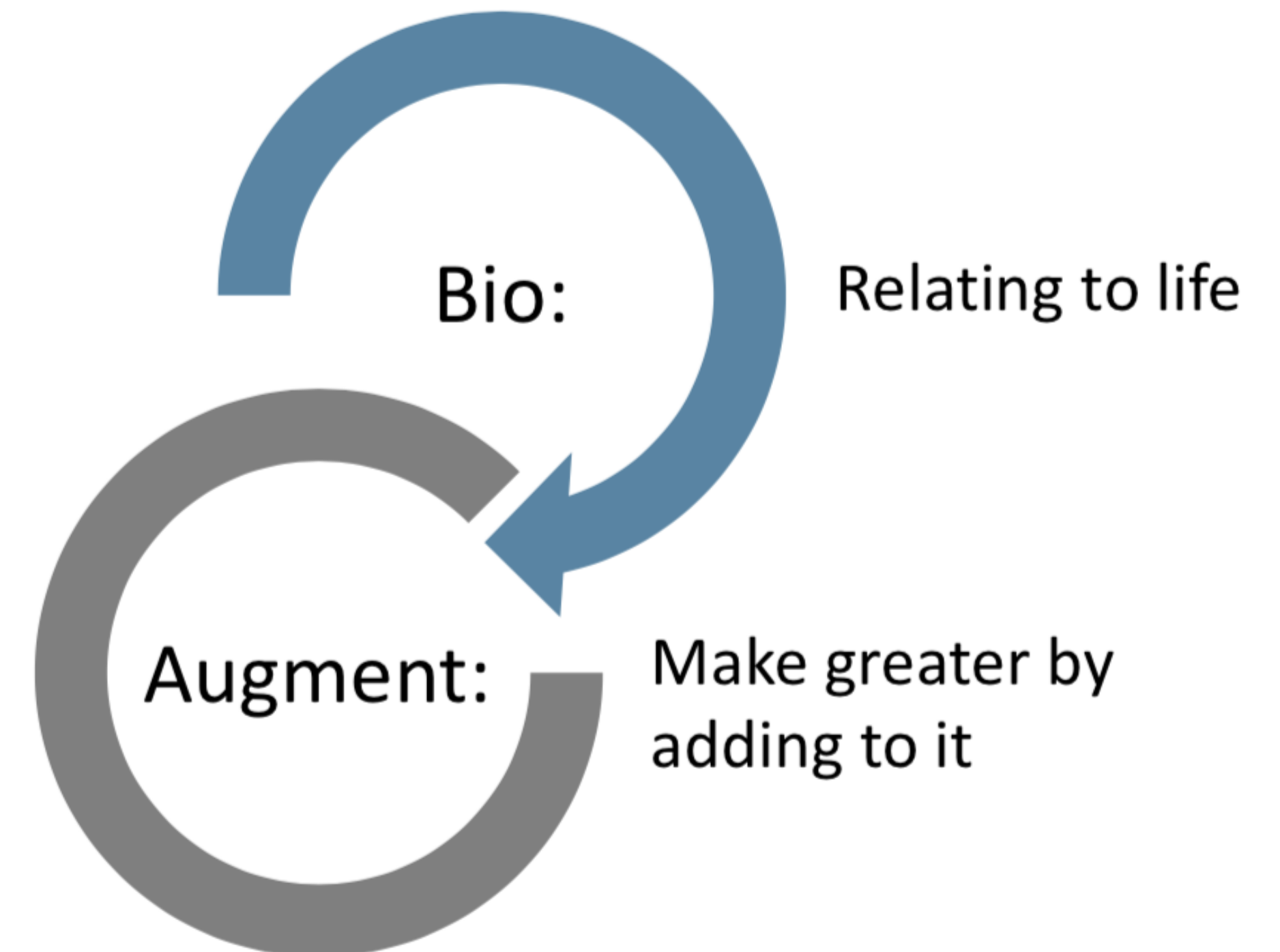
# Bio augmentation...what is it?

What if we could add to our biology to improve it's function?

## Bioaugmentation

### Definition:

“The process of adding **scientifically selected organisms** into a **microbial community** in order to **enhance that community.**”





“People are literally out here eating microbes on purpose”

- Kanye West

# Applications for Bio Augmentation

- Human Probiotics
  - \*39 Trillion human cells
  - 100 trillion microbes
- Agricultural Probiotics
- Bioremediation
- Landfill - PET biodegradation
- Wastewater Treatment



\*There are a lot of strains of bacillus  
- Paul Krauth

[B. acidicer](#)  
[B. acidicola](#)  
[B. acidiproducens](#)  
[B. acidocaldarius](#)  
[B. acidoterrestris](#)  
[B. aeolius](#)  
[B. aerius](#)  
[B. aerophilus](#)  
[B. agaradhaerens](#)  
[B. agri](#)  
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[B. akibai](#)  
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[B. aminovorans](#)<sup>[2]</sup>  
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[B. butanolivorans](#)  
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[B. cecembensis](#)  
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[B. centrosporus](#)  
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[B. dipsosauri](#)  
[B. drentensis](#)  
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[B. ehimensis](#)  
[B. eiseniae](#)  
[B. enclensis](#)  
[B. endophyticus](#)  
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[B. lautus](#)  
[B. lehensis](#)  
[B. lentimorbus](#)  
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[B. licheniformis](#)  
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[B. pycnus](#)  
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[B. qingshengii](#)  
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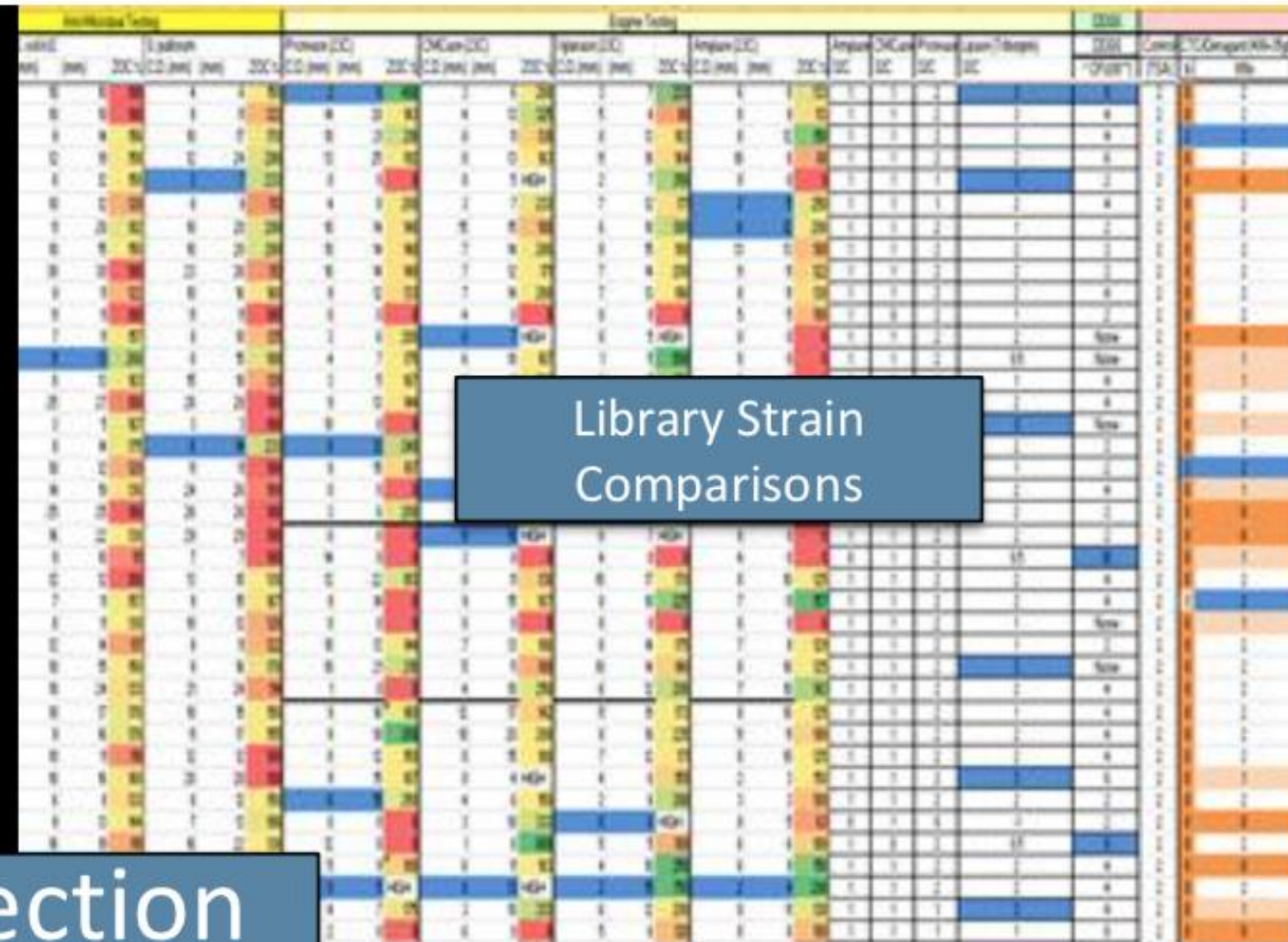
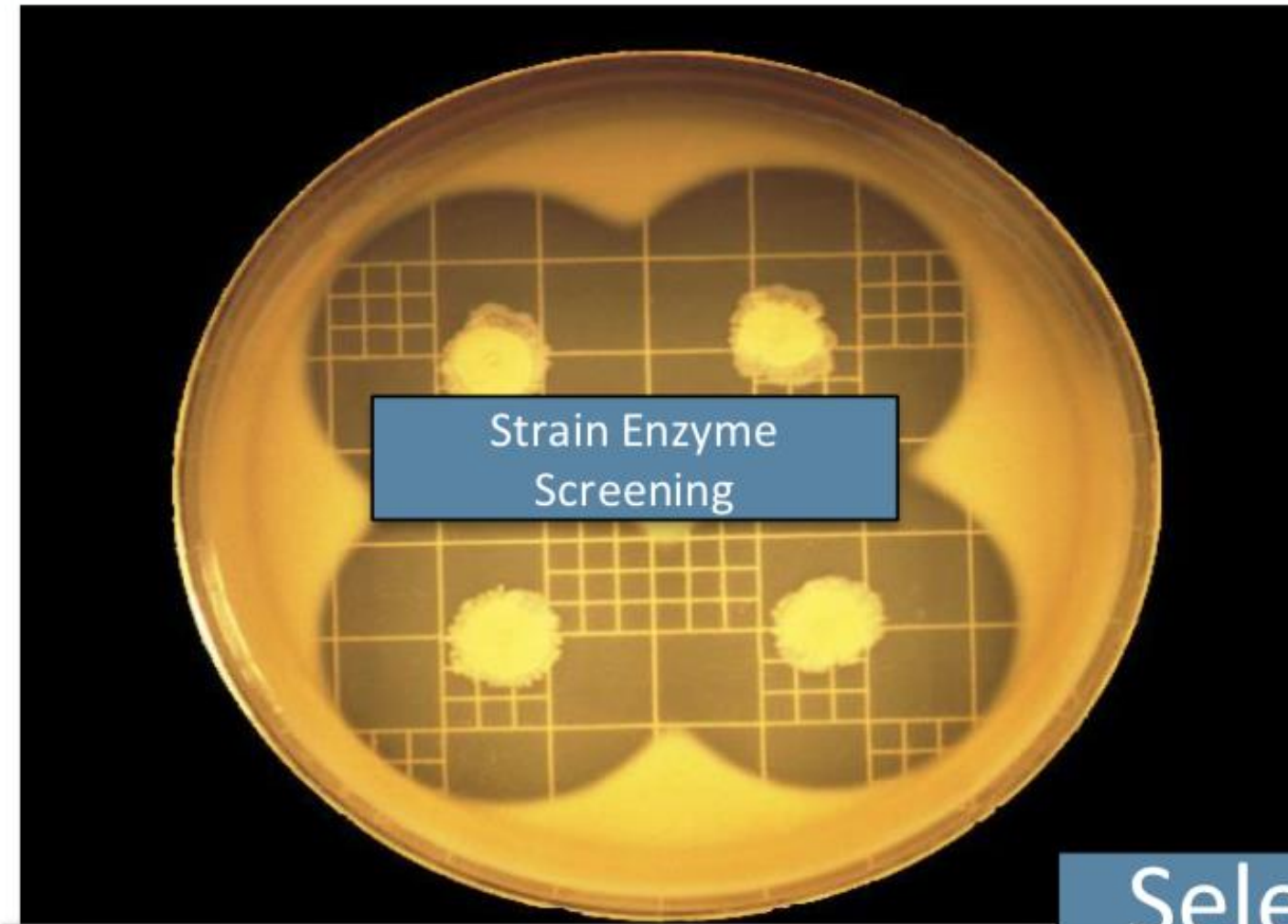


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[B. isronensis](#)  
[B. jeotgali](#)  
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[B. kobensis](#)  
[B. kochii](#)  
[B. kokeshiiformis](#)  
[B. koreensis](#)  
[B. korlensis](#)  
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[B. krulwichiae](#)  
[B. laevolacticus](#)  
[B. larvae](#)  
[B. laterosporus](#)

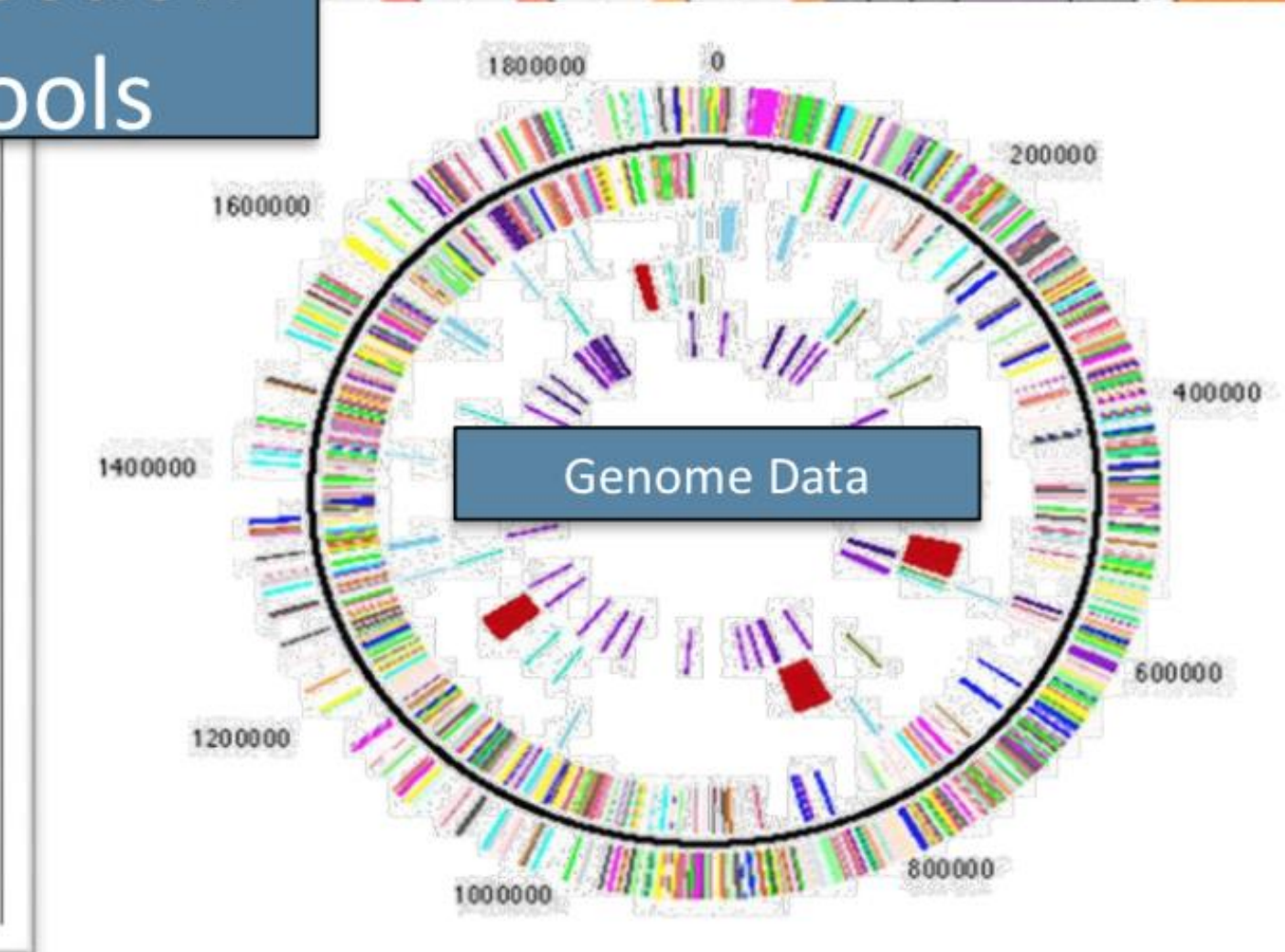
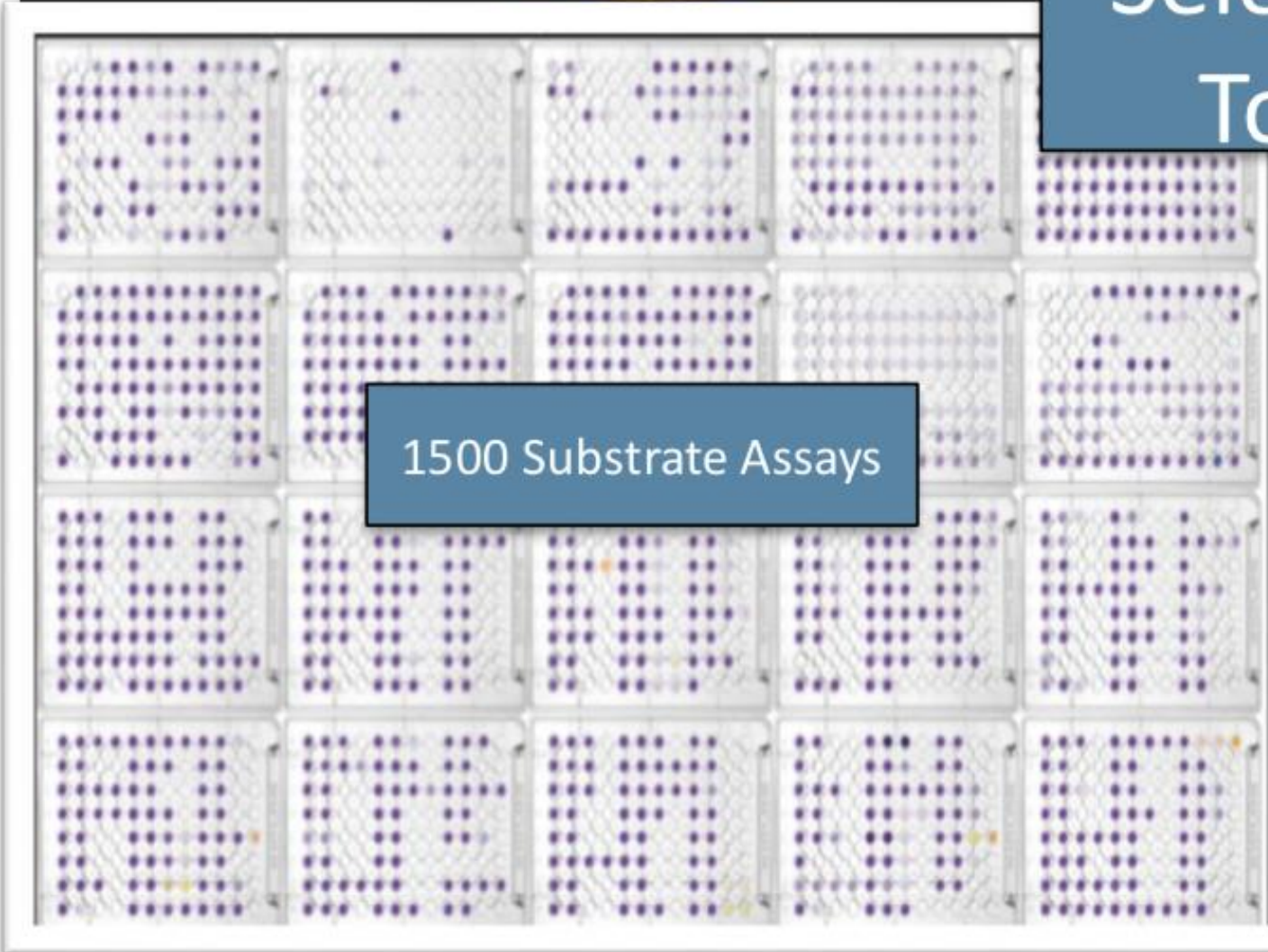
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[B. polymyxa](#)  
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[B. arcticus](#)  
[B. rophilus](#)  
[B. ylovorans](#)  
[B. enulatus](#)  
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[B. velezensis](#)  
[B. vietnamensis](#)  
[B. vireti](#)  
[B. vulcani](#)  
[B. wakoensis](#)  
[B. xiamenensis](#)  
[B. xiaoxiensis](#)  
[B. zhanjiangensis](#)

# Tools for selecting strains



## Selection Tools







- \*A GENOME is an organism's complete set of DNA, including all of its genes. Each GENOME contains all of the information needed to build and maintain that organism.



# SOME BIG WORDS/ACRONYMS

- **\*RT-qPCR**
- **\*FISH**
- **\*ELISA**
- **\*CRISPR CAS9**
- **High-throughput pyrosequencing of 16S rRNA amplicons**



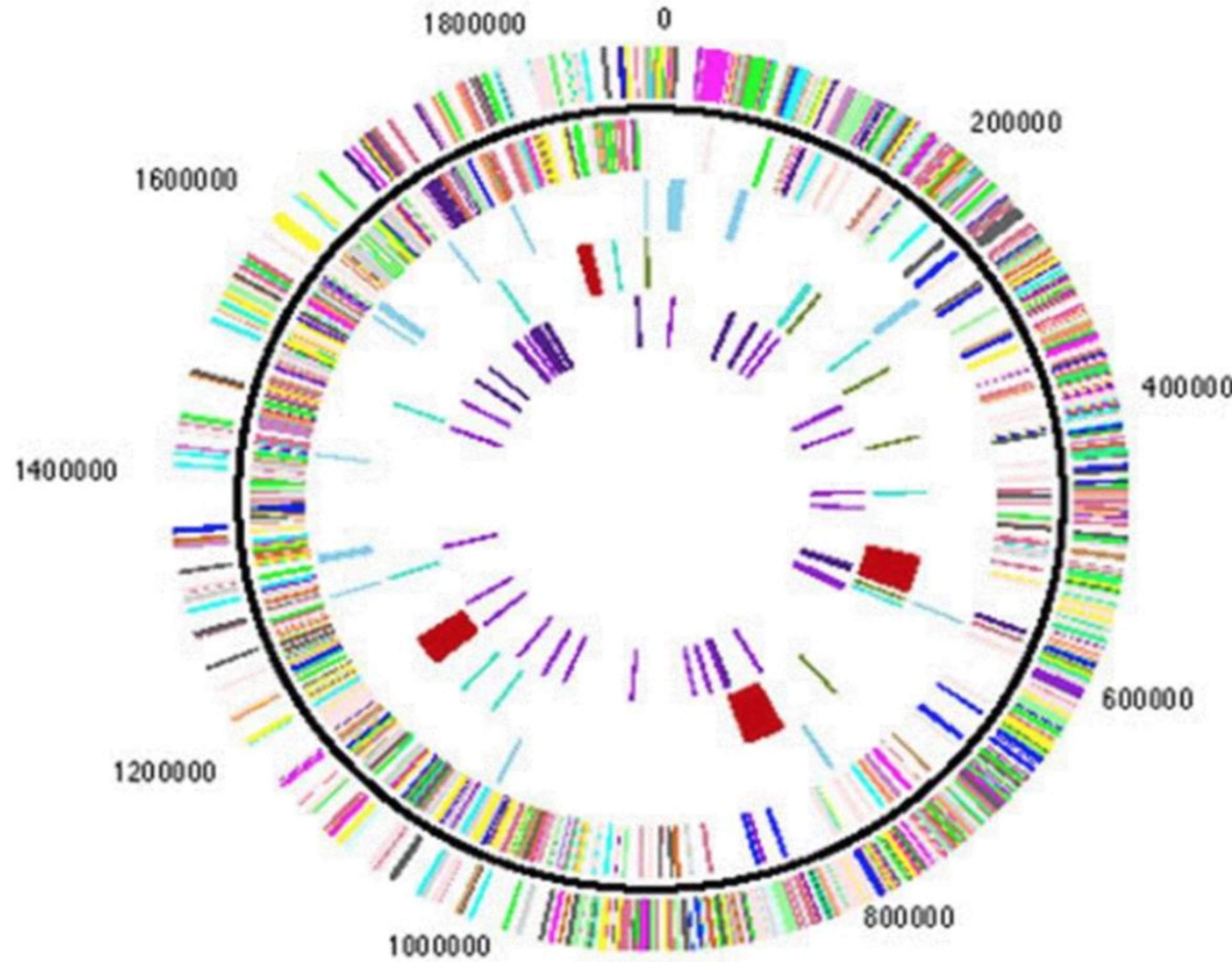
“Life finds a way”

*-Dr Ian Malcolm,  
Jurassic Park*

- \*How much did it cost to map the human genome and how long did it take?
  - \$2.7 Billion
  - 15 years
- \*How about today?
  - \$1000
  - Hours

**WELCOME  
TO THE FUTURE** 

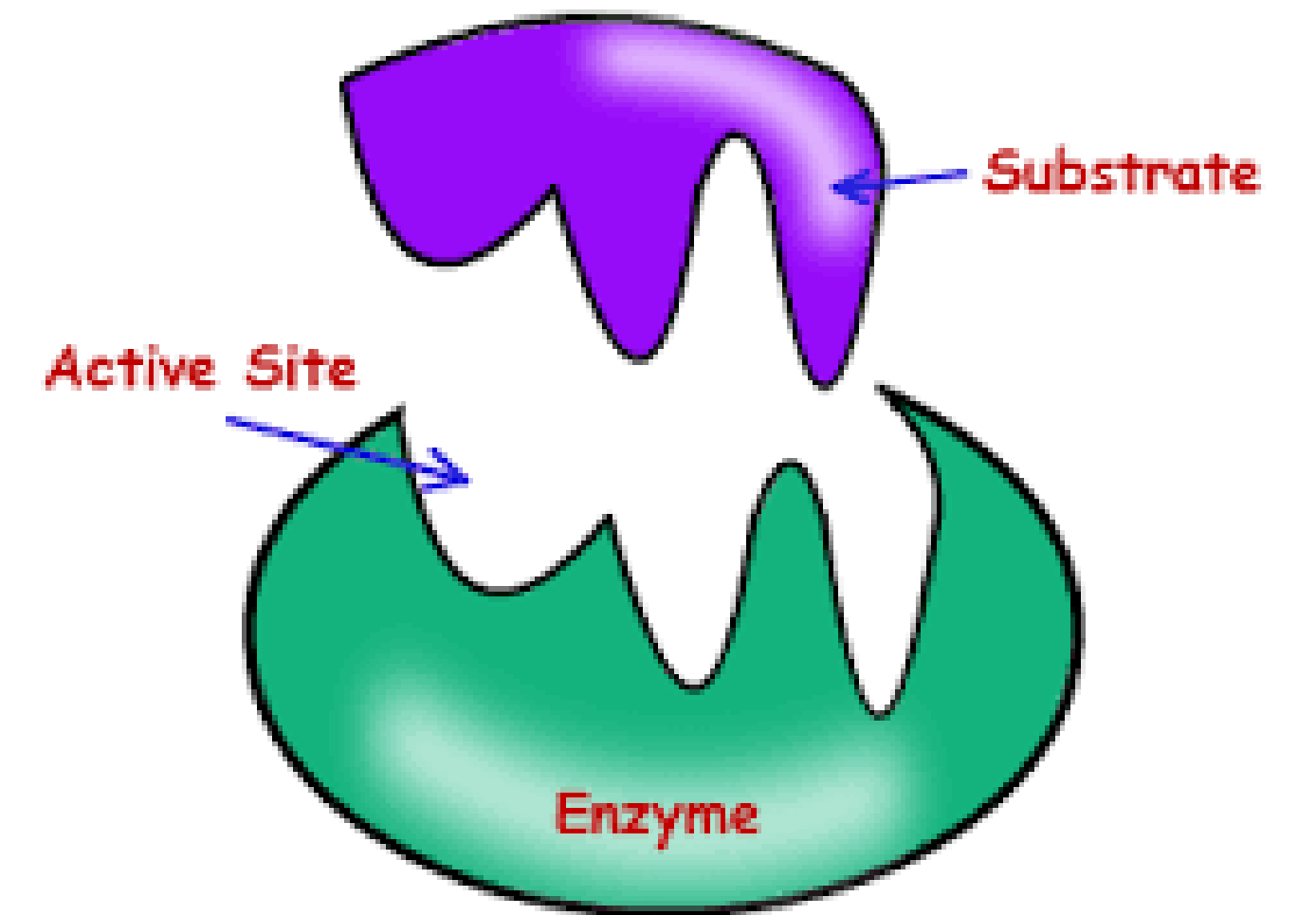
# The Genome of a Microbe



- 502 genes for protein and amino acid metabolism
- 385 genes for carbohydrate metabolism
- 75 genes for lipid and fatty acid metabolism
- 36 genes for sulfur metabolism
- 25 genes for ammonia & nitrogen metabolism
- 20 genes for phosphate metabolism



- \*ENZYMES are biological catalysts which accelerate chemical reactions for specific metabolic pathways
- Specificity is IMPORTANT
  - The right enzyme for a specific substrate for a specific metabolic pathway





# Broad Functional Mechanisms

## WHAT MAKES YOUR BUGS SO SPECIAL?

### Population Growth and Stability

- Promotes surrounding population's growth (more workers = more work)
- Survival across broad range of environments & substrates (spore former)

### Enzymes Production

- General: Amylase, protease, xylanase, cellulase, glucosidase, lipase, esterase, urease
- Specialty: Ligninase, laccase, arabinoxylanase, mannosidase, phytase, monooxygenases, galactosidase, and pectatatelyase

### Biochemical Processes

- Ammonia assimilation, sulfur utilization, phosphate assimilation, aromatic hydrocarbon utilization
- Genes involved with arsenic & cyanide

### Metabolism Methods

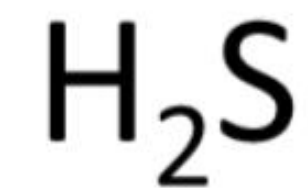
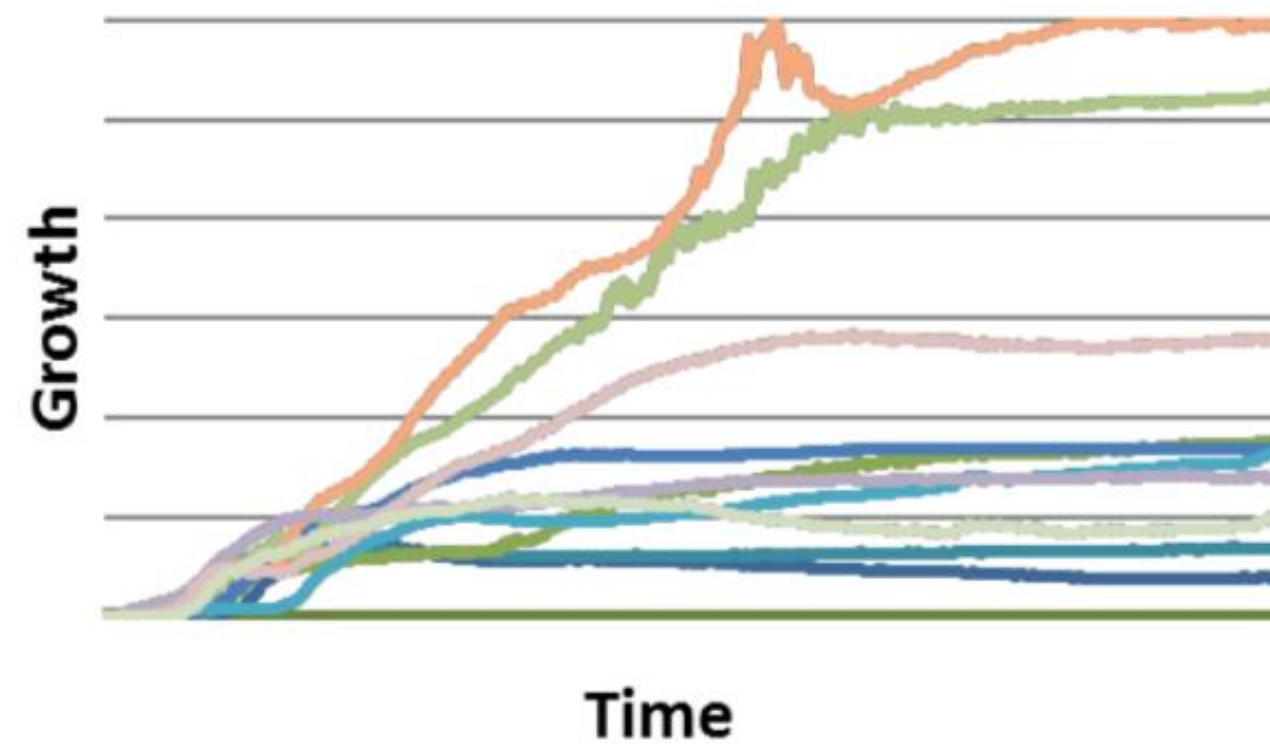
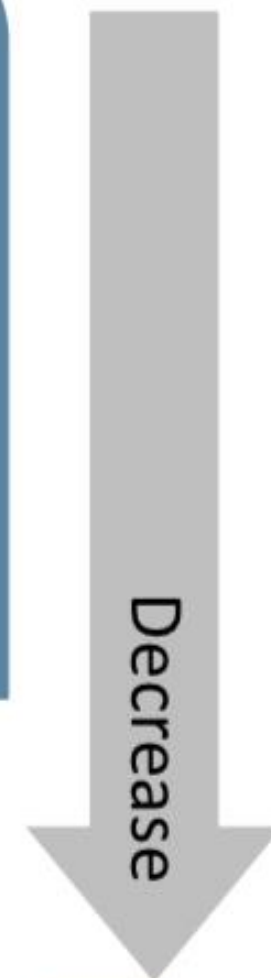
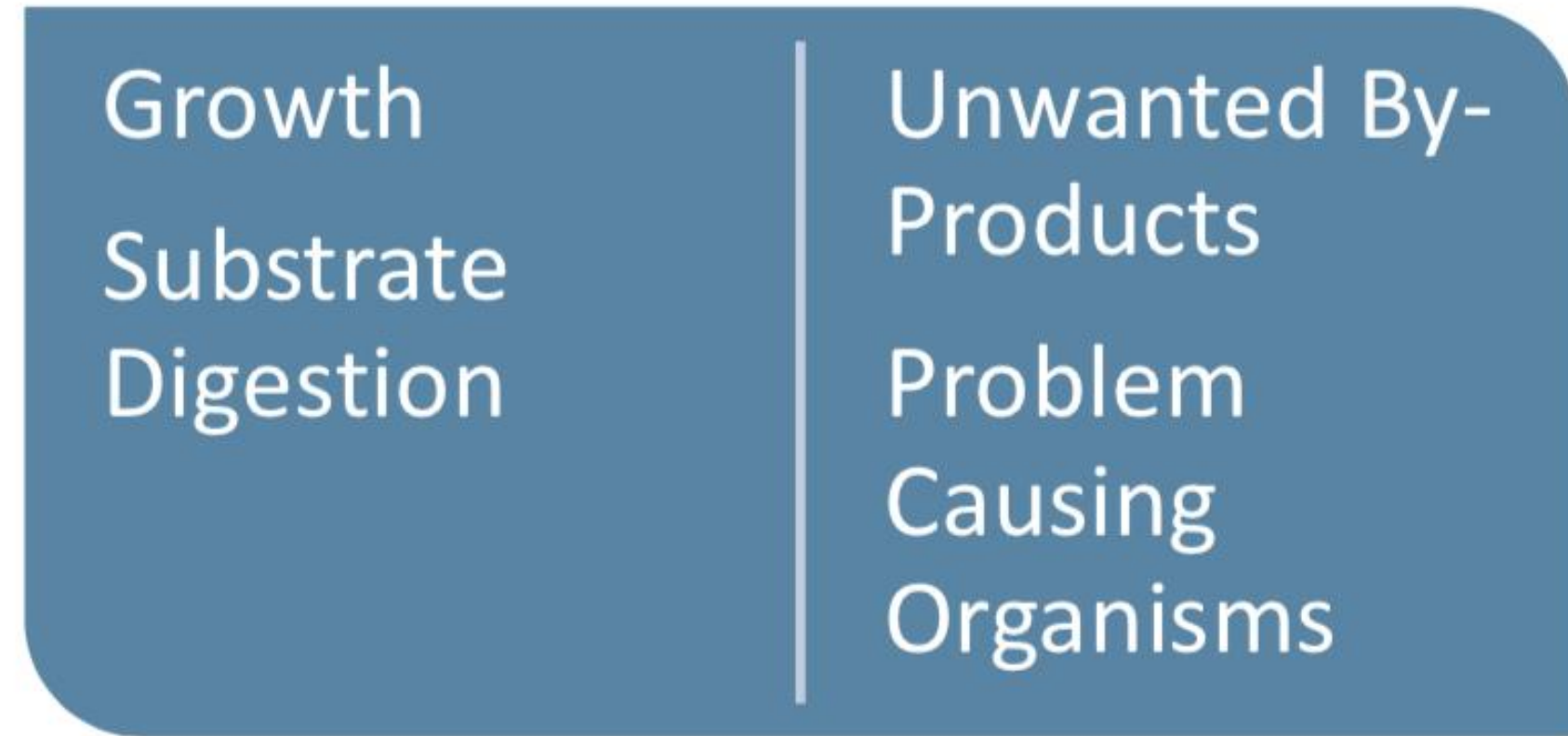
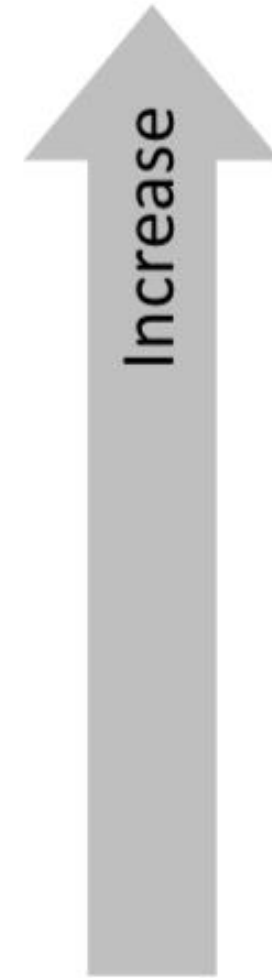
- Aerobic, anoxic and anaerobic growth



# THE ENZYMES

- STARTING TEAM
- AMYLASE
- PROTEASE
- XYLANASE
- CELLULASE
- GLUCOSIDASE
- LIPASE
- ESTERASE
- UREASE
- SPECIAL TEAMS
- Laccase
- Arabinoxylanase
- Mannosidase
- Phytase
- Monooxygenase
- Galactosidase
- Pectatellyase
- Ligninase

# Community Enhancement

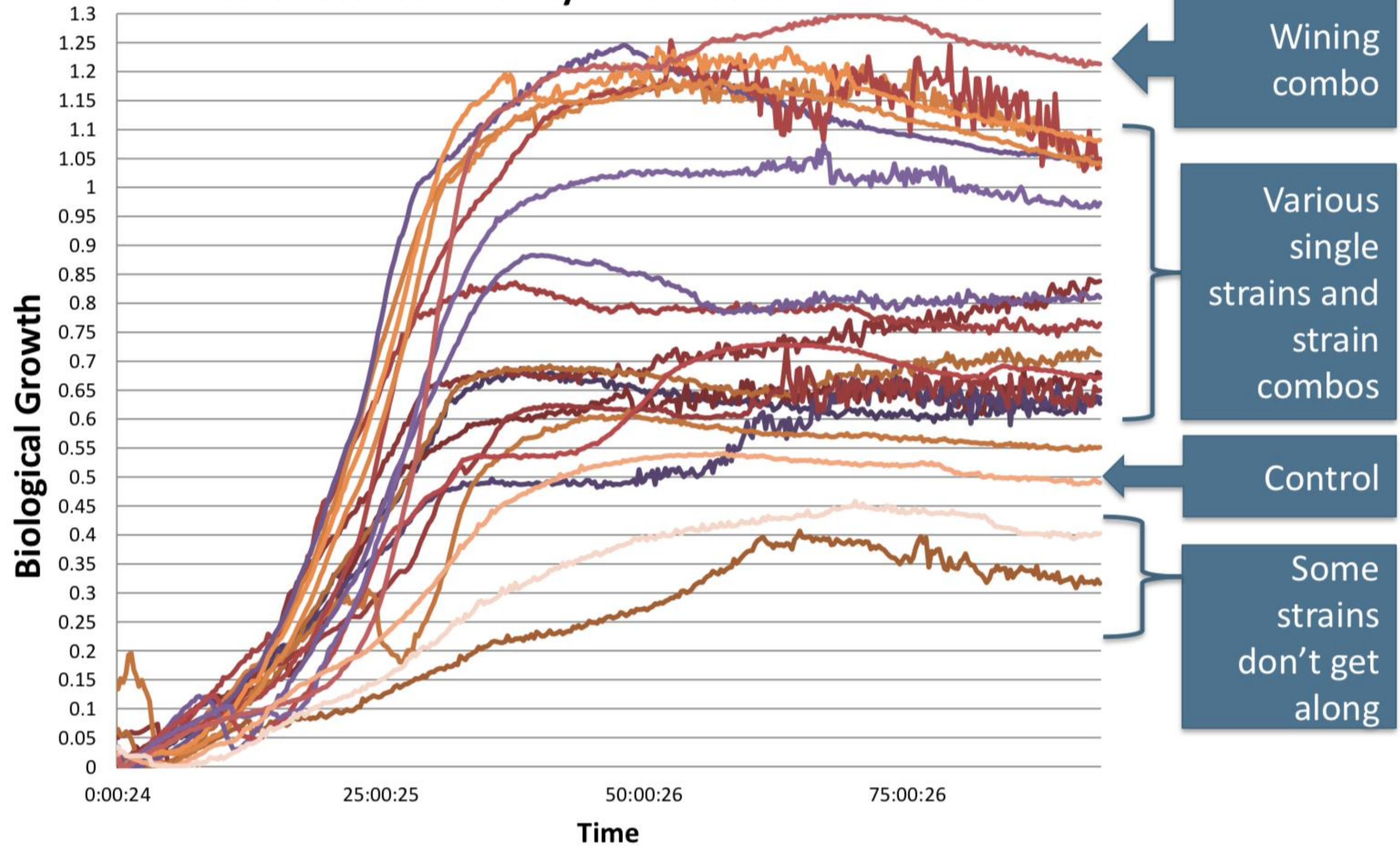




# Strain Combos and Ratios



Wastewater Community + *Bacillus* Strain Combos

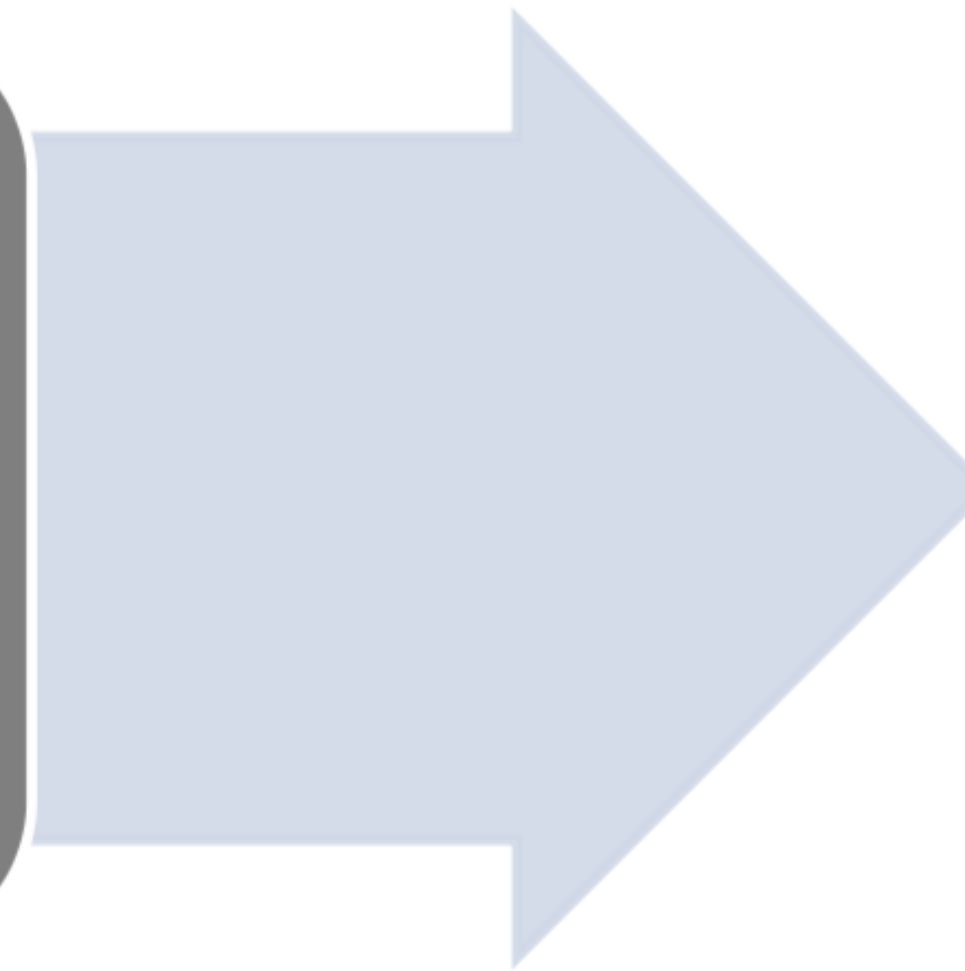




# Reality Check....

*So these are **SUPER BUGS?***

Apply  
anytime,  
anywhere, no  
matter what?



**Just like any other worker, the correct environment is needed to perform.**



# Practical Considerations for Use

## Environmental Tolerance

- pH 4.5-9.0
- Temp: 4-50° C
- Toxicity Resilience
- Facultative

## Product Stability and Reliability

- Shelf life (2 years\*), consistently manufactured

## Ease and Safety of Use

- GRAS
- No Coliforms or Salmonella

# Lift Station Pain Points



Fats, Oils & Grease (FOG) build up

- Layer of FOG over ~70% of surface

Bad odors

- 8 (1-10 scale)
- Lowest - Highest)

Pumped out every 2 weeks

- \$2,200/pump out

Magnesium hydroxide:

- For odor control
- Pricing \$25,000/Year



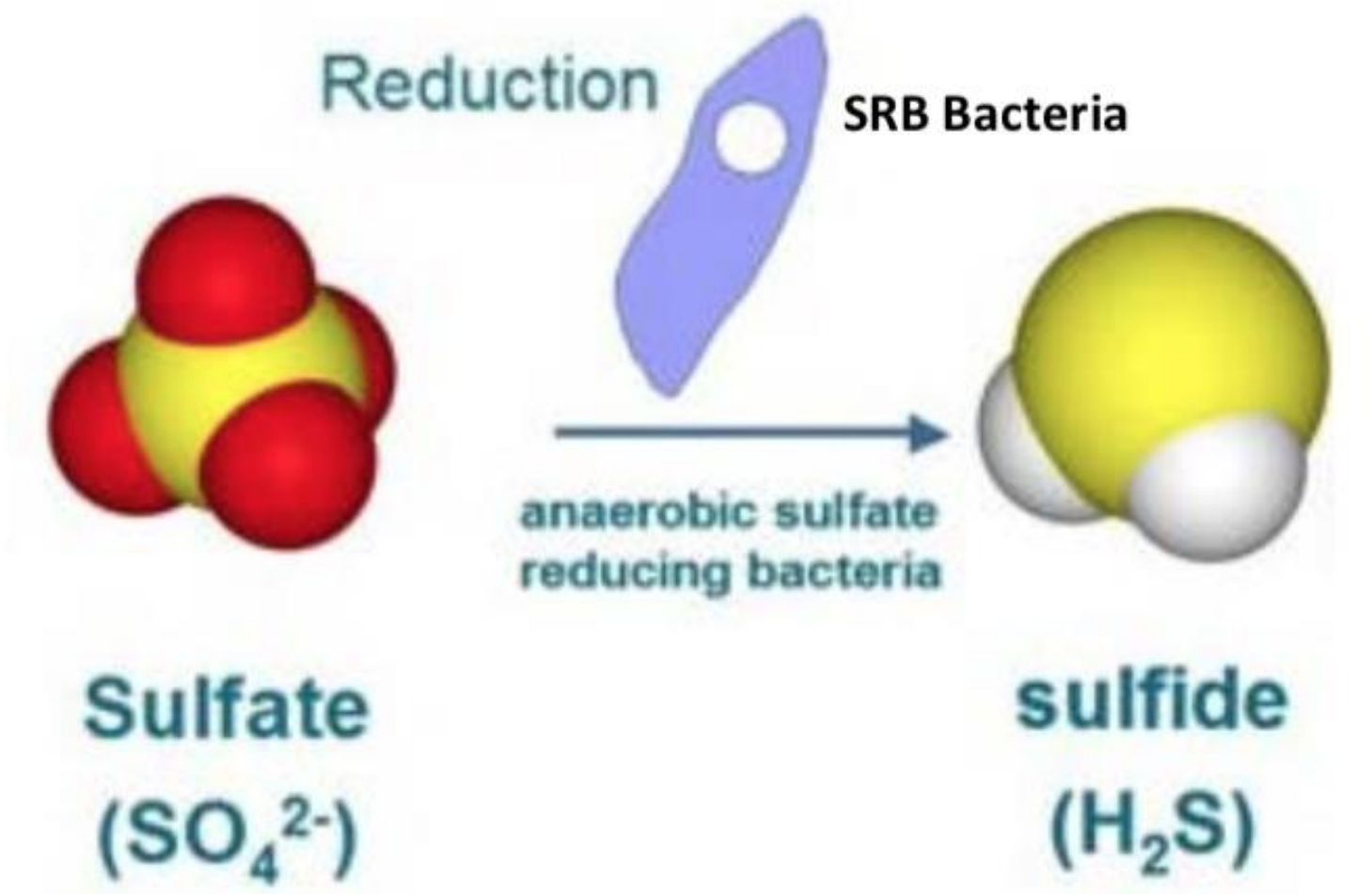
# Odor



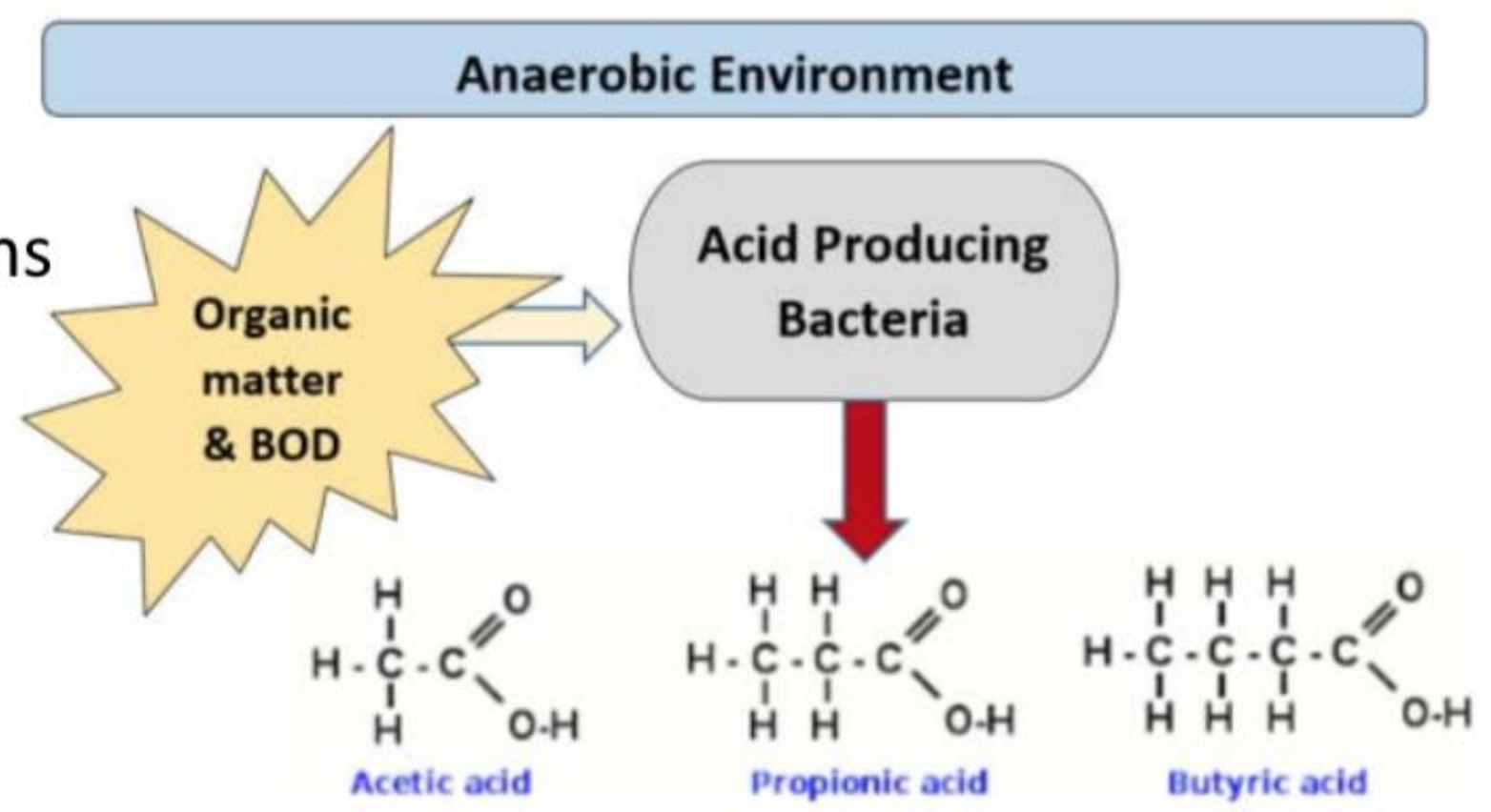
“H2S Smells Bad”

- Abraham Lincoln

- **Hydrogen Sulfide (H<sub>2</sub>S)**
  - **Formed by sulfate reducing bacteria (SRBs)**
    - Use sulfate (SO<sub>4</sub>) as an energy source with the byproduct of H<sub>2</sub>S
    - Live in biofilms and sludge while feeding on volatile fatty acids (VFA's)
    - Thrive in anaerobic conditions



- **Volatile Fatty Acids (VFA)**
  - Acid producing bacteria sometimes require anaerobic environments and similar to SRB's tend to live in biofilms & sludge.
    - The VFA's are odorous themselves but also feed SRB's and indirectly produces H<sub>2</sub>S




# Odor Challenges

- **Hydrogen Sulfide (H<sub>2</sub>S)**

- Hazardous
  - Extra safety and personnel required
- Highly corrosive to equipment
  - Failure & replacement costs
- Discomfort to the community
  - Complaints/fines & business losses

- **Volatile Fatty Acids (VFAs)**

- Promote H<sub>2</sub>S production
- Discomfort to the community (Complaints/fines & business losses)



“We’ve completely replaced calcium nitrate while practically eliminating H<sub>2</sub>S and FOG buildup, at a net cost savings”

Chris Jackson,  
Saratoga Springs UT



“We haven’t had a single odor complaint since starting this program”

Tyler Lowe,  
Payson UT

# Bio Augmentation Impact to Odors

- **Sulfur utilization**
  - Our bacteria required sulfur for growth
    - Less sulfur available for conversion to H<sub>2</sub>S
- **Removal of biofilms and sludge housing SRB and APB**
  - As our bacteria digest sludge and break up biofilms the environment that harbored the SRB & APB is no longer available.
- **VFA and FOG utilization pathways**
  - VFA and FOG are nutrient sources for our *Bacillus*
    - Limits VFA addition to odors as well as food source to SRBs

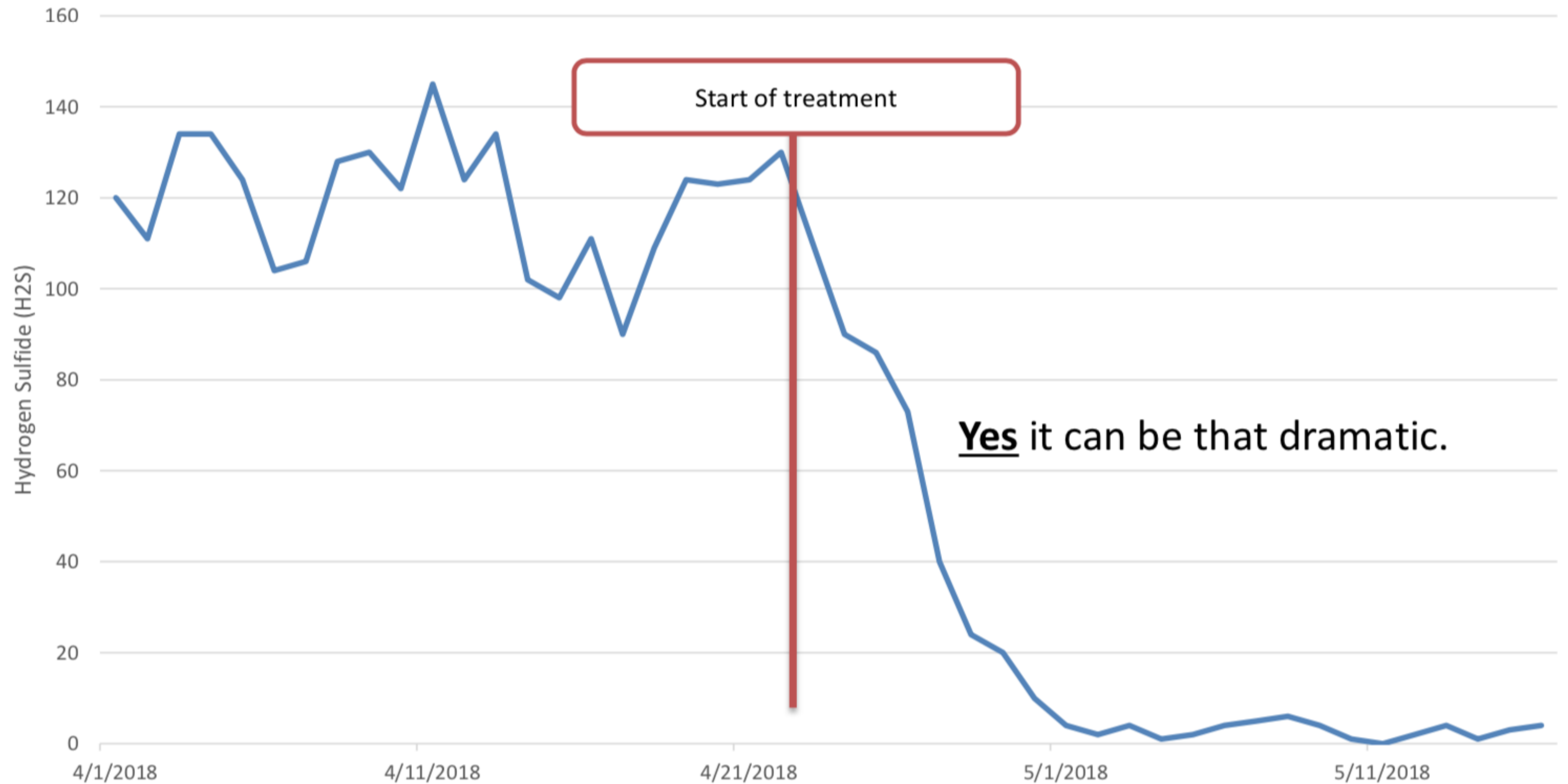
# H2S Reduction



“These [expletive] microbes work pretty [expletive] great”-

Unnamed  
Wastewater  
Superintendent  
Payson Utah

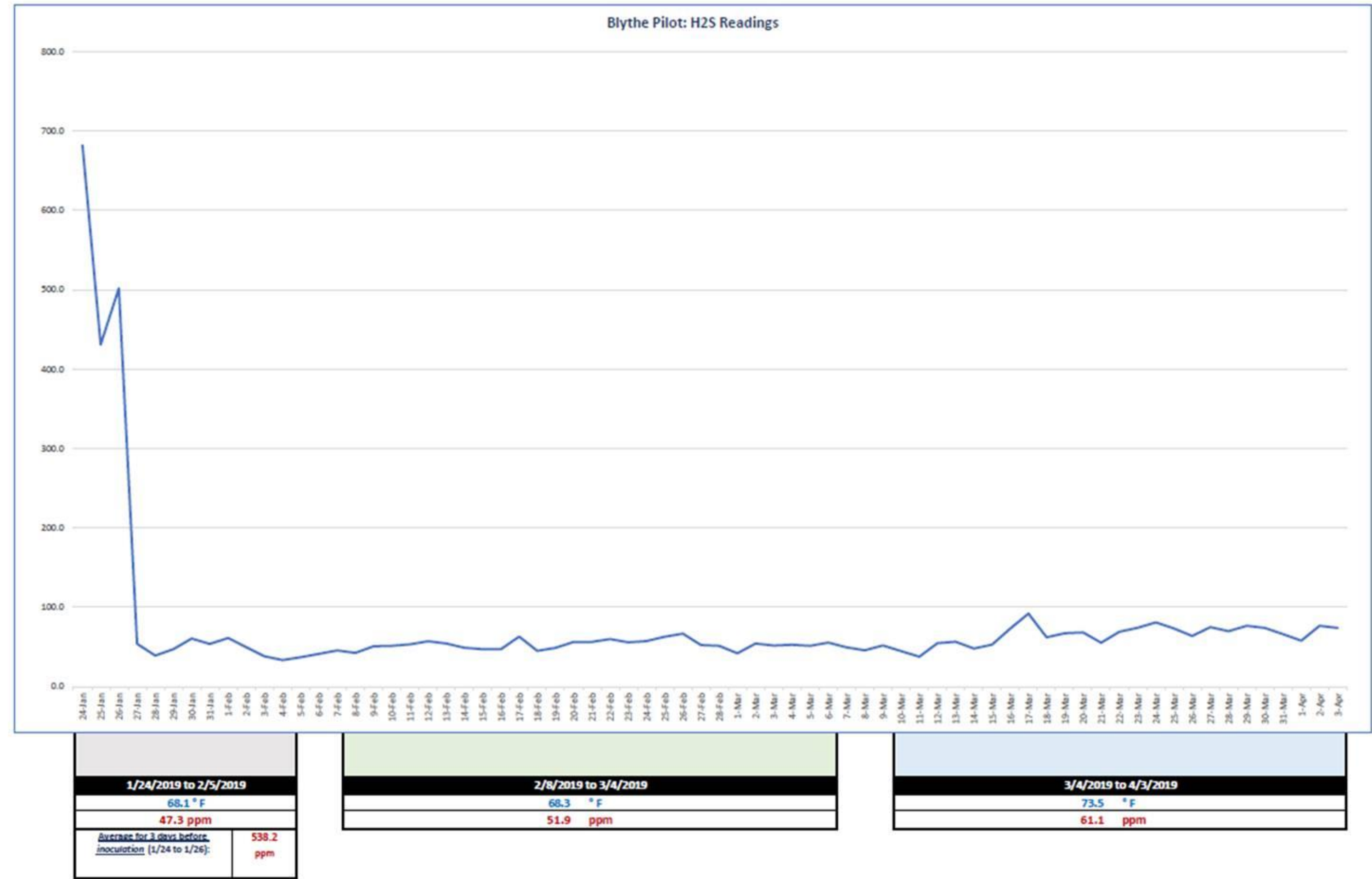
Hydrogen Sulfide Over Time



**Yes** it can be that dramatic.



# Middle of Nowhere, California



# Fats, Oils & Grease (FOG)



## Vacuum, Jetting and Clean-outs

- High cost & labor to remove
- Enzyme/Degreaser products just liquefy and don't remove, push FOG downstream

## Equipment Failure

- Corrosion
- Pump back ups
- Manual labor to fix

## Fines and Complaints

- Biological odors cause discomfort the community
- H<sub>2</sub>S is hazardous and can lead to fines





# How Bacillus Strains Remove FOG

## The Ability

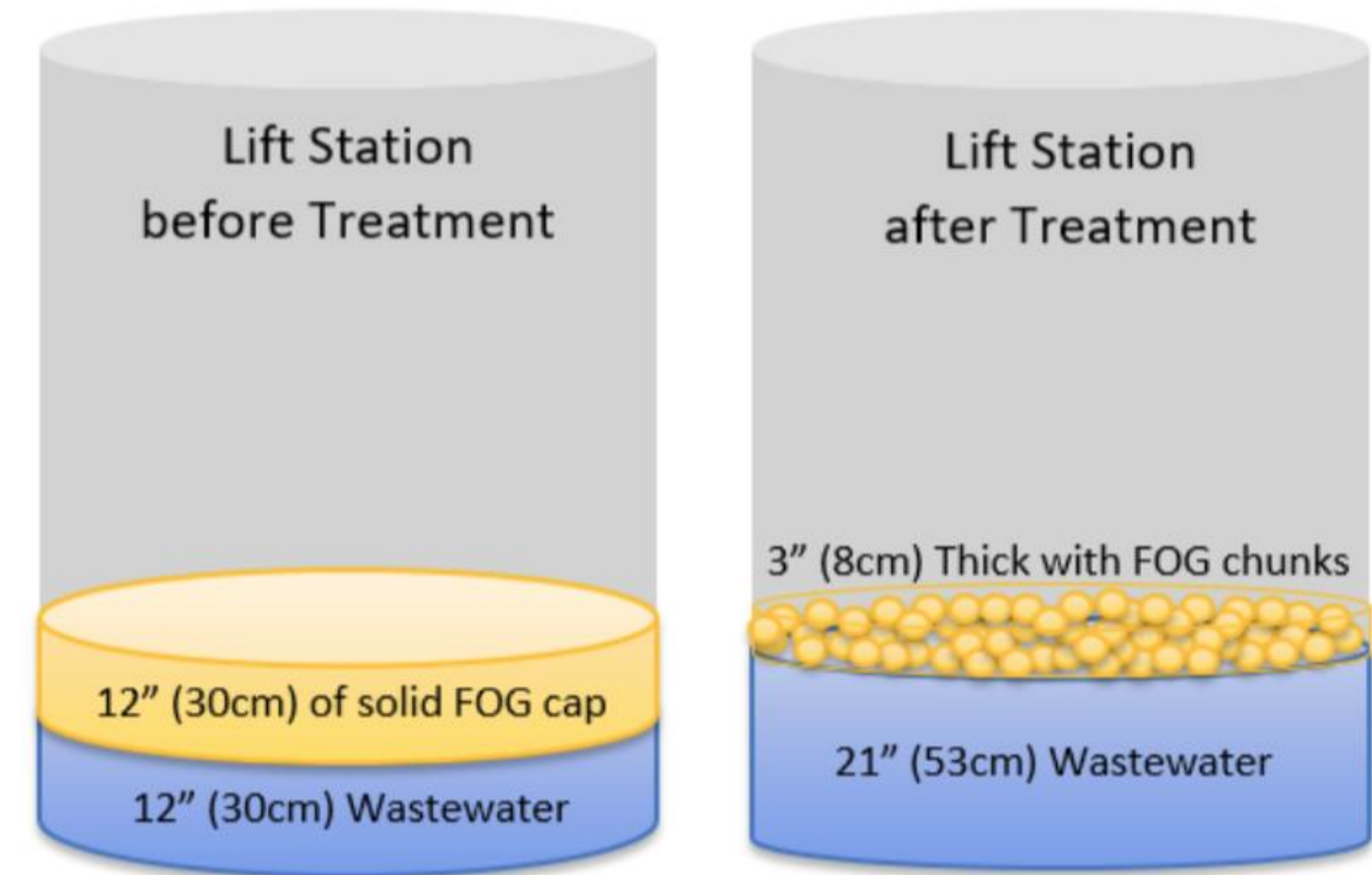
- **Useful Genes:** Our strains have many genes for lipid and fatty acid metabolism

## The Tools

- **Enzymes:** Lipases, esterases, and many others involved in long chain fatty acid & hydrocarbon digestion
- **Micronutrients:** Key for enzyme activation and microbial growth

## The Access

- **Bacteria produce bio-surfactants**
  - Helps overcome hydrophobic/hydrophilic interface of FOG & Water



Before

After 3 weeks



We aren't the only ones in the game!



# Comparing Alternative Solutions



“I wasn’t sure  
what to say  
right here”

*Greg*

- Metal Salts (Ferric)
- Magnesium Hydroxide
- Calcium Nitrate
- Sealing/rubbernecking Manholes
- Vapor Phase filtration
- Mechanical ventilation



**THIS IS THE LAST SLIDE**  
**Feel free to forget everything you just**  
**heard**

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