Learning Objectives CEE6580 (end of semester)

May 2018

Topic: Metabolism:

1. Define the nuanced differences between biodegradation, bioremediation, biocatalysis, biotechnology and biogeochemistry.
2. Be able to draw black boxes around metabolism at multiple scales.
3. Explain the difference between mechanisms and summary reactions.
4. Trace the flow of carbon and electrons in common microbial metabolisms (chemolithoautotrophs, photoautotrophs, chemoorganoheterotrophs (“heterotrophs”).
5. Recommend strategies for enrichment culturing of specific catabolic capabilities.
6. Explain why it is more difficult to enrich for organisms that PRODUCE compounds of interest than those that biodegrade them (note exceptions - e.g. antibiotic production).
7. Recognize the differences between central (intermediate) metabolism and secondary metabolism - and explain how secondary metabolism relates to biodegradation & biocatalysis
8. Be able to balance coupled redox reactions AND predict yield of cell growth given metabolic summary reactions
9. Describe eeq, COD and coulombs. Convert easily amongst them.

Enzymes & common metabolic pathways

1. Calculate ΔG0’ ~~and E~~~~0~~~~’~~ values for coupled redox reactions and adjust ΔG0’ ~~and E~~~~o~~~~’~~ for “real” concentrations of substrates & products (~~know how to use the Nernst equation~~)
2. Trace the net flow of carbon & electrons in cells that are:
   1. Respiring
   2. Fermenting
3. Utilize thermodynamics/bioenergetics calculations for organisms with different physiologies to predict growth Yield. (i.e. via the “A” equation in Gossett’s handout)
4. Describe the roles of ATP & NADH/NADPH/FADH as “currencies” in metabolism
5. List and briefly explain the 6 functional categories of enzymes (“OTHLyIL”). Given an enzymatic reaction, determine which class it belongs to.
6. Explain what is meant by primary, secondary, tertiary and quartenary structure of enzymes.
7. ~~Recognize the difference between apoenzymes and holoenzymes~~
8. Effectively use online enzyme/pathway databases (KEGG, Brenda, eawag Biodeg & Biocatalysis website) to obtain information on enzymes and to incorporate kinetic parameters into models
9. Predict degradation pathways for chemicals by examining molecular structure coupled with general “rules” for biochemistry (eawag B&B Pathway Prediction)

Kinetic models and reactor mass balances

1. Recognize the difference between reaction mechanisms and summary reactions w/respect to microbial metabolism and that reaction kinetics are determined by mechanism of rate-limiting reactions.
2. Understand (and sketch plots illustrating) how specific substrate utilization rate varies with Substrate for the following types of kinetics:
   * zero, first, ~~second and half order~~
   * Monod/Michaelis-Menten (basic and with variations):
   * Haldane model of substrate inhibition
   * Competitive inhibition and noncompetitive inhibition
3. Describe different examples of suspended and fixed film bioreactor types
4. Perform material balances to determine substrate and biomass for the following reactor configurations and Monod kinetics: Batch, CSTR and PFR with suspended biomass only
5. Utilize modeling software to model S and Xa in bioreactors.

Biodegradation and Bioremediation

1. Recognize common aerobic and anaerobic biodegradation pathways for different chemical classes
   1. Aromatics & alkanes
   2. Organohalides (chloro- and flouro- organics) (e.g. TCE, PCBs, chlorobenzenes)
   3. Polymers
   4. Metals/radionuclides
2. Discuss reasons for a delay between a microbial communities’ exposure to a toxic chemical and observable compound biodegradation (=Acclimation period)
3. Discuss the general steps in site remediation at a particular contaminated site
   * Remedial characterization /Feasibility study
   * Selection of strategy (=Record of Decision)
   * Implementation and monitoring (Remedial Design/Remedial Action)
   * Closure of site
4. Describe the difference between in situ and ex situ bioremediation
5. Explain the differences between the following regarding *in situ* bioremediation: natural attenuation, and two types of enhanced bioremediation (biostimulation and bioaugmentation).
6. List the three overlapping lines of evidence required to document that *in situ* bioremediation is occurring at a site. Be able to describe some modern tools to provide this evidence

Genetics/ Molecular Techniques

1. Discuss the functions of the three major informational macromolecules in biology (DNA,RNA,protein).
2. Describe the processes which lead to slow (mutations) and fast (HGT) evolution of enzymes/pathways.
3. Understand various molecular methods that target DNA, RNA and Protein, respectively.
4. Know the broad level difference between phylogenetic and “functional” gene targets (in biodeg’n often synonymous with “catabolic”). Know what features are important to each type of gene.
5. Know details of the following techniques: PCR, sequencing (Sanger, Illumina), restriction analysis, gel electrophoresis, Fluorescent In Situ Hybridization, TRFLP, reverse-transcription PCR, quantitative PCR.
6. ~~When shown the techniques employed in a method, to be able to determine whether that method is quantitative or qualitative/semi-quantitative.~~
7. Describe the roles of the following molecular biology enzymes and the molecular techniques that utilizes them: DNA Polymerase, reverse transcriptase, restriction enzymes, ligases.

Diagnostic Env’l Biotech

1. Recognize key criteria for good bioindicators/biomarkers (BMs) of e.g. bioremediation or disease or fecal source tracking.
2. Think critically regarding the causes of false negatives and false positives when using BMs (sensitivity and specificity)
3. Understand common assays for DNA and RNA biomarkers (including the important concept of “hybridization”)
4. Compare the pros and cons of DNA BMs versus RNA/protein BMs
5. Describe Biosensor basics (recognition, signal transduction, detection and readout).
6. Specifically discuss Sensitivity and Specificity of BMs/Biosensors

Response mechanisms in cells and regulation of gene expression

1. List 3 points of regulation (transcriptional, post-transcriptional, post-translational)
2. Describe the key difference between inhibition by feedback inhibition versus transcription level inhibition
3. Describe the following types of transcription-level regulation: negative regulation (induction, repression), positive regulation (activation) of transcription.
4. Understand the following terms and how they relate to transcriptional regulation: activator binding site, operator, promoter, operon, sigma factor
5. Describe the general process of Quorum Sensing and some phenotypes that are linked to quorum sensing regulons.
6. Define signal transduction and briefly outline a simple example of signal transduction by two-component regulatory systems (HK/RR systems).

Wastewater treatment and Waste-to-Energy:

1. Recognize the various embedded energy in domestic wastewater (heat, nutrients, and organic carbon) and that most of the COD, and nutrients come from excreta (urine and feces)
2. Describe the elements of a functioning centralized sanitation system (User Interface, conveyance, centralized treatment system, disposal and/or reuse of treatment products)
3. Recognize sanitation options that don’t rely on centralized treatment (e.g. composting toilets, urine diverting dehydrating toilets, septic systems, constructed wetlands, biogas reactors)
4. Sketch out a typical US wastewater treatment plant and recognize the two greatest electricity demands (aeration of activated sludge and pumps to move wastewater)
5. Recognize the key benefits of anaerobic digestion of wastewater organic matter to “biogas” (sludge reduction, production of useable biofuel, opportunity for co-digestion of wastewater organics with local food industry wastes - e.g. brewery wastes, food scraps, manure, cheese and yogurt whey)
6. Describe the overall biological conversions and key microorganisms for the following emerging/advanced biological treatment reactors
   1. UASBs, EBPR, Anammox, GSBR, ~~MEC/~~MFC, Anaerobic membrane bioreactors (MBRs), algal ponds
7. Describe the following chemical treatment to recover nutrients from wastewater
   1. Struvite precipitation and ammonia stripping

Omics:

1. Describe the following “omics” and how they inform us about microbes’ catalytic activities and capabilities:
   * 1. Genomics/metagenomics
     2. Transcriptomics
     3. Proteomics
     4. Metabolomics
     5. Explain the general process of genome Assembly and Annotation and what is an inherent danger of simple Bioinformatics-based annotation
2. Use genomic web resources (Integrated Microbial Genomes; Comprehensive Microbial Resource; NCBI) to investigate genomes and find “homologous” genes in other genomes
3. Explain how genomic sequence data enables other “omics” – i.e. transcriptomics and proteomics
4. For Microarrays:
   1. Describe what a microarray is and what a microarray measures.
   2. Explain what information is gleaned from: spot intensity values and the log ratio data
5. For Proteomics:
   1. Explain the general components of a tandem mass spectrometer (an ionization source, two MS detectors with a collision cell between them)
   2. Describe strategies for reducing complexity in proteomics characterization (Cell separation, gel separation of proteins, 2 dimensional LC separation of peptide pools)
6. Explain how omics can be applied to aid environmental biotechnology in the following fields:
   1. Green Chemistry (biomining for novel enzymes or enzyme products)
   2. Bioremediation ((discovery of biodegradation pathways)

Bioproducts (Biofuels and biochemicals):

1. Recognize the challenges to biocatalysis for production of bioenergy and biochemicals of interest
   * 1. IDing of orgs/genes capable of specific biosynthetic capabilities
     2. maximizing yields and minimizing side reactions
     3. product recovery and purification
2. Discuss the pros, cons and challenges to microbial bioenergy processes and products that are being explored for biofuels (methane, MFCs, ethanol, butanol, H2, biodiesel)
3. Discuss the challenges of lignocellulosic ethanol *as a large scale replacement for petroleum derived liquid fuels.*

*Systems Biology:*

1. *Define systems biology*
2. *Describe top-down versus bottom-up approaches for describing a biological system*
3. *Explain the general framework of the systems biology approach ­ including the interplay of “wet” experiments and “dry” experiments*
4. *Explain how proteomics and metabolomics are cornerstones of systems biology.*
5. *List three general applications of systems biology.*
6. *Explain the general difference between deterministic (mechanistic) models such as flux-balance models and probabilistic models*