QUORYM

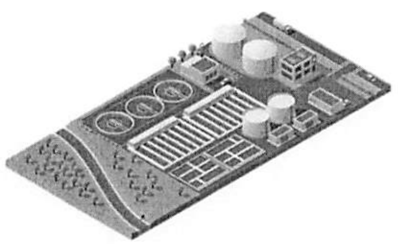
1

Biological Metabolic Processes and Nutrient Removal

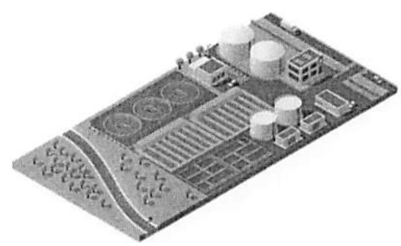
Presented by QUORYM

Innovator of Biological Treatment Systems

2



3



4

Activated Sludge Process Control Parameters

- DO
- PH
- Specific Oxygen Uptake Rate (SOUR)
- Mixed Liquor Suspended Solids
- Mixed Liquor Volatile Suspended Solids
- MLSS/MLVSS Ratio

5

Organic Compounds Removed

- Fat, Oil, and Grease (Fatty chain acids)
- BOD
- Nitrogen
- Phosphorus (Biological Uptake)

6

Organic Compounds Added

- Sludge (Biomass)
- Oxygen
- Media (Surface Area)

7

Byproducts of Biological Removal

- N2 (Gas)
- CO2 (Gas)
- H2S (In the absence of alternative e- acceptor)

8

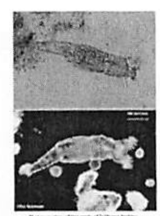
Microbial Environment

- Protozoa
- Bacteria
  - Gram Positive and Gram Negative
- Long Filamentous (Scaffolding)
- Enzymes (Biological byproduct)

9

Protozoa

- Characterization evaluates microbial biodiversity
- Qualitative Analyses useful in determining control parameters
- Not responsible for nutrient metabolism



10

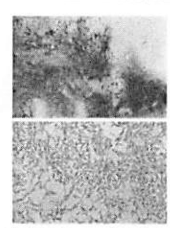
Enzymes vs. Bacteria

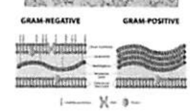
- Enzymes
  - Act as a catalyst for redox reactions
  - Not living, and are a biological byproduct
  - Efficient at liquifying solids (ie. FOG)
  - Similar to chemical treatment
  - Will not metabolize pollutants
- Bacteria
  - Living organisms
  - Produce enzymes to help break down solids and process carbon sources
  - Bacteria will fully metabolize carbon sources

11

Bacteria Taxonomy

- Gram Positive
  - Cell wall comprised of primarily Peptidoglycan
  - No outer membrane
  - Stain purple in a Gram stain procedure
- Gram Negative
  - Cell wall comprised of outer membrane, periplasmic space, and cytoplasmic phospholipid membrane
  - Stain red in Gram stain procedure





Photos courtesy of the Center for Disease Control

12

Types of Cellular Metabolism

- Autotroph
  - Does not require carbon source from environment
  - Photoautotroph
    - Requires Sunlight (anabolic pathway)
  - Chemoautotroph
    - Requires energy from inorganic oxidation (catabolic pathway)
- Heterotroph
  - Requires Carbon source from environment
  - Photoautotroph
    - Requires Sunlight (anabolic pathway)
  - Chemoautotroph
    - Requires energy from inorganic oxidation (catabolic pathway)

13

Biochemical Properties

- Chemolithoheterotroph
  - Most efficient in intermittent DO levels (Aerobic and microaerobic concentrations, SBR process)
  - Grow rapidly in Aerobic environment
  - Denitrification occurs in microaerobic and anaerobic conditions
  - Capable of lipid metabolism
  - i.e. nirS-type betaproteobacteria, firmicutes, pseudomonas
- Chemolithoautotrophs
  - Typically obligate aerobes
  - Convert ammonia to nitrate
  - Not capable of fatty acid chain metabolism
  - Only viable in liquid phase
  - Includes some Methogens, Halophiles, Sulfur Reducers, Nitrifiers, and Thermoacidophiles
  - i.e. Nitrosomonas, Nitrobacter, etc.

14

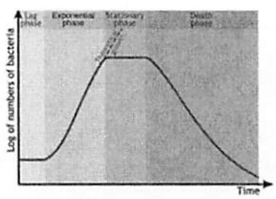
Biochemical Properties

- Anaerobic Chemoheterotrophs
  - Includes Methane producing bacteria
  - Includes Sulfide reducing bacteria
  - Require carbon source
  - Obligate anaerobes (require <0.2 ppm DO)
- Aerobic Chemoheterotrophs
  - Highest rate of carbon source metabolism
  - Capable of lipid metabolism
  - Abundant in aerobic treatment chambers

15

Bacterial Growth Curve

- Lag Phase
  - Bacteria is adjusting to environmental factors
- Exponential Phase (Log Phase)
  - Cells grow at an exponential rate
  - Binary growth function $N=N_0 2^n$
  - Grow by doubling (2,4,8, etc.)
  - Healthiest cells, optimal nutrient and carbon uptake
- Stationary Phase
  - Cells are limited by nutrients, carbon source, or space
  - Stop binary fission, activate genes to prepare for starvation
- Death Phase (Decline Phase)
  - Cells lose viability, Cell density decreases as cell die
  - Will not reproduce if reinoculated



16

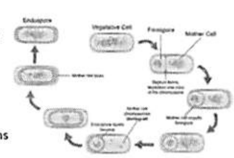
Vegetative Cell Growth

- Vegetative cells incapable of sporulation will follow cell growth
- High uptake of nutrients
- High uptake of carbon source
- Goal of continuous and fed-batch reactor growth is to maintain vegetative log state
- Most gram negative cells or strictly vegetative
- Cannot be stored for long periods of time at room temperature (<2 weeks on Agar medium)

17

Sporulation/Germination

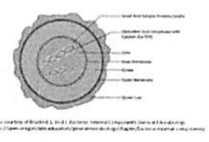
- Sporulation is a survival mechanism
- Ability to sporulate only expressed in Gram Negative
- Induced by nutrient exhaustion
- Produces highly resistant endospore
- Sporulation induced at end of log phase
- After endospore is produced, nutrient uptake stops
- Cells capable of Sporulation ideal for dynamic systems with large nutrient range
- Upon environmental change suitable for vegetative state cells will germinate back to vegetative state



18

EndoSpore

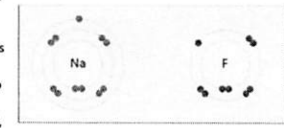
- Highly resistant survival mechanism
- Resistant to a large range of:
  - Temperatures
  - pH
  - Volume limitation
  - Nutrient depletion
- Capable of long storage time 1-3 months (@ room temp)
- Contains DNA to return back to vegetative state



19

What is Redox?

- Redox means "Reduction-Oxidation Potential"
- Two atoms (or molecules) that are unstable share an atom to increase the stability of the resulting molecule
- Unstable atom with an extra electron needs to give it away to be stable (Base)
- Unstable atom lacking an electron needs to take an electron to be stable (Acid)
- When they react the base oxidizes the acid, and the acid reduces the base



20

What is Redox?

- The "potential" for a redox reaction is dependent on the strength of the reactant
- Strong oxidizers (bases/caustics) like Sodium Hydroxide have a large potential
- Strong acids (acids) like Sulfuric Acid have a large potential
- The higher the potential of the reaction, the more heat will be produced
- Redox potential is similar to magnetism
  - Low potential is comparable to a refrigerator magnet
  - High potential is comparable to a crane lift magnet
  - High potential will increase the "speed" that the reaction will take place

21

Redox Biological Metabolic Processes

- Carbon Metabolism
  - $C_6H_{12}O_6 + 6O_2$ to $6CO_2 + 6H_2O$ + heat
- Nitrification
  - $2NH_4^+ + 3O_2$ to $2NO_2^- + 4H^+ + 2H_2O$
  - $2NO_2^- + O_2$ to $2NO_3^-$
- Denitrification
  - $NO_3^- + 2H^+ + 2e^-$ to $NO_2^- + H_2O$
  - $NO_2^- + 2H^+ + e^-$ to $NO + H_2O$
  - $2NO + 2H^+ + 2e^-$ to $N_2O + H_2O$
  - $N_2O + 2H^+ + 2e^-$ to $N_2 + H_2O$
- Methanogenesis
  - $CO_2 + 4H_2$ to $CH_4 + 2H_2O$ (via Hydrogen gas partial pressure)
  - CH_3COOH to $CH_4 + CO_2$ (via acetate pathway)
- Sulfide Reduction
  - $SO_4^{2-} + 4H_2$ to $H_2S + 2H_2O + 2OH^-$

22

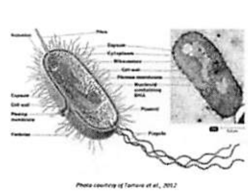
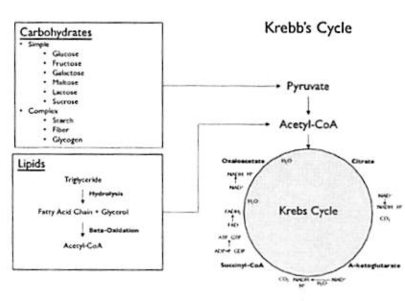


Photo courtesy of Tamura et al., 2012

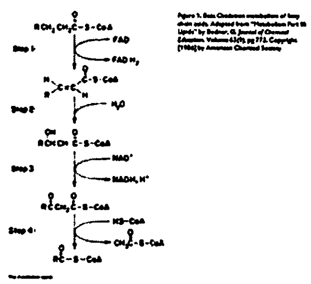
Metabolic remodelling during biofilm development of Bacillus subtilis, Pisithkul T., et al., 2019

23

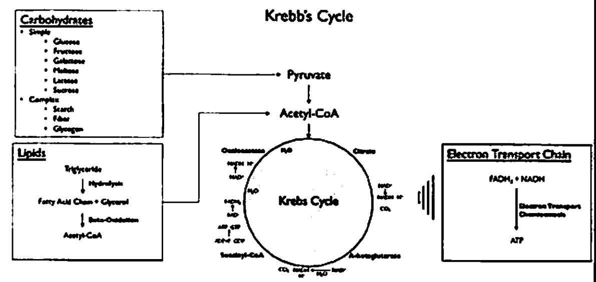


24

Beta-Oxidation Mechanism

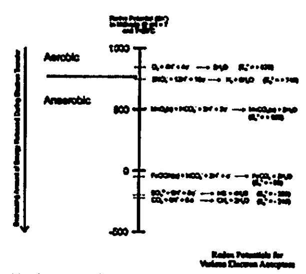


25



26

Electron Transport Chain
Reduction Oxidation Potential



27

Importance of Oxygen Transfer

- Increased Redox Potential responsible for increased metabolic rate
- Oxygen availability is dependent only on the dissolved portion of oxygen
- Oxygen transfer is the amount of oxygen dissolved during an oxygen bubbles contact with water
- Parameters of oxygen transfer:
  - Surface area of air bubbles (small bubbles = high surface area)
  - Temperature
  - Contact time
- Course air will have very low oxygen transfer
- Fine air will have very high oxygen transfer

28

Benefits of Aerobic and Anaerobic Treatment Systems

- Aerobic (Activated Sludge and Biofiltration)
  - Increased BOD rate of removal
  - Will function better then anaerobic systems in low pollutant concentrations
  - Increased stability
  - SBR systems have greater Nitrogen removal rates
  - Increased surface for biofilm production (biofiltration)
- Anaerobic
  - 95% of Carbon is released as byproduct (CH4 and CO2) and only 0.05 contributes to biomass
    - Reduced sludge waste
  - Production of Methane for energy use
  - Excellent pretreatment option for high pollutant wastewater concentrations

29

Solving Problems Using Cellular Metabolism

- Nitrogen (Ammonia, Nitrate)
- Phosphorus
- BOD
- FOG
- H2S
- Settleability

30

Collection System Biological Activity

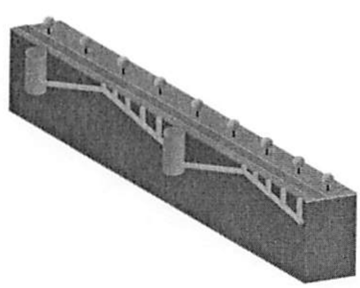
- Surface Area
- Dissolved Oxygen
- Nutrients (N & P)
- BOD
- FOG
- Detention Time
- Biofilm Biodiversity

31

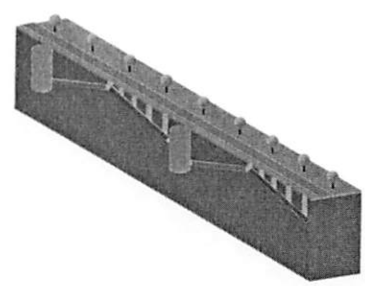
Using Cellular Metabolism to Enhance Collection System Efficiency

- Biofilm Production
  - Increase cell concentration for metabolic processes
- FOG (fatty acid chain) metabolism
  - Reduces grease collection in sewer and lift stations
- BOD Metabolism
  - Uptake of carbon sources for energy production
  - Reduce Carbon source from potential Sulfide producing organisms
- Aerobic/Microaerophilic Conditions
  - Introduce alternative electron acceptors for increased biodegradation and decrease Sulfide gas byproduct

32



33



34

WWTP and Collection System Cell Troubleshooting

- Alternative electron acceptor (O2, N, Fe, MnO2)
  - Increase O2, N, Fe, MnO2
    - Decrease Hydrogen Sulfide (H2S)

35

WWTP and Collection System Cell Troubleshooting

- Cell Metabolism
  - Increase Cell Concentration
    - Decrease N
    - Decrease P (Bio P membrane uptake)
    - Decrease BOD
    - Decrease FOG

36

WWTP and Collection System Cell Troubleshooting

- Nitrifier
  - Increase Nitrifier
    - Increase Nitrate
    - Decrease Ammonia

37

WWTP and Collection System Cell Troubleshooting

- Denitrifier
  - Increase Denitrifier
    - Increase N2 gas (Inert)
    - Decrease Nitrate

38

WWTP and Collection System Cell Troubleshooting

- FOG
  - Decrease FOG
    - Increase Filamentous density
    - Increase Settleability

39

WWTP and Collection System Cell Troubleshooting

- O2
  - Increase O2
    - Decrease denitrification
    - Increase Metabolism
    - Increase Colony Growth
    - Remove Obligate Anaerobes
  - Decrease O2
    - Increase denitrification
    - Decrease metabolism
    - Decrease Colony growth
    - Remove Obligate Aerobes (<1ppm)

40

Biological Pretreatment Troubleshooting

- Carbohydrates/BOD
- Fatty Acid Chains/FOG
- Hydrogen Sulfide
- NBOD (Nitrogenous Biological Oxygen Demand)
- SSO (Sanitary sewer overflow)
- CSO (Combined sewer overflow)
- Nitrogen/Nitrate/Ammonia
- Corrosion

41

References

[illegible]

42

Questions?

43