Follow the electrons to understand microbial metabolism, find pollution problems, and energy opportunities

• Removing electrons from a material is oxidation, adding electrons is reduction, and the shorthand name in Redox.

• Redox reactions almost always must be catalyzed, which is why the microorganisms are able to do them.
  – They get the energy to grow and sustain themselves
  – We get the service from their enzymes
Follow the electrons -- fuel microbial life

• The transfer of electrons from a donor to an acceptor yields the energy the fuels life.
  – For example, oxidation of glucose yields 24 e⁻ eq and 41.35 kJ of free energy per e⁻ eq.
    \[ \text{C}_6\text{H}_{12}\text{O}_6 + 12\text{H}_2\text{O} \rightarrow 6\text{H}_2\text{CO}_3 + 24\text{H}^+ + 24\text{e}^- \quad (\Delta G^\circ = -41.35 \text{ kJ/e}^-\text{eq}) \]
  – Transfer of those 24 e⁻ eq to O₂ gains 78.72 kJ/e⁻ eq)
    \[ 6\text{O}_2 + 24\text{H}^+ + 24\text{e}^- \rightarrow 12\text{H}_2\text{O} \quad (\Delta G^\circ = -78.72 \text{ kJ/e}^-\text{eq}) \]
  – The overall redox reaction is the sum of the half reactions, or
    \[ \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{H}_2\text{CO}_3 \quad (\Delta G^\circ = -120.07 \text{ kJ/e}^-\text{eq}) \]
  – On the other hand, transferring the 24 e⁻ to produce CH₄ is much less energy lucrative for the cells:
    \[ \text{C}_6\text{H}_{12}\text{O}_6 + 3\text{H}_2\text{O} \rightarrow 3\text{CH}_4 + 3\text{H}_2\text{CO}_3 \quad (\Delta G^\circ = -17.82 \text{ kJ/e}^-\text{eq}) \]

Follow the electrons -- make biomass

• Electrons are biomass (aka, sludge)
  – E.g., 20 e⁻ eq in the C of C₅H₇O₂N
  – Another 8 e⁻ eq in N if it has to be reduced from NO₃⁻.
• Investing electrons in biomass is expensive (positive \( \Delta G^\circ \)), particular for autotrophs, whose C source is the most oxidized possible, H₂CO₃.
• The electrons not going to the acceptor (e.g., O₂ consumed or CH₄ produced) mostly end up in biomass, or excess sludge production.
Follow the electrons -- Pollutants when misplaced

• Reduced pollutants are just misplaced electron donors. This is why the most traditional water pollutants are called oxygen demand, or OD.
  – On the one hand, oxidation of OD actually consumes dissolved oxygen in the water environment, a severe problem
  – On the other hand, OD just means electron equivalents, with 8 g OD/e⁻ eq.

\[ 0.25O_2 + H^+ + e^- \rightarrow H_2O \]  

(0.25 mol = 8 g O₂ = 1 e⁻ eq)

Follow the electrons -- Energy opportunity

• Electrons are energy!
  – 8 e⁻ eq/mol CH₄
  – 30-40% energy capture as electricity using conventional combustion methods
  – 2 e⁻ eq/mol H₂
  – ~55% energy capture as electricity using H₂ and a conventional PEM fuel cell
  – ~65% energy capture as electricity directly with a microbial fuel cell.
Criteria for success for an environmental biotechnology

• It must be practical at a large scale. The relevant units normally are huge: 1,000s of m³/d for flow and tonnes/d for mass.
• It must operate reliably for continuous use. We cannot turn off the flow if something goes wrong. We cannot get a “time out” if the conditions change.
• It must be economical to build and operate. Environmental biotechnology provides essential bulk services that cannot be priced outside society’s reach.
• It must be relatively simple to operate, largely self-correcting without a lot of human intervention.

Services we can or ought to achieve

• Remove reduced contaminants, or oxygen demand -- common but needs improvements in reliability and costs
• Remove oxidized contaminants, like nitrate, perchlorate, selenate, chromate, and chlorinated solvent -- emerging except for nitrate
• Capture resources, particularly energy from renewable organic sources -- common with methane, but very novel with biohydrogen gas or the microbial fuel cell for bioelectricity
Activated Sludge Treatment Example

- In use since around 1920 and the most widely used wastewater treatment technology worldwide
- Still undergoing improvements to enhance reliability and treatment options
- Standard/routine goal: remove organic BOD (CBOD)
- Ancillary benefit: reduction of pathogens
- Additional/advanced goals:
  - Remove ammonium (NH$_4^+$) and NBOD
  - Remove total N (NH$_4^+$ + NO$_2^-$, + NO$_3^-$)
  - Remove total P (to a large degree phosphates)
  - Remove hazardous chemicals
Activated Sludge Components

- Aerobic reactor (aeration basin) to hold a high concentration of flocculated biomass (mixed liquor)
- Aeration system (e.g., blower) to supply dissolved oxygen, usually from air
- Settling tank (or secondary clarifier) to capture the flocculated biomass for biomass retention and producing a clear effluent (secondary effluent)
- Recycle line (RAS) to return the captured biomass from the settling tank to the aeration tank
- Biomass waste line (WAS) to control the concentration of biomass in the mixed liquor and the solids retention time (SRT)

Schematic of activated sludge
Picture of an aeration basin

Diffused-Aeration Options

Ceramic Disc

Top centerline diffuser mounting prevents cantilever or torque forces from being transmitted to piping system.

Sanitary BDM membrane material provides greater durability and flexibility.
Settling Tank Pictures

Aerial View of a Large Activated Sludge Plant
Picture of a small activate sludge plant with components closely integrated

Activated Sludge Schematic and SRT

The SRT (solids retention time) is the average time that active biomass stays in the system. It is a measure of the “age” of the biomass.

To remove only CBOD, the SRT can be around 5 days.

To remove NH$_4^+$, the SRT must be longer, around 15 days, to accommodate the slow-growing nitrifying bacteria.

SRT >> HRT The point!

$\text{HRT} = \theta = \frac{V^{AB}}{Q^o}$

$\text{SRT} = \theta_x = \frac{X_a V^{AB}}{Q^e X^{e_a} + Q^w X^{w_a}}$
Typical Performance (1)

• Influent quality to AS:
  – CBOD -- 400 mg/L (total, not BOD₅)
  – TN (at reduced NH₄⁺ level) -- 50 mgN/L
  – NBOD -- 230 mg/L
  – NBOD + CBOD -- 630 mg/L
  – Suspended Solids -- 150 mgSS/L
  – Phosphorus -- 10 mgP/L

Typical Performance (2)

• Influent quality to AS:
  – CBOD -- 400 mg/L (total, not BOD₅)
  – TN (at reduced NH₄⁺ level) -- 50 mgN/L
  – NBOD -- 230 mg/L
  – NBOD + CBOD -- 630 mg/L
  – Suspended Solids -- 150 mgSS/L
  – Phosphorus -- 10 mgP/L

• Effluent with "conventional" SRT ~ 5 days
  – CBOD -- 20 mg/L (total, not BOD₅)
  – TN (still at reduced NH₄⁺ level) -- 30 mgN/L
  – NBOD -- 140 mg/L
  – NBOD + CBOD -- 160 mg/L
  – Suspended Solids -- 15 mgSS/L
  – Phosphorus -- 7 mgP/L
Typical Performance (3)

- Influent quality to AS:
  - CBOD -- 400 mg/L (total, not BOD₅)
  - TN (at reduced NH₄⁺ level) -- 50 mgN/L
  - NBOD -- 230 mg/L
  - NBOD + CBOD -- 630 mg/L
  - Suspended Solids -- 150 mgSS/L
  - Phosphorus -- 10 mgP/L

- Effluent with nitrifying SRT ~ 15 days
  - CBOD -- 15 mg/L (total, not BOD₅)
  - TN (now at oxidized NO₃⁻ level) -- 30 mgN/L
  - NBOD -- ~0 mg/L
  - NBOD + CBOD -- 15 mg/L
  - Suspended Solids -- 10 mgSS/L
  - Phosphorus -- 8 mgP/L

Typical Performance (4)

- Effluent with nitrifying SRT ~ 15 days and then adding an anoxic region to denitrify (reduce) the NO₃⁻ to N₂ for total-N removal
  - CBOD -- 15 mg/L (total, not BOD₅)
  - TN (now at oxidized NO₃⁻ level) -- 5-10 mgN/L
  - NBOD -- ~0 mg/L
  - NBOD + CBOD -- 15 mg/L
  - Suspended Solids -- 10 mgSS/L
  - Phosphorus -- 7 mgP/L
Typical Performance (5)

- Effluent with nitrifying SRT ~ 15 days plus an anoxic region for denitrification + an anaerobic region for enhanced biological P removal
  - CBOD -- 15 mg/L (total, not BOD₅)
  - TN (now at oxidized NO₃⁻ level) -- 5 - 10 mgN/L
  - NBOD -- ~0 mg/L
  - NBOD + CBOD -- 15 mg/L
  - Suspended Solids -- 10 mgSS/L
  - Phosphorus -- 1 mgP/L

Membrane BioReactors (MBRs)

- A new form of activated sludge in which the settling basin is replaced by a membrane separator.
- Able to achieve almost zero effluent SS, which improves effluent quality and process reliability. For example, for 15-day activated sludge:
  - CBOD -- 10 mg/L (total, not BOD₅)
  - TN (now at oxidized NO₃⁻ level) -- 30 mgN/L
  - NBOD -- ~0 mg/L
  - NBOD + CBOD -- 10 mg/L
  - Suspended Solids -- ~ 0 mgSS/L
  - Phosphorus -- 8 mgP/L
- Also allows higher mixed liquor suspended solids concentration and smaller HRT for the same SRT.
Biofilm Processes

• Also developed around 1920 and in widespread use today.
• Instead of retaining the biomass by capturing flocs in a settling basin, the biomass is naturally retained by attachment to a solid material.
• It is possible to do with a biofilm process anything that can be done with activated sludge.
Trickling Filters

• The original (1920s) process, still in use today.
• Uses large rocks (1 - 4 inches) in a bed about 1 m deep.
• Water “trickles” over the rocks, leaving room for air to move through the bed for aeration.
• TFs are simple, but take up a lot of land.

Historic photos of a small rock trickling filter.

Notice how the rotating arms distribute the water over the rocks.

This fellow is (the now famous) Perry McCarty.
Biological Towers

- The advent of strong, lightweight plastics in the 1970s lead to a “plastic trickling filter.
- Modular plastic could be up to 12 m tall, reducing the land area a lot.

Figure 8.3 Close-up of a module of corrugated plastic media.
Source: With permission from Brainwood Industries.

Plastic modules alone, in place, and in operation in a biological tower
Performance

- Trickling filters and biological towers give performance similar to activated sludge for removing CBOD and NBOD.
- Costs also are roughly similar.
- Biological towers have been adapted for total-N and total-P removal, although it is not common.

Other Biofilm Processes

- New biofilm processes are constantly being developed. For the traditional treatment objectives (described so far), new biofilm processes include:
  - Biologically aerated filters with gravel-sized carriers
  - Fluidized beds of sand-sized carriers
  - Circulating beds of lightweight carriers
  - Environmental Biotechnology describes them.
- The membrane biofilm reactor (MBfR) is a special case that deserves a look.
The Membrane Biofilm Reactor (MBfR)

- A technological breakthrough that allows us to use H$_2$ gas as the electron donor to reduce NO$_3^-$ and a very large range of other oxidized contaminants: perchlorate, chromate, selenate, bromate, chlorinated solvents, and more.
- H$_2$ is delivered efficiently and safely by diffusion through a “bubbleless” membrane.

![Diagram of Membrane Biofilm Reactor](image)

- **Composite membrane:**
  - Microporous hydrophobic polyethylene support
  - Dense polyurethane inner layer
- Pore size: 0.1-0.15 µm
- Water
- Biofilm
- NO$_3^-$, ClO$_4^-$
- A bundle of clean hollow-fiber membranes
- The membrane with biofilm having grown on it
The MBfR allows us to gain the benefits of using $H_2$ as an electron donor for microbial reductions

- $H_2$ is the “universal donor” for microbially catalyzed reductions; we are able to reduce many or all oxidized contaminants.
- $H_2$ is the lowest-cost donor we can buy in large quantities.
- $H_2$ is non-toxic to humans.
- $H_2$ leaves no residual in the water.
- $H_2$ supports autotrophs, which have low biomass yield and, therefore, generate little excess biomass.
- Using $H_2$ is consistent with a $H_2$-based energy economy.

Capturing Renewable Energy

- Three possible outlets for transforming the energy value in biomass to a useful form:
  - Methane -- $CH_4$
  - Biohydrogen -- $H_2$
  - Electricity -- electron current, $i$
- Biomass sources can include waste sludges (e.g., waste activated sludge), animal feedlot wastes (e.g., pig manure and urine), agricultural residues (e.g., corn stover), and agricultural energy crops (e.g., water hyacinths).
Methane

- Methanogenesis is a long-standing process for digesting wastewater sludges and stabilizing the biodegradable organic matter to CH₄ gas. \[ \text{C}_6\text{H}_{12}\text{O}_6 + 3\text{H}_2\text{O} \rightarrow 3\text{CH}_4 + 3\text{H}_2\text{CO}_3 \]
- The CH₄ gas is collected, cleaned, and combusted to produce heat that can be used directly or converted to electricity.
- The electricity-capture efficiency is 30-40%, and air pollutant include NO (always), CO₂ (always), and SO₂ (depending on H₂S content of the gas).

Methane Digesters

- The classic methane digester has a sludge input and a large volume to allow for hydrolysis of the input solids.
- For typical sludges, the conversion for input solid biomass to CH₄ is 50 - 70%, depending on the input material and operation.
Other Methanogenesis Options

![Digesters](image)

**Photo 13.1** Mesophilic anaerobic sludge digester with a floating cover.

**Photo 13.5** Egg-shaped anaerobic sludge digester.

![Diagram](image)

**Figure 13.1** Typical anaerobic reactor configurations. Source: Speece, 1983.
Biohydrogen (BioH₂) -- The good news

- H₂ is another gaseous outlet for the electrons and energy in biomass. \( \text{C}_6\text{H}_{12}\text{O}_6 + 12\text{H}_2\text{O} \rightarrow 12\text{H}_2 + 6\text{H}_2\text{CO}_3 \)
- Like CH₄, the H₂ could be combusted for heat and electricity.
- But, the real goal is to use the H₂ in a fuel cell, which generates electricity without combustion.
- This can improve the electrical-energy capture efficiency to about 55% and eliminates the combustion air pollutants!! We can see why H₂ has become a “hot topic.”
BioH₂ -- The bad news

• It is so far not possible to capture all 12 molH₂ per molC₆H₁₂O₆.
• Most are happy with 2 mol/mol, and 4 mol/mol is considered a grand success.
• The fundamental problem is the H₂ is the “universal donor,” and microorganisms that oxidize H₂ compete very strongly to oxidize the H₂ before we can harvest it.
• BioH₂ is a major research effort worldwide. We have ideas to do better, but we have a long way to go.

Microbial Fuel Cell (MFC)

• This is the most recent entry to capture renewable energy in a useful form.
• It is a special case of fuel cells, which produce electricity directly, without combustion.
• This avoids all combustion pollution and can have an energy-capture efficiency up to 65%.
• It is made possible by the recent (< 10 years) discovery that some bacteria transfer electrons to solid phases, including an anode.
MFC Basics

• Bacteria living as a biofilm on an electrode remove electrons from an organic fuel and transfer them to the electrode and through the circuit.

• Fuel-cell technology now can use renewable organic fuels from wastes and fuel crops.

This overcomes the main limitation of conventional fuel cells: The only viable fuel today is H₂.

But, H₂ hardly comes from renewable sources, since BioH₂ is not close to viable yet, and H₂ is not a stored energy source on Earth.

H₂ is a huge industry today (~ 10⁸ m³/year in the USA), but 99% of it comes from reforming of fossil fuels!!

MFC Status

• MFC research is picking up worldwide, particularly in the USA and Korea.

• We can reliably produce electricity from a range of organic fuels, including wastewater.

• The size is very small.

• The current density (amps/m² of anode) is too small to be commercially interesting.

• We need to learn a lot about how bacteria transfer electrons to an anode and how to make the current density much higher, at least 100 times higher.
**In situ Bioremediation (optional topic)**

- One of the big challenges is to deal with contamination that has been leaked or spilled on the soil or underground.
- The biggest problem comes from fuel leaks, especially gasoline, which is stored underground everywhere. Benzene, toluene, ethyl benzene, the xylenes, and MtBE are the most common groundwater contaminants.
- Other widespread problems are the chlorinated solvents, dry-cleaning solvents, perchlorate, and nitrate.
- More specialized (but still major) problems are metals (Cr, Hg), radionuclides (Pu, U), explosives (TNT), selenate, and arsenic.

**Natural Attenuation**

- In some cases, natural biodegradation can biodegrade or immobilize the contaminants without human intervention.
- This is called *natural attenuation*, and it is popular when it works.
- However, the major contaminants for which natural attenuation is likely are BTEX (benzene, toluene, ethylbenzene, and the xylenes).
- Natural attenuation is possible for some other contaminants, but not usual.
- In all cases, natural attenuation must be carefully monitored and documented.
Engineered *In Situ* Bioremediation

- The idea is to stimulate microbiological activity to destroy or immobilize the contaminants.
- This is accomplished by adding the missing substrate or nutrient, just like in regular treatment.
  - Add O$_2$ for aerobic biodegradation of reduced contaminants, like BTEX
  - Add an electron donor to reduce oxidized contaminants, like nitrate, perchlorate, selenate, and others.
  - Occasionally, we also add specialized bacteria to make sure that they are present.

Engineered *In Situ* Bioremediation

- The challenge is that getting the material to the bacteria in all places where they need to work is very difficult, since the sub-surface is heterogeneous and not easily accessible; it is underground!
- Many techniques are used to get the stimulants to the right places.
For aerobic biodegradation

Bio-venting (sucking air through) and air-sparging (blowing air through) are most popular.

For reducing contaminants *in situ*

- The most successful approach is to inject a “slow release” organic material that is molasses like in texture and slowly hydrolyzed to release organic compounds that can be fermented to $H_2$ to fuel microbial reductions.
  - Molasses itself (simple carbohydrates)
  - Vegetable oil (aliphatics)
  - Chitin (complex carbohydrates)
  - Hydrogen Release Compound (HRC®) (poly-lactate)
- In principle, we can use $H_2$ directly with an *in situ* version of the MBfR.
Questions?

Discussion?

Other topics you wish to broach?

Environmental Biotechnologies
Sessions EB5 - EB7

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