ABE 580: Process Engineering of Renewable Resources

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M/W/F 10:30 am – 11:20 am

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W 5:00 pm – 6:00 pm

POTR 220

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Syllabus: <syllabus-spring19.pdf>

About the course:

* 3 midterm exams
* ~ 8 homework assignments
* No final exam!!!
* Seminar/survey course after spring break

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# January 7, 2019

## Lecture Notes

* [lect1-introduction.pdf](Lecture_Slides/2019-01-02_lect1-introduction.pdf)

## Introduction

### Biotechnology

* “Any technology application that uses biological systems, living organisms, …”

### History of Biotechnology

* Humans gathered in larger numbers to be able to ferment beer/wine sustainably
* Egypt, Korea, etc.
* Term invented after Louis Pasteur
* Roots in food fermentation
* Alexander Fleming
  + Ernst Boris Chain and Edward Abraham at Oxford University translated penicillin form laboratory curiosity to practical therapeutic by isolating the active ingredient and developing the method to purify it in a stable form.
  + Discovered in 1928 by Fleming: *Penicillium* bacteria
  + First patient treated in 1942
    - Time gap: first time ever, someone tried to scale up industrially a product where the product is not made by humans but by an organism.
* Scale up of penicillin
  + March 14, 1942, first patient treated. This consumed ½ of global supply of product
  + Peoria, Illinois: as far from oceans as possible to avoid Japanese bombers from destroying the secret project, lab studying diseases and livestock already in place there
  + Before this, more people died in wars from disease than directly from fighting
  + 2 British spies hid penicillin under coat collars and rode a cruise to the US and smuggled the project/invention to America from England
  + June 1942, scaled-up production produced enough penicillin to treat 10 patients
  + Used milk bottles with rags to keep out contamination. Filled with agar broth, grow fungus and then scrape off the fungus to put in larger vats to create penicillin
  + Surface area 🡪 volume
* “Moldy” Mary Hunt
  + Bacteriologist at USDA research lab in Peoria
  + Scaled from surface area to voluminous production
  + Found that the fungus could be grown in liquid media instead of on flat agar
  + Pfizer got involved with production: scaling up production of penicillin, using submerged culture in large stir reactors: John Smith, executive was worried about was it worth it? Losing a 2000-gallon tank vs one flask
  + By June 1945, 646 billion units produced per year
* Margaret Hutchinson Rousseau
  + Designed first commercial penicillin production plant in 1943-1944
  + First female of AIChE

## Biotechnology

* Color-coded
  + Red: Medical biotechnology
  + Green: plant biotechnology for agriculture
  + White: industrial biotechnology; biofuels, biopolymers, biochemicals; biorefinery concept
* Industry size: $400 billion/year, 5 million US jobs
* New biotech vaccines or drugs approved per year has increased consistently
  + Drugs: can draw structure, know molecular weight, etc. (small-molecule); purity is important; regulated on identity, purity
  + Biologic: substance that has therapeutic value that is complex and is not necessarily worth knowing the exact structure/weight (i.e. proteins); purity not as important; regulated on process for identification
* US Pharm sales
  + small molecule therapeutics are still the largest part of the industry, but biopharmaceuticals are slowly taking out larger pieces of the industry
  + The industry is growing at a consistent rate.
* Phase III clinical trials
  + Therapeutic proteins are largest part
  + Insulin is a smaller piece
  + Antibody-based trials are increasing in popularity especially for cancer-based drugs
  + Number of trial has increased more than 50-fold over the last two decades (as of 2014)
* First GMO Crops
  + FlavrSavr Tomato, 1994 (failure)
  + BT corn and cotton and Round-Up ready soybeans, 1995 (still in use)
    - BT: poisonous to insects (moths, butterflies, beetles) that eat crops
    - Round-Up: herbicide that kills plants (allows plants to grow in the presence of Round-Up while the weeds are killed)
  + Adoption of genetically engineered crops in the US has increased in corn, cotton, and soybeans to about 80%
  + Insecticide use on cotton and corn has decreased since 1995
  + Number of releases of genetically engineered organism varieties approved by APHIS peaked in about 2002 and has decreased by about half since then
  + Approval is complicated in US because there is overlap between FDA and USDA jurisdiction
  + Relevance of genetic engineering traits:
    - Herbicide/insecticide tolerance are largest
    - Product quality/agronomic properties are increasing in in popularity
      * Water/drought tolerance, length of day change, spoilage time, increased nutrition etc.

## Homework due January 23, 2018

* [HW1-Monod\_growth-2019-01-07.pdf](Homework/HW1-Monod_growth-2019-01-07.pdf)

# January 9, 2018

## Institutions having most authorized permits and notifications

* Monsanto is number 1
* Followed by Pioneer (now part of DuPont), Syngenta, USDA/ARS, AgrEvo, Dow AgroSciences, Dupont, ArborGen, Bayer CropScience, Seminis (as of 2013)
* Pioneer is a hybrid seed company that has been around for nearly a century
* Dow AgroSciences purchased Syngenta recently
* Dow and DuPont merged and have spun off the biotech arms as a new company called Corteva AgroScience, biggest competition to Monsanto

## White Biotechnology: Biorefinery Concept

* Biomass feedstocks
* Bioprocessed things
* Use for corn has changed from 60% feed, 20% export, 14% food and industry, 7% fuel to 40% feed and 40% fuel, 9% food and industry, and 14% export
* Feed, export, and food/industry use has stayed about the same (in bushels), but fuel ethanol has grown about 4x 2000 to 2015!!!
* Corn production has grown 3.5% year by year since 1975 on about the same amount of land
* Bioproducts today
  + Polyols
    - Foam for car or bus seats
  + PHA
    - Plastics made from natural polymers from sugars made by bacteria as an energy storage
  + Polyactic acid
    - 1990s: Cargill and DuPont commercialized process, thought use would be for clothing and grocery bags
    - Mostly used for 3D printers (temperature is right!) and carpets
  + Enzymes and biobased solvents for cleaners
    - Detergent for clothes
    - SimpleGreen, citrus cleaners for kitchen cleaners
  + SmartStrand with DuPont Sorena renewably sourced polymers
    - Carpets and other building materials
    - Textiles, but not clothing
* BioProducts via Biotechnology
  + Products go from fairly low value (i.e. food and industrial chemicals) with high price elasticity (flat price curve) to high product value (i.e. pharmaceuticals) with low price elasticity (steep price curve)
    - Elasticity: change in price determines how much a consumer will buy
      * Elastic: price-sensitive (i.e. Ramen noodles)
      * Inelastic: not price-sensitive (i.e. medicine)
    - Trend about linear (log-log scale): things that are supplied at higher volume tend to be lower priced
  + Engineering the processes to make these products is very different

Chapter 4: Microbial Fermentation

* [chapter4-microbial\_fermentations.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Lecture_Slides/chapter4-microbial_fermentations.pdf)

### Fermentation

* Microbial processes that produce various products via biochemical action
  + Foods
  + Pharmaceuticals
  + Small molecules
  + Proteins
  + “biologics”
* Vs. cell culture (mammalian or plant)
  + Unicellular mammalian or plant cells are cultured
  + Bacteria and yeast are fermented
* Control
  + Temperature – how fast the reaction is, which reactions occur/whether the reactions occur at all
  + Nutrients
  + pH – cells function in a narrow range of pH
  + O2 (anaerobic? – term looser in industry, so there could be aerobic fermentation) - some processes may require oxygen or require no oxygen, some don’t care, some depend upon amount of oxygen;

### Industrially Important Microorganisms: Table 4.2

* Industrial chemicals
  + *S. cerevisiae* (yeast) – ethanol from glucose (beer, wine, bread)
  + *C. acetobutylicum* (bacterium) – acetone and butanol (solvents)
  + *A. niger* (mold) – citric acid (preparation for yeast fermentation, laundry detergent)
* Amino Acids and Flavor-Enhancing Nucleotides and Acids
  + *C. glutamicum* (bacterium) – L-Lysine (essential amino acid, mammals must consume, we cannot produce it; rate-limiting nutrient for milk production); 5’ Inosinic Acid and 5’ Guanylic Acid; Glutamate (MSG)
* Vitamins
* Food and Beverages
  + *S. cerevisiae* (yeast)
  + *C. milleri* (Yeast) – sourdough bread
  + *L. sanfrancisco* (bacterium) – sourdough bread
  + *L. sp.* (bacterium) – yogurt
  + *P.* *shermanii* (bacterium) – swiss cheese
  + *G. suboxidans* (bacterium) – vinegar
* Polysaccharides
  + *X. campestris* (bacterium) – xanthan gum
* Pharmaceuticals
  + *P. chrysogenum* (mold) – penicillin
  + *Streptomyces* (bacterium) – amphotericin *B*, Kanamycin, Neomycin, Streptomycin, Tetracyclines, and others

### Fermentation Processes Must Provide Nutrients: Table 4.1

* For *E. coli* fermentation
* Major constituents of the cell, broken down elementally
* Most abundant elements are C, H, O, N, P, S
  + Carbon: half of the mass of what makes up a cell (not counting water!)
  + Top 6 make up about 94.5% of cell
    - Other 5.5%: trace elements such as metals (calcium, potassium, manganese, cobalt)
    - Proteins use the trace elements to help with enzymatic reactions
* Most abundant constituents
  + Proteins – enzymes, muscle, etc.
  + RNA
  + DNA
  + Lipids – fats and oils; cell membranes
  + LPS – lipopolysaccharide; cell walls of some bacteria (gram negative)
  + Peptidoglycans – cell wall
  + Polyamines – cell wall
* Hydrolysis takes out H/O atoms

### Fermentation Media

* Defined media
  + Aka minimal media – chemically defined
  + Know exactly how much of each chemical compound is in the media
    - Lots of glucose or similar sugar (primary source of C/H/O)
    - Ammonium sulfate next most abundant (nitrogen)
    - Trace elements next most abundant that also have P/S
    - Then vitamins are least abundant added to make products more efficiently
      * Nicotinic acid (N in RNA/DNA)
  + Fairly expensive
  + Used because you know exactly what went in, so you can account for everything and where it goes
    - Important in pharmaceuticals so that they can say exactly what is in their drugs
* Rich media
  + AKA complex media or undefined media
  + Partially processed plant, animal, or microbial derived material
  + Complex mixture of carbohydrates, minerals, vitamins, and biomolecules
  + Laboratory media: Luria broth, trypticase soy agar, yeast extract with peptone, etc.
* Industrial undefined media
  + Carbon source: sugar/carbs
    - Molasses
    - Malt extract
    - Whey
    - Dextrose (AKA glucose)
    - Starch
  + Lipids (rarely used; carbon source, metabolically slower than above sources)
    - Soy oil
    - Fish oil
  + Nitrogen source
    - Urea
    - Diammonium phosphate (DAP, most common)
    - Corn steep liquor (by-product of processing corn into other products, i.e. wet-milling—pile corn in large reactor, circulate water, sulfur dioxide to digest protein in corn kernels to separate starch, oil, proteins, etc.)
    - Yeast extract (nutrients left by dead yeast cells for other yeast cells to use)
    - Soy meal
  + Vitamins and minerals
    - Corn steep liquor
    - Yeast extract
    - Specialty products (too numerous to list)

# January 11, 2019

## Rich Media Components

* Can find analysis of components for certain common rich media
  + Yeast extract
  + Blackstrap molasses
  + Corn Steep Liquor
* FAM = free-amino nitrogen

## Bioreactor basics

* Sealed
* Made from metal for sterilization
* Agitation
* pH monitor
* Temperature monitor
* Heating and cooling (typically water) – surface area vs. volume problem at large scale!
  + 1 L vs 40 L vs 300 L vs 20,000 L fermenters and beyond (several hundred thousand L – 1 million gallons)
  + Really large: circulate media through heat exchangers because the fermenters are too big to justify a water jacket; agitators can’t handle the force to mix, so it circulates like a fountain pumping the liquid up and down and through heat exchangers

## Modeling

* How is the model to be used?
  + What are you trying to accomplish?
  + What decisions or insights will model give you?
  + Leads to next question…
* What level of detail is required?
* How accurate must your model be (error)?
  + What are you trying to estimate?
  + What order of magnitude is being modeled?
* Does your model need to be predictive or just descriptive?
  + Descriptive: needs data
  + Predictive: needs data, but more robust, insights beyond tested situations
    - Be sure not to extrapolate too far!
    - Expresses mathematically the scientific principles behind what is happening
* How robust does your model need to be?
  + Robust: how easily can we break the model?

### Modeling Fermentation

* Growth of cells
  + Unstructured, unsegregated model
    - Unstructured: mechanism is not described mathematically
    - Unsegregated: lump parameters together for simplicity (i.e. mass transfer/heat transfer, etc.)
* Phases of fermentation
  + Lag phase: cells need to synthesize enzymes, reconfigure metabolism to take advantage of nutrients
    - Not always included in model
  + Exponential phase: rapid phase of growth
  + Stationary phase: growth levels off (run out of nutrients, mechanisms to recognize crowding i.e. quorum sensing, polluting environment from by-products of metabolism)
  + Death phase: without removing polluting or adding more nutrients, cells die from starvation or poisoning
* Cell growth
  + Cells reproduce through binary division
* Modeling cell division
  + How much time passes between cellular divisions?
  + Doubling time, td
    - * G = number of generations
      * T = time that has passed
      * Td = doubling time
    - Assumption: all cells are the same and have the same doubling time and grow at the same rate etc.
      * This assumption is not always true!!!
* Modeling binary division
  + - X = number of cells
    - Xo = cells at initial conditions
  + µ = specific growth rate

### Monod Equation

* + Where X = cell concentration (g/L)
  + µ = specific growth rate

#### Research on the Growth of Bacterial Cultures

* Jacques Monod at University of Paris in 1941 (in the middle of WWII)
  + 1st thesis: research on the growth of bacterial culture (dioxic growth)
    - Bacteria will eat one sugar at a time if given a mix of sugars
  + Developed mathematical models for dioxic growth
  + Then worked to figure out what is going on: discovered *lac* operon

### Assumptions

* Homogeneous
* Exponential growth
  + Cells grow as quickly as possible
* Balanced growth
  + All cells are equal metabolically/physiologically
  + All cells grow at same rate: “synchronization of cell division” – Campbell 1957
  + **Because all cells are the same, we can assume that the mass of each cell is the same**
    - X = # cells/L becomes X = g cells/L
* Substrate controlled
  + Some nutrient is the primary driver of how fast cells can grow
  + Assume that it is the carbon source, but could also be oxygen
* Saturated vs. limited growth
  + Fundamental discovery that Monod pointed out in dissertation work was that cells, like enzymes, have a saturation
    - MM enzyme kinetics: 1917, a couple decades before Monod
    - There is some maximum growth rate for cell division; give more food and don’t grow any faster
    - Below that threshold, they will grow, but more slowly because there aren’t enough resources for them to grow!
* Monod Equation:
  + Growth rate not constant
  + Subject to constraints
    - Substrate-limited:
      * S = substrate (g/L)
      * µmax = maximum growth rate
      * Ks = monod constant
        + S where mu = ½ mumax
      * Similar to enzyme (M-M) kinetics

# January 14, 2019

## Monod Equation

* µ units are inverse time
* Unlimited growth
* Ks vs Km mean different things (different assumptions) but we don't care about that right now
* Means concentration of substrate where µ is 1/2 maximum growth rate

## Coupling Cell Growth to Substrate Use

* Yield coefficient: Yx/s = delX/delS = dX/dS (\* dS/dt = dX/dt)
  + "Yield of cells (X) per utilized substrate (S)"
  + Units = g (cells)/g (substrate) [dimensionless]
* Assumptions that go into saying that the yield coefficient × dS/dt is part of Monod equation (part in parentheses above):
  + Accumulation = in - out + generation - consumption
  + = generation
    - No consumption == no death (not valid... Slow down)
    - for now, only assuming closed system, cells don't die, but this won't always be the case
* Y subscript is a hint to which is the numerator vs denominator
* Impose physical limitations in excel code

## Modeling Product Formation

* How is product formed (biochemistry)?
* How is product formation related to call growth?
* How is product formation related to substrate consumption?
* Are there any other factors that affect product formation?

### Metabolic Context

* Metabolism: all biochemical processes into a cell
* Catabolism: break down larger molecules into smaller molecules to produce energy
  + Electrons to make bonds
  + Gibbs free energy
* Anabolism: build up molecules to provide raw materials for growth and division (require precursors)
* Bridge between: ADP, NADP+, NAD+ <-> ATP, NADPH, NADH
* Generally, electron rich, Gibbs poor ... Have more electrons than Gibbs Free Energy
* Cells need to get rid of electrons!!!
* Aerobic processes dump electrons into oxygen
* Without oxygen, electrons dumped into weird places to
  + Fermentation
  + Metals

## Fermentation Classification

* Aerobic vs Anaerobic vs Microaerobic
  + Is oxygen required?
  + Is oxygen necessary?
* Fermentation type
  + Relationship between cell growth, substrate utilization, product formation
  + Related to metabolic pathway responsible for product
    - For now, using lumped parameter for this
    - Need some alignment between metabolism and structure of fermentation type
* Oxidative
  + Efficient at ATP formation
  + Cell yield high
  + Secondary product formation little to none
  + Scale up of oxygen transport is major part of design
  + Rate of feedback energy is heat or cells
  + Heat production is about half the value of the feedstock
* Non-oxidative metabolism
  + Low ATP yield
  + Low cell yield
  + High secondary product yield
  + Oxygen transport not an issue or needed
  + Fate of energy... Most goes to organic products
  + 10% of feedstock heating value heats up the cell
* Generation of ATP is most important
* Oxidative and non-oxidative metabolism can be combined, but not without decreasing product yield.
* Converting a large fraction of the feedstock mass and energy to organic products requires that most or all metabolism proceed non-oxidatively

### Type I Fermentation

* Substrate consumption proportional to rate of growth
* Product Formation proportional to cell growth
* Example
  + Ethanol fermentation
    - Glucose consumed by yeast
    - Electrons dumped into ethanol
    - Cells grow
    - Product proportional
  + Lactic acid
    - Electrons dumped into lactic acid
  + Anaerobic fermentation of pyruvate
  + Acetone
* Cells grow because they consume substrate, just make product
* Product directly from central metabolism

### Type II fermentation

* Substrate and growth proportional
* After some time, product formation proportional but not equal,
* Product not firefighting central metabolism
* Secondary or intermediate
* Examples
  + Secondary metabolites necessary for growth but not the main product of anabolism
    - MSG metabolism
    - Citrix acid
    - Glutamate
    - Lysine

### Type III fermentation

* No relationship between product formation and cell growth
* Examples
  + Antibiotics: want to kill competitors so they make it regardless of growth stage
  + Things that just needed t o maintain what you have to survive stages
  + Can engineer cells to perform this process
* Two stagesprocess
  + Tropophase populated bioreactor with catalyst
  + Idiophase uses the catalyst to form product
  + need to engineer a balance by decoupling with a trigger to switch the phases

# January 16, 2019

Chapter 5: Modeling Fermentation

* [chapter5-modeling\_fermentations.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Lecture_Slides/chapter5-modeling_fermentations.pdf)

### Derivation of Monod Model

* Empirical model
  + Fitting measures data to curve
* Theoretical approaches
  + Cellular energy balance (Heijnen and Remein)
  + Cellular redox balance (Jin and Bethke)
  + Coupled transport/reaction (Merchuk and Asenjo)

### Monod Equation

* Empirically derived curve fitting of microbial growth data
* Constants have direct relationships to physical and chemical phenomena
  + - X = cell concentration [g/L]
    - µmax = maximum specific growth rate
    - S = concentration of limiting nutrient
    - KS = Monod coefficient

### Specific growth rate vs glucose concentration

* Finding Ks = half of µmax

### Linearizing the Monod equation

### Inverse plot (Lineweaver - Burk): 1/mu vs 1/S

* y Intersection gives 1/µmax
* Slope is Ks/µmax
* X intersection gives -1/Ks
* Problem: small error multiplied as inverse

# January 18, 2019

## Coupling Substrate Use to Products (including cells)

* Yield coefficient
  + “yield of cells (X) per utilized substrate (S)”
  + Units = g (cells)/g (substrate)

## Cell Concentration [g/L] and Glucose Concentration [g/L] vs. Time [min]

## Concentration [g/L] and Cell Concentration [g/L] vs. Fermentation Time [hrs]

## Model: Material Balance on Cell

* Assume sugar is limiting nutrient
* Treat cell as “black box”
* True mass balance?
  + Type I fermentations most amenable to mass balance approach
  + Type III fermentations least amenable
* Sugar 🡪 Yeast 🡪Ethanol and CO2 (C, H, O), Minor waste products (glycerol, etc.) (C, H, O), Change in Cell mass (C, H, O, N, P, S)

### Table 4.1 Total Weights of Monomer Constituents which Make up Macromolecular Components in 100 g Dry Weight of *E. coli* K-12 cells (adapted from Battley, 1991)

* Monomers from
  + Proteins
  + RNA
  + DNA
  + Lipids
  + LPS
  + Peptidoglycans
  + Polyamines
  + Water
* C, H, O make up 76%
* N makes up an additional 15%

## Generalized Mass Balance

* A (CaHbOc) (carbon source) + B (O2) (oxygen (optional)) + D (NH3) (nitrogen source) 🡪 M (CαHβOγNδ) (cell mass) + N (C’αH’βO’γN’δ) (product) + P (CO2) (waste) + Q (H2O) + Heat
* Note: this is on a molar basis (need to convert mass of cells to number of cells to moles of cells using Avogadro’s number, 6.022 x 1023

### Cell Yield

* + R = CHON composition of cells (approximately 0.91) or CHO composition of cells (approximately 0.766)

### Product Yield

* + Units = g product/g substrate
* Sugar 🡪 Yeast 🡪 Ethanol + CO2, change in cell mass (+Gibbs Free Energy (ATP), + Reducing Equivalents (e-))

### Energy

* ATP = carrier of Gibbs Free Energy
* Energy also needed for cell growth
* Sugar 🡪 Yeast 🡪 Ethanol + CO2, change in cell mass, + Gibbs Free Energy (ATP) + Reducing Equivalents (e-)

## Luedeking-Piret Model

* + Substrate consumption = growth associated + non-growth associated
* , or other function!
  + α = YS/X = Yield coefficient
  + β = me = maintenance coefficient

### Modeling Constants

* *S. cerevisiae*, 100 g glucose/L
  + µmax = 0.27 hr-1
  + YP/S = 0.46 g/g
  + YX/S = 0.055 g/g
* *S. cerevisiae*, industrial, 20 g glucose/L
  + µmax = 0.29 hr-1
* *S. cerevisiae*, 424A (LNH-ST), 20 g xylose/L
  + µmax = 0.21 hr-1
* *S. cerevisiae*, Unidentified
  + KS = 250 mg/L
* *S. cerevisiae*, ATCC 4226
  + KS = 315 mg/L
* *Z. mobilis*
  + µmax = 0.37 hr-1
  + YP/S = 0.49 g/g
  + YX/S = 0.028 g/g
* *E. coli*
  + KS = 2-4 mg/L

## Inhibition

* Waste products often inhibitory to growth and metabolic function
* Disrupt cell membrane
* Inhibit cellular functions
* Inhibit enzymes

### Fraction of Uninhibited Ethanol Productivity vs. Ethanol concentration [g/L] i

### Ethanol Inhibition of Xylose Fermentation

* Extent of Inhibition [Vspecific/observed/Vspecific/control] vs Ethanol concentration [g/L]
  + Athmanathan, A.; Sedlak, M.; Ho, N.W.Y.; Mosier, N.S. “Effect of Product Inhibition on Xylose Fermentation to Ethanol by *Saccharomyces crevisiae* 424A (LNH-ST). “Biological Engineering 3(2): 111-124 (2011).

### By-Product Inhibition Summary

* Table 5-2: fermentation product, Concentration at high inhibition, and inhibition mechanism
* Kobs vs. CR
  + At n = 1, the curve is a straight line with a negative slope
  + As n increases, the curve has a high negative slope at first and tapers off until C\*R is reached
  + As n decreases, the curve starts with a small slope and then gets steeply negative until C\*R is reached
  + Beyond C\*R, the reaction stops

# January 23, 2019

## Homework 1 – Due Today

* [HW1-Monod\_growth-2019-01-07.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Homework/Homework-1/HW1-Monod_growth-2019-01-07.pdf)
* [Atherton-Homework-1.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Homework/Homework-1/Atherton-Homework-1.pdf)
* [Atherton-Homework-1-code.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Homework/Homework-1/Atherton-Homework-1-code.pdf)

## Inhibition

* Slows the rate of growth of cells and production

### Ethanol Inhibition of Xylose Fermentation

### By-Product Inhibition Summary for *S. cerevisiae*

* Table 5-2: fermentation product, Concentration at high inhibition, and inhibition mechanism
  + Ethanol
    - 70 g/L = inhibition
    - Membrane stability/porosity
  + Furmic acid
    - 2.7 g/L = inhibition
    - Chemical interference with cell maintenance functions
  + Acetic acid
    - 7.5 g/L = inhibition
    - Chemical interference/pH
  + Lactic acid
    - 38 g/L = inhibition
    - Chemical interference
  + Propanol
    - 12 g/L = inhibition
    - Chemical interference
  + Methyl-1-butanol
    - 3.5 g/L = inhibition
    - Chemical interference
  + 3-Butanediol
    - 90 g/L = inhibition
    - Chemical interference
  + Acetaldehyde
    - 5 g/L = inhibition
    - Chemical interference

## Kobs vs. CR

* The observed rate depends on the actual rate and the CR\*
* N > 1 is more common than n < 1
* Linear relationship as n = 1

### Extended Monod Kinetics for Substrate, Product, and Cell Inhibition

* By Octave Levenspiel, Oregon State University, August 6, 1987
* Modeled cell production, product inhibition, substrate inhibition, cell inhibition

## Using Levenspiel Equation

## Maiorella Ethanol Model

* + E = efficiency of cell mass production
  + Specific ethanol production rate
  + Substrate concentration
  + νmax = maximum specific production rate
  + Km = Monod constant
  + n = toxic power constant
  + Pmax = maximum product concentration
* Productivity of product is primary equation, everything else is linked to that

## Other Factors that Influence Growth

### Temperature

Growth constant, k [hr-1] vs. 1,000/T [K]

* Up to a certain point, a cell will grow faster until they die
  + 39oC is optimal for growth
* Semilog plot
  + Inverse time vs. inverse temperature
* Arrhenius equation: slope of line gives activation energy
* Hump in graph because of cell growth and cell death
  + After 39oC, cell death exceeds cell growth

## Continuous Stirred Tank Bioreactor

* Chemostat, turbidostat
  + Concentration of a certain molecule or product stays constant
  + Cell concentration stays constant
* Tank content is homogeneous in
  + Temperature
  + Composition (concentrations)
  + pH
  + etc.
* Inlet material instantaneously mixed into tank contents

### CSTR vs. CSTBR

* Growth of cells (biocatalysts) in CSTBR
  + Dynamics around catalysts (cells grow and die, so catalyst concentration does not remain the same)
* Difficulty in strict stoichiometry in biochemical reactions
* Otherwise, basic equations very similar

### Uses of CSTBRs

* Rare for industrial applications – why?
  + Agitation can be difficult for cells to grow
  + If there is a problem, throw out everything since last good test as you don’t know where things went badly
  + Pharmaceuticals require better handle on quality of product
  + Really only use for things where quality doesn’t totally matter
    - Ethanol, commercial solvents, etc.
* Research and development applications:
  + Study effect of changes in substrate concentration on cell growth/product formation
  + Study effect of environmental parameters such as pH and temperature
  + Selective culturing method for strain development
  + Sample for metabolic flux analysis of cellular metabolism (fill in the “black box”)

### CSTBR parameters

* X – concentration of cells (coming into the tank, in the tank, leaving the tank)
* S – concentration of substrate (coming into the tank, in the tank, leaving the tank)
* P – concentration of product (coming into the tank, in the tank, leaving the tank)
* V – volume of tank
* F – volumetric flow rate into tank, leaving tank
* Assumptions:
  + Perfect and instantaneous mixing: homogeneous assumption, concentrations in tank and leaving tank are equal
  + Operates continuously and at steady state, so volumetric flows must balance
  + No appreciable cell death within reactor

## Derivation of Growth Expression

* Continuity equation: Accumulation = in – out + generation – consumption
  + Mass is conserved
  + Accumulation = VdX
  + In = FXodt
  + Out = FXdt
  + Generation = VµXdt
  + Consumption = 0 (assume no appreciable death)
  + Divide by Vdt
  + - Dilution rate: how quickly are you replacing the volume of the reactor?
  + Assume that feed is sterile—no new cells in
  + “Net difference between µ and D, times X)
  + Cell growth – cells leaving
  + At steady state, dX/dt = 0
    - cell growth = cells leaving

# January 25, 2019

## Derivation of Growth Expression Continued

* – derived from continuity equation
* Steady state assumption:

## Derivation of Substrate-Growth Coupling Expression

* + Assume that generation = 0
    - Not generating substrate, but not always the case
    - Could feed the reactor a different substrate to generate the substrate to make the final product
    - Think about which terms are useful for each situation
  + Consumption: rate of cells growing multiplied by yield coefficient
    - Don’t include dilution because it doesn’t affect substrate
  + Divide by volume, dt; Dilution factor stays the same as growth expression
  + Net flux of substrate
  + Assume steady state
  + Use what was found above; know that µ = D
  + Ideally get equations into this form:
    - Y = dependent variable – things we don’t control, result of choices we make (i.e. S)
    - X = independent variable – things we have control over (i.e. So)
    - Independent variable = S
    - Dependent variable = D
    - Not exactly y = mx + b, but can segregate knowns from parameters to discover
    - Substitute in S from above
    - YX/S = 1/YS/X
  + Fix one = D, vary other = So to look at response
  + format!
  + Slope = YX/S
  + Independent variable = So
  + B = last term
* Take data at steady state, constant dilution rate, vary substrate concentration at feed
  + Expect that points should fall on a line, slope should give us YX/S
  + Helps to find what one of our parameters is
  + Data obtaining is long and painstaking
  + Yield coefficient can vary from beginning to end of fermentation process, can be hard to identify trends in the data immediately
* Cells consume substrate because they are growing *and* because they are alive – apparent yield coefficient in data, not actual yield coefficient!!!
  + In a batch reactor:
    - YS/X, app = observed disappearance of S per appearance of X
  + In a CSTBR at steady state µ = D
    - Y = mx+b format!
    - Slope is maintenance coefficient (me), intercept is true yield coefficient

## Constant Feed [Substrate]

* Keep Substrate concentration in feed constant and vary D
* Steady state concentration of cells starts out high when D is low
* Increase the flow rate, concentration of cells decreases slowly at first and then drop off to zero quickly
  + High D is where dilution is higher than maximum growth rate, so cells cannot grow fast enough to replenish themselves
  + At steady state, wash out and make reactor sterile
  + Precarious condition to operate a reactor in, so any slight change in pH and temperature can cause a washout
* Conversely, concentration of substrate vs. dilution rate
  + Concentration of substrate starts at zero and then increases exponentially (eventually plateau at feed concentration of substrate at washout condition)

# January 28, 2019

## Washout Condition

* Nothing happens in the reactor because there are no cells to do anything!
* Assume steady state, assume no uncontrolled behavior in transient
* Reactor is most efficient at critical condition, just before washout!

## Steady State to Find Critical Condition

* + (critical dilution rate)
  + When So >> Ks, µ = DC = µmax
* Graphing biomass output (concentration of cells per hour) vs. D
  + Biomass output = DX
  + Constant S­o
  + Critical point is peak, just before washout when productivity goes to zero
* Zooming in on the roll off point – critical point doesn’t quite meet the maximum productivity

### Maximum productivity

* Rate of cell output = R = DX

### Dilution rate at Maximum Productivity

### Maximum Cell Concentration

* if So >> KS
  + Maximum cells = yield coefficient \* substrate available
* Cell concentrations rapidly plummet to zero if dilution rate fluctuates over critical
  + Usually want to run just below in case of fluctuations

Chapter 6: Aerobic Fermentations

* [chapter6-aerobic-fermentations.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Lecture_Slides/chapter6-aerobic%20fermentations.pdf)

## Aerobic Fermentations

* Amino acids
* Antibiotics
* Recombinant proteins (pharmaceuticals)

## Effect of Dissolved Oxygen on Cell Growth

* + µ ~ µmax

## 2,3 Butanediol

* Manufacture of butadiene rubber, plastics
  + (ABS – Lego bricks)
* Byproduct
* Run compound over a catalyst in the presence of hydrogen, strip out water, get butadiene
  + Synthetic rubber
* Made from petroleum all today, but could be made from renewable resources

### *Klebsiella oxytoca* ATCC 8724

* Related to *Klebsiella pneumoniae*
* Han Christian Gram invented “Gram stain” to differentiate between *Klebsiella* and *Streptococcus pneumoniae*
  + Treated in different ways because one is gram positive and gram negative
    - Gram positive killed by penicillin
    - Gram negative not affected by penicillin
* Of industrial interest
  + Readily ferments xylose and glycerol
    - Lower market value – less competition than glucose
  + Makes 2,3 butanediol
* Facultative anaerobe
  + Can grow with or without oxygen
  + Oxygen is not poisonous

### Cellulosic Biomass: Major Constituents

* Lignin: 15% - 25%
  + Complex aromatic structure
  + High energy content
  + Resists biochemical conversion
* Hemicellulose: 23% - 32%
  + Xylose is the second most abundant sugar in the biosphere
  + Polymer of 5- and 6-carbon sugars, marginal biochemical feed
* Cellulose: 38% - 50%
  + Most abundant form of carbon in biosphere
  + Polymer of glucose, good biochemical feedstock

### Xylose (Wood Sugar)

* Xylose is a carbohydrate without much of an industrial use
  + Found in every plant cell wall, not useful to human digestion
  + Second-most abundant sugar
* C5H10O5
  + Pentose: 5-sugar
* In grasses (corn, wheat, rice, etc.), 40% of carbohydrate of inedible plant material
  + Fairly “young” plants

#### Xylose Fermentation

* In: xylose
* Out:
  + Respiration: if O2 is available
    - Generates CO2­, ATP
  + Fermentation: if no O2 is available
    - Generates CO­2­, ATP, 2,3 butanediol
  + Cell mass
    - Uses ATP

#### Fermentation pathways in Klebsiella pneumoniae and strategies for constructing the 2,3-butanediol-producing base strain

* Simplified glycolysis
* Electron shed between glyceraldehyde 3-phosphate and phosphoenol-pyruvate
* In processing of pyruvate, need to deal with electron
  + Make ethanol, lactate
  + Don’t want lactate, took out gene to make it
  + Alternative pathway: from pyruvate to acetolactate, acetoin, 2,3 butanediol
    - Consumes half an electron per one 2,3 butanediol
    - Need more electrons: supply just enough oxygen to supply the electrons, but not enough to perform respiration

### 2,3-Butanediol

* Plot xylose, 2,3-butanediol concentration, ln(cell weight) vs hours.
  + Ln of cell dry weight because cells grow exponentially, but ln makes it appear linear!
  + When no 2,3-butane diol, just enough oxygen to respirate
  + When cell dry weight goes off of the linear, oxygen deprived so 2,3-butanediol was formed
  + Rate of aeration constant throughout experiment
  + When rate of oxygen consumption exceeds rate of oxygen supply 🡪 fermentation occurs

## Modeling Approach

* Cellular function is ATP constrained
  + “Gibbs Free Energy is electron-rich”
* ATP use is prioritized: cell maintenance then cell growth
  + Cells try to stay alive first, then divide
* Metabolism is regulated to maximize ATP production
  + Cells make as much ATP as they can, given environmental constraints
* O2 is required for maximum ATP generation
  + Respiration is preferred until O2 deficiency
* When O­2 is limiting, anaerobic metabolism is induced to make up the ATP deficiency
  + Cell growth is scaled to make up for deficiency

# January 30, 2019

## Homework 2 – Due Today

* [Hw2-ethanol-model.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Homework/Homework-2/Hw2%20-%20ethanol%20model.pdf)
* [atherton-homework-2.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Homework/Homework-2/atherton-homework-2.pdf)
* [SIMBAS\_homework2.xlsm](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Homework/Homework-2/SIMBAS_homework2.xlsm)

# February 1, 2019

## 2,3 Butanediol Phases of Fermentation

* Aerobic fermentation
* Transition from aerobic to oxygen limiting conditions
* Oxygen limiting conditions – where 2,3 Butanediol is made
  + Microaerobic or Anoxic fermentation

### Terminology

* QS: rate of substrate utilization **(consumption)** via specific metabolism
* In batch fermentation:
  + No in, no out, no generation
  + Each Q represents a metabolic pathway that consumes substrate S
  + Written in molar format, so need to use molecular weight to convert to mass form of ODEs
* Example:
  + QS,tot = QS,A + QS,R + QS,F­
    - QS,A = cell mass
    - QS,R = respiration
    - QS,F = fermentation

### Stoichiometry of Respiration

* Xylose 🡪 5 CO2 + 10 NADH + 10/3 ATP
* ½ O2 + NADH + H+ 🡪 2 ATP + H2O
* Xylose + 5 O2 🡪 5 CO2 + 70/3 ATP

# February 4, 2019

## Stoichiometry of Fermentation

* Stoichiometry doesn’t quite balance because omitting water as biochemists do
* Determine when fermentation pathway kicks in after respiration
  + Assumptions:
    - Xylose consumed at some rate dS/dt
    - Batch reactor model (in = out = 0; acc = gen – con)
      * Consumption of S is due to respiration, fermentation, cell mass
    - Cycling of ATP <-> ADP so fast in comparison to dS/dt such that dATP/dt = 0
    - Cycling of NAD+ <-> NADH so fast in comparison to such that dNADH/dt = 0
    - 2,3 butanediol produced at some rate dP/dt
    - Cells grow at some rate dX/dt = S \* µmaxX/(KS + S)
      * KS is so small that dX/dt= ~ µmaxX
    - Rate at which cells uptake oxygen doesn’t change quickly

## Phase I – Aerobic Growth

## Phase II – When is O2 limiting?

* Xylose + 5 O2 🡪 5 CO2 + 70/3 ATP
* Limiting when OUT > Oxygen Transfer Rate
* 5 QSR > kLaC\*
  + H = Henry’s law constant
  + KL = lumped transfer coefficient
  + A = surface area of bubble
  + P = partial pressure
  + C = concentration of O2 in the water/bubble
  + High temperatures = low solubility of a gas
  + Concentration decreases as distance from bubble increases
  + At the limit, no oxygen in solution, rate of mass transfer = C\*
    - Once molecules go into solution, bacteria uptake them
* Metabolism is balanced so that oxidative phosphorylation (electron transport system, ETS) is saturated
  + NADH + ½ O2 + H+ 🡪 ATP + NAD+
    - dNADH/dt = 0
      * generation = consumption
    - consumption = QETS = 2 kL­aC\*
    - generation = 10 QSR + 5/6 QSF
    - Assumption: cell will self-optimize to make the most ATP possible
      * Only do fermentation out of desperation
    - QETS = dNADH/dt
    - QETS = -2kLac\* + 10 QSR­ + 5/6 QSF
  + Maximize ATP generation!
    - QSR = 1/10 (Q­ETS – 5/6 QSF)
    - Consumption = µmax X/YATP + meX
    - dATP/dt = 0 🡪 generation = consumption
    - generation = 10/3 QSR + 5/3 QSF + 2 QETS
    - QSF = 18/25 (µmax­X/YATP + meX – 7/3 QETS)

## Phase III - Fermentation

* QETS = 2 kL­aC\*
* QSF = 18/25 (µmax­X/YATP + meX – 14/3 2 kL­aC\*)
* QSR = 1/10 (2 kLaC\* - 5/6 QSF)
* Q­SA 1/120 µmax­X
* dX/dt = µmax­X - kdPX
* dP/dt = 5/6 QSF MW2,3butanediol
* MW2,3butanediol = 90 g/mol
* dS/dt = -(QSR + QSF + QSA) MWxylose

### Table 6.2

* differences in parameters for oxygen sufficient and oxygen insufficient conditions
  + Y­ATP not much different, but better in oxygen sufficient
  + µmax has no change
  + me nearly triple in oxygen sufficient conditions
    - cleaning up free radicals
  + kLaC\* and Kd are not valid in oxygen sufficient conditions
* parameters are better in oxygen sufficient conditions because oxygen is very reactive
  + oxygen relates to DNA damage, mutations, cancer

# February 6, 2019

## Aeration

…

# February 8, 2019

## Exam 1 Today

<cheatsheet.pdf>

# February 11, 2019

## Surface Area of Bubbles

* a = total volume of bubbles / total volume of broth \* area of bubble / volume of bubble
  + = nFotb/V \* πD2/(πD3/6)
    - N = number of orifices in a sparging tube
    - Fo = bolumetric air flow rate per orifice
    - Tb = residence time of bubble in liquid
    - D = average diameter of air bubble
  + = Vb/Vl \* πD2/(πD3/6)
  + = H(6/D)
* V = πr2height
  + H = (ha – ho)/ho
    - Introducing air to the volume of liquid adds volume (ha is liquid + air volume, ho is volume of liquid without air)
    - H = ratio of volume of bubbles to volume of liquid

## Bioreactors/Fermenters

* Mechanical Agitation
  + Affect on kLa
  + No electrical bill for mechanical agitation
* Bubble columns
* Loop reactors

### Power for Agitation

* Want mass transfer
* Pay for it with mechanical agitation
  + Pg = gassed power, horsepower
  + V = volume of gas-liquid dispersion (aerated solution, L)
  + Vs = superficial gas velocity (cm/s)
* Note non-linear (important for scaleup)
* Note ratio of P/V – power per unit volume
  + Pg­ = gassed power, HP
  + Po = ungassed power, HP
  + N = rpm of impeller
  + D = impeller diameter, feet
  + Q = gas flow rate in ft3/m
  + Different powers to spin when there are bubbles vs. when there aren’t
    - Less power when aerated

### Michel and Miller

* Depends on types and number of impellers
  + Turbine impeller
    - Length
  + Marine impeller
    - Length
    - Curvature
    - Direction – multiple gives cross-flow
  + Rushton impeller
    - length

### Fermenters

* “cylindrical-ish”, may have conical/rounded bottom for drainage
  + For ease of manufacture
* Height : Diameter = 2:1 or 3:1
  + Animal cell reactors often 1:1
  + Not flag poles, more like a can of soda or beer in ratio
* Constructed from stainless steel to prevent corrosion
  + Plant and animal cell – 316L (L = low carbon, no iron)
  + Using water and salt – not a good combination for iron or steel
  + Stainless steel has hundreds of grade – name is pure marketing, no iron!
* Foaming is an issue in aerated reactors
  + Working volume is usually 60-75% of total volume to account for this
  + Foam comes from liquid + gas + emulsifier/surfactant (protein!!!)
  + Vents have a filter, foam plugs filters – turns reactor into a pressure vessel

### Stirred Tank Bioreactors

* Flexible operation
* High kLa
* High power
  + Up to 5 kW/m3
* 400 m3 (400,000 L) max
* Baffles – stir on outside
* Impellers – stir in center
* Design considerations
  + Height
  + Diameter
  + Size of baffle/impeller
  + Working volume
  + Height/thickness of impellers/baffles
  + Height from bottom of impellers/baffles
  + Number of impellers/baffles and spacing
  + Bottom shape
* Design parameters
  + Power input, P
  + Volume, V
  + Impeller Rotation, N
  + Impeller Diameter, D
  + Density of fluid, ρ
  + Viscosity of fluid, µ
  + Gas flow rate, QG
* Problems with design
  + Flooded reactor
    - Residence time is low
    - Air flow too high or rotation too low
  + Loaded reactor
    - Intermediate
    - May be preferred configuration
    - Good residence time, dispersion
  + Fully dispersed reactor
    - Good mixing, long residence time
    - Bubbles carried around in eddy currents – stay in liquid, don’t disperse out
    - Could cause a lot of foam, but shouldn’t be a problem for reasonably sized reactors

#### Similar to Distillation Operation

* Improper liquid flowrate or vapor flowrate
  + Weeping – liquid percolates through holes where vapor should come through
* Vapor flowrate too high
  + Foaming, no liquid mixing (Weir crest)
  + Low contact time

#### Rushton Impeller Flooding Limit

* + Fr = Froude number = N2D/g = ratio of the inertial to buoyancy forces
    - Inertial is forces from mixing (down, side to side)
    - Buoyant is forces from floating up
  + ND3 = pumping rate of impellers = volume of liquid pushed by impellers = Q
  + Left = ratio of volumetric gas flowrate from pump to flowrate caused by mixing
    - Mixing of reactor itself acts as a “pump”
  + Right = ratio of diameter to height

### Scale-Up Rules of Thumb

* Constant P/V = Constant kLa
* Constant N\*D = Constant Shear Rate
  + Cavitation bad for cells and mechanical components
  + High concentrations of salts, corrode impellers
* Constant N = Constant mixing times
  + How long does it take to fully disperse in reactor?

# February 13, 2019

## Table 9 – Different Criteria for Bioreactor Scale-Up

* Scale up for 2L batch reactor to 20L plant reactor
* T = height of tank
* Keep H/width ratio same
  + What other parameters do we keep constant (C) or change?
* P/V = power
* πNT = shear rate
* Re = Reynolds Number
* KLa = mass transfer rate

## Mixing in Large Scale Bioreactors

* Mixing – for O2 transfer and nutrient and pH dispersion – is the critical issue with scale up
* Mixing rate (time) is proportional to mixing speed (N)
* Power requirement quickly outpaces ability to mix reactor

### Estimating Mixing Time

* [W/Kg]
* As reactor gets larger in size, mix time increases

#### Plotting performance ratio vs. oxygen transfer rate for different types of tanks

* Use buoyant forces of air to mix contents of reactor
* Trade-offs on what it takes to agitate oxygen in a reactor
* Higher is better on X axis
* Y axis considers electrical motor that powers impellers and shaft and fan/pump that pumps air into reactor
* Lower is better on Y axis
* Agitator is good because long contact time between bubbles and fluid, bubbles are broken up so better surface area

### Airlift Loop Reactors

* Low shear
* Lower kLa than mechanically stirred reactor
* Good for mammalian cells
* Reasonable oxygen transfer
* Example: wastewater treatment plant aerobic reactors
* Advantage of external loop airlift reactor:
  + Separation of gas and fluid easier
  + Fluid velocity profile decreases when increasing volume of tube == calmer surface
  + Less likely to make foam
* Want lots of tiny bubbles == lots of surface area
  + Will probably get mixture of bubble sizes
  + Churn-turbulent: likely to happen: turbulent flow, cannot assume spherical shape; larger bubbles form when smaller bubbles hit each other
  + Don’t want slugging where giant slugs of bubbles that pass through reactor with low surface area; splash fluid everywhere, not good mass transfer

#### Plot PGUsg2 vs. PLULr2

* + Usg = superficial gas velocity
  + ULr = superficial liquid velocity
  + Shows areas where different bubble phenomena occur for different gas and liquid velocity. Want high liquid flow rate and low gas flowrate

#### Plot superficial gas velocity JG vs. Colum diameter D

* + Know volumetric flowrate
  + Know volume of gas in reactor
  + Divide out to get superficial gas velocity
  + Want homogeneous bubbly flow: low gas velocity

## Antibiotics: Background for Homework 4

Chapter 7: Enzymes

## Enzymes

* All enzymes are protein (not all proteins are enzymes)
* All proteins composed of at least one linear polymer of amino acids
* The sequence of amino acids in the polymer is encoded in DNA
* The 3D structure of the protein, which is a function of the solution thermodynamics of the polymer, determines the function
  + In aqueous solution!
  + Thermodynamics and shape change if the solution changes (i.e. acetone)

### Amino Acids

* Chiral organic molecules
  + Carbon that has four bonds to four different things
  + All except one amino acid because it has H instead of R
* Amine + carboxylic acid
  + Amine = NH2
  + Acid = COOH
  + R = side chain
    - Acidic, basic, hydrophilic, hydrophobic, charged, uncharged, etc.
    - There are infinite side chains that we can synthesize that aren’t found in nature

#### Charged characteristics of amino acids

* Charge depends on pH of solution
  + Low pH = + charge
  + High pH = - charge
  + Neutral pH = balanced charges (neutral molecule)
* Rs can also be charged and then can change the charge of the molecule based on pH
* Proteins vary in charge over pH
* Different dominant forms at different pHs
  + Solubility of proteins tend to be lowest at pI
    - When they lose charge, they come out of solution

### Amino Acid Polymerization

* Condensation reaction
  + Water is removed from 2 adjacent amino acids to form a peptide bond
  + Take out the OH form the acid and an H from the amine ALWAYS
  + Always from non-R chain amine or carboxylic acid

### Proteins

* Proteins are linear polymers of amino acids
  + Directionality
  + N-terminus (amine) 🡪 C-terminus (carboxylic acid)
  + Order of amino acids determines structure
* Structure and function of protein determined by 3D structure that polymer assumes in water

# February 15, 2019

## Side Chains

* 20 naturally occurring side chains
* Five groupings
  + Acidic and amide
  + Aromatic
  + Basic
  + Aliphatic
    - Glycine – only non-chiral amino acid
  + Sulfur and hydroxyl

## Proteins

* 3D structure a function of lowest energy conformation
  + Hydrophobic groups associate away from water
  + Hydrophilic groups associate toward water
  + Opposite charges can associate
  + Disulfide bonds
* Protein function
  + Enzymes catalyze biochemical reactions
  + Structure and support
    - Collagen
  + Communication between cells (hormones)

### Fred Sanger

* Godfather of Bio-sequencing
* Nobel Prize in Chemistry – 958
  + Developed a chemical reagent to label N-terminus of polypeptide
  + Used chromatography to isolate and identify specific amino acid
  + Enabled 1o structure of proteins to be determined
* Nobel Prize in Chemistry – 1980
  + Developed di-deoxy nucleotides to act as terminators to PCR
  + Used electrophoresis to determine which nucleotide (C, G, A, or T) what last in the chain
  + Enabled DNA to be sequenced
* Only person to be awarded Nobel Prize in Chemistry twice
* Died in 2013 at 95 years old – “just a chap who messed about in the lab”

### Protein Structure

* Primary structure (order of amino acids)
* Secondary structure (beta pleated sheet and alpha helix form due to interactions of individual amino acids)
* Tertiary structure (how secondary structures interact)
* Quartinary structure (how proteins associate together to make more complex structures)

#### Alpha Helix

* Hydrogen bond interactions that are close together in chain sequence
* Weak bonds

#### Beta Sheets

* Hydrogen bond interactions but further apart in chain sequence
* Weak bonds
* Look like accordion pattern

#### Disulfide Bonds

* Adjacent sulfurs coming together
* Covalent bonds, stronger
* Usually two cystines

##### Example: Thrombospondin antiangiogenic protein

* “anti” – against
* “angio” – chest pain
* “clot-busting drug”
  + Used for heart attacks or strokes
  + Breaks down blood clots
* Three di-sulfide bonds
  + Amino acids are not close spacially

##### Example: Insulin

* Protein messenger (hormone)
* Stimulates the uptake of glucose from the blood by liver, muscle, and fat tissues
* Protein Sanger studied when developing the sequencing method
* Highly conserved protein
  + Used in lots of animals
  + Sequence homology is very similar between humans, chimps, mice, rabbits, frogs, and zebrafish
  + Important function
* Synthesized as continuous peptide and then insulin is actually two fragments held together by disulfide bonds
* Insulin made by companies is made by bacteria that don’t necessarily know how to fold them correctly
  + Control redox potential in solution to ensure disulfide bond forming
  + Lots of purity separation

## Enzymes

* Biochemical catalysts
* Specificity
  + Small number of molecules able to participate in catalyzed reaction???
  + “lock and key” analogy
  + Value: catalysts are very specific, only react with one compound and not others
  + Some more specific than others
* Selectivity
  + Small number of reactions catalyzed
    - NA = Desired product
    - NB = Undesired product(s)
    - Good number? Enzymes have massive numbers! 50,000 or 9,000,000!

### Basis of Specificity

* Substrate binding – multiple interactions
  + 2-3 interactions to “recognize” substrate

### Basis of Selectivity

* Enzyme active site structure
  + Example: glycoside hydrolase retaining mechanism
    - (sugar, adding water)
    - Bond acted upon is close to catalytic reaction area
  + Example: Chymotrypsin
    - Cleaves water out of Phe, Trp, Try
  + Example: Trypsin
    - Cleaves water out of Arg, Lys

## Enzyme Nomenclature

* “traditional” or common names
  + Like “homo sapiens” vs. people
* Enzyme code
  + Developed by IUBMB, affiliated with IUPAC
  + International Union of Biochemistry and Molecular Biology (IUBMB)
* Family classification
* <http://www.chem.qmul.ac.uk/iubmb/enzyme/>

### Common Name

* Suffix –ase
* Root word = substrate
* Amylose hydrolyzing enzyme = amylase
* Protein hydrolyzing enzyme = protease
* Most enzymes are reversible! So what is the substrate and what is the product?
  + Depends on point of view
  + Enzyme that converts ethanol to acetaldehyde and vice-versa = ethanol dehydrogenase
    - Originally studied in rat livers to turn ethanol into acetaldehyde
    - Yeast do the opposite reaction, still called the same!
      * Make ethanol to steal food from competitors and kill competitors
      * Make ethanol faster than they grow to win competition
    - Yeast have a different enzyme to do the rat liver reaction!
* Common name rules are broken pretty often!

### Enzyme Code

* Based on reaction catalyzed
* E.C. 2. 7. 1. 1 (hexokinase, phosphorylates hexose (i.e. glucose), first step in glycolysis)
  + First number = class
    - 1 = oxido-reductase – redox reactions
    - 2 = transferase – transfers functional groups
    - 3 = hydrolases – hydrolysis reaction
    - 4 = lyases – adds something to a bond
    - 5 = Isomerases – isomerize
    - 6 = ligases – tie things together
    - 7 = actively transport molecules across membranes by hydrolyzing ATP (new as of August 2018, first since 1961)
  + Second number = subclass
  + Third number = sub-subclass
  + Specific enzyme
  + Kind of like Kingdom, Phylum, etc.
* Formal naming convention that tells what kind of bond is reacted on, where the bond is in the substrate, and the reaction type

### Family Classification

* Based upon relatedness between primary amino acid structure
* Structural classification
* Gene-related
* Evolutionary relationship
* Least useful classification for reactions!

## Enzyme Database

* Online databases of biological data (bioinformatics) are useful resources
* Enzyme-specific database is BRENDA
  + Braunschweig Enzyme Database
  + University of Cologne
  + Started as book series in 1996
  + <http://www.brenda-enzymes.info/>
    - Can search for enzymes if you know the substrate structure
    - Can search by name or code
    - Gives you information about organisms that use the enzyme
    - Gene information
    - Papers where data is harvested from
    - Substrates
    - Cofactors
    - Km, Kcat, Ki values for different organisms

# February 18, 2019

## Example I – Laundry Enzyme

* [chapter7-brendaexamples.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Lecture_Slides/chapter7-brendaexamples.pdf)
* Create a model for the production of α-amylase (3.2.1.1) from *Bacillus lichenformis*
* An additive to remove starch-based stains
* Important considerations
  + Cofactors – anything that you need in addition to having the protein itself for it to function (i.e. ATP, NADH, etc.)
    - May have to provide in reaction
  + Activity (kcat, km)
    - Kinetics of enzyme reactions
    - Constants
    - kinetic rate of catalysis
      * Turnover number, how quickly substrate turned into product
    - Concentration where rate is half the maximum
      * Don’t want too large so that it goes quickly at relatively low concentration
  + Inhibitors
    - What might inhibit in our application?
  + Possible conditions
    - Temperature
    - pH
* <https://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.1&Suchword=&reference=&UniProtAcc=&organism%5B%5D=Bacillus+licheniformis&show_tm=0>
  + Give a lot of strains of bacteria species
  + Get gene ontology number
  + Reaction definition
  + Substrates can matter!
    - Natural substrates = not laboratory synthesized substrates
  + Metals and ions
    - Stimulate enzyme
  + Inhibitors
    - Some detergents!
    - Dissolve the proteins and don’t allow for function
  + Optimum pH
    - 5 – 9.5 for different variants
  + Temperature optimum
    - Again, different variants
  + Km and Kcat

## Example II – Lactose Removal

* Finding an enzyme that does the following reaction: lactose 🡪 glucose + galactose
  + Hydrolysis 🡪 3
  + Glycosidic bond 🡪 3.2
* Which one is better?
  + Parameters
  + Conditions
  + Availability
  + Express on another organism?
* Lactase: 3.2.1.108: <https://www.brenda-enzymes.org/enzyme.php?ecno=3.2.1.108>
  + Lactose galactohydrolase
  + Β-galactosidase
* Homo sapiens
* Km values
  + Pay attention to substrate
  + 5.1-30
* Optimum temperature:
  + 20oC – why not 37?
    - See *E. coli*

## Enzyme Commission (Code)

* [chapter7-enzymecodeexamples.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Lecture_Slides/chapter7-enzymecodeexamples.pdf)

### Code defined by

* Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)
* <http://www.sbcs.qmul.ac.uk/iubmb/enzyme>

### How EC Works and How to Use it

* Hierarch of classification (similar to Linnaeus taxonomy – kingdom 🡪 phylum 🡪 class, etc.)
* Class 🡪 sub-class 🡪 sub-sub-class 🡪 serial identifier
* Example
  + EC 3.1.21.4
    - 3 = hydrolase (inserts water into bond)
    - 1 = acts on ester bonds
    - 21 = endodeoxyriboneclease producing 5’-phosphomonoesters
    - 4 = type II site-specific deoxyribonuclase

#### Classes

* 1. Oxidoreductases: redox reactions
* 2. Transferases: transfers chemical group from one molecule to another
* 3. Hydrolases: cleaves bond by addition of water
* 4. Lyases: removes a group from a molecule leaving a double bond or adds a group to an existing double bond
* 5. Isomerases: isomerizes molecule
* 6. Ligases: joins two molecules together

### Example of using EC to find enzymes

* Semi-synthetic derivatives of penicillin can increase antimicrobial activity, lower harmful side-effects, and overcome antibiotic resistance
* You’ve been charged with searching for potential enzymes that could catalyze reactions with penicillin that would enable large-scale manufacture of antibiotics that affect resistant bacteria

#### Antibiotics

* Specific chemical substances derived from or produced by living organisms that are capable of inhibiting the life processes of other organisms
* Various mechanisms are known
  + Interfere with protein synthesis
  + Interfere with key enzymes needed for synthesizing cell wall

#### Gram (+) vs Gram (-) Bacteria

* Gram (+) bacteria: thick cell walls of amino acid cross-linked polysaccharides
  + *Bacillus antracis*
  + *Clostridium perfringens, defficile*
  + *C. diptheriae*
  + *Listeria monocytogenes*
  + *Staphylococcus*
  + *Streptococcus*
  + *Shigella*
  + Penicillin (and other beta-lactam antibiotics) covalently binds to the active site of the enzyme that makes these peptide bond cross-links
    - Structure
    - Naproxin (Aleve)
* Gram (-) bacteria: thin polysaccharide cell wall coated with a lipid layer (lipopolysaccharides, LPS)
  + *E. coli*
  + *Klebsiella*
  + *Salmonella*
  + *Shigella*
  + *Yersini*
* Pathogenic forms of both are known

#### Penicillin

* Penicillin G (natural fermentation product) + Naproxin (nonsteroidal anti-inflammatory drug: NSAID)
* Step 1: prepare penicillin G
  + Penicillin acylase: 3.5.1.11
    - 3 = hydrolase
    - 5 = acting on C-N bond, other than peptide
    - 1 = acting on linear amides
    - 11 = penicillin amidase (preferred name)
* Step 2: conjugation
  + Unknown enzyme
    - What is the EC for the enzyme?

# February 20, 2019

## Penicillin

* Step 2: conjugation
  + Use Enzyme code to find class of enzymes to test in the lab
    - 6 = ligases: joining 2 molecules together
    - 6.3 = forming C-N bonds
    - 6.3.1 = acid-ammonia or amine ligases (amide synthases)
    - 6.3.1.??
      * Based on enzymes in this category, likely requires ATP to drive Gibbs free energy!

Chapter 8: Enzyme Kinetics

* [chapter8-enzyme\_kinetics.pdf](https://d.docs.live.net/a6815758e2b5dbf9/ABE_580/Lecture_Slides/chapter8-enzume_kinetics.pdf)

## Enzyme Kinetics

* Similar derivation to all chemical kinetics
  + Fundamentally: same as all chemical kinetic reactions
* Enzyme kinetics has some special considerations
  + Aqueous environment
    - Usually, but some exceptions
    - If water is produced by the reaction, it does not significantly change the concentration of water (omit water from concentrations – constant)
    - Same if water is a reactant
  + Catalyst reaction
  + Multiple substrates in single reaction common
    - Sometimes the order that the substrates interact with the enzymes matters
    - Interaction of substrates with enzyme changes how substrates interact with each other

### Mechanistic Reaction Models

* Focus on mechanism of reaction
  + Values describe fundamental interactions!!!
  + Inhibitors, activators, substrates, products
* Interaction of substrate(s) and catalyst(s)
* Interactions of product(s) and catalyst(s)

#### Use of Mechanistic Models

* Accurately captures interactions between enzymes and substrates
* Elucidates dynamics of enzyme binding and dissociation
  + Helps understand the mechanism, how to test/diagnose mechanism theory

## Kinetic Theory: Collision Theory of Reaction Rates

* A + B 🡪(k) 🡪 C
* K = (ZAB)(F)
  + K = kinetic constant
  + ZAB = frequency of collisions
    - Function of temperature and pressure of system
  + F = Boltzman factor (fraction of collisions giving rise to reactions)
    - Not all collisions are in correct orientation, have enough energy to give rise to reactions
* Developed for dilute (low pressure) gases
  + Violates all the assumptions made for enzyme reactions!!!
* Reactions in liquid (or aqueous!) phase incoherent based upon first principles estimations for gases
* However, still widely used for developing mathematical expressions of biochemical reactions
  + Condensed-phase, aqueous reactions still modeled with this

### Deriving and Using this Theory

* Accumulation = In – Out + Generation – Consumption
  + Assuming mass is conserved in A + B 🡪 C
* How does C accumulate in our system? – assume batch reaction, C is a concentration, no consumption of C
  + dC \* V = Fin C0 dt – Fout C dt + kAB V dt – 0
    - Batch: flow terms are zero
    - Second order reaction
    - Enzymes: there isn’t an order, or if there is an estimated one it’s not an integer (i.e. 1.792)
    - From Law of Mass Action

## Michaelis Menten Kinetics

* Background
  + German professor of organic chemistry in 1917
    - Beginning of WWI, invited Maud Menten to his lab
  + Maud Menten was the first Canadian woman to get a PhD in medicine
    - Couldn’t get a job in Canada, joined German lab
  + Interested in how enzymes function, specifically invertin (invertase)
    - Breaks down sucrose into glucose and fructose
  + Jointly published paper that described their studies and how they developed the Michaelis-Menten expression
  + She left Germany and came to the US
  + Menten joined Johns Hopkins
    - Scarlet fever vaccine
    - Diabetes
    - First woman head of pathology
  + Michaelis stayed in Germany
    - 1922: left Germany because he was Jewish
    - Joined University of Tokyo for three years
    - Left and came to the US, worked in medical research at different universities
* Mechanistic model
* Based upon Law of Mass Action
* Key assumptions – important to understand so you know when they are violated
  + Homogeneous reaction
    - Enzymes aren’t isolated in the cell – usually not the case!
  + Substrate in excess of enzyme [S] >>> [E]
    - Not bad assumption – definition of catalyst is that there is a small amount of catalyst
  + (pseudo) steady-state or equilibrium
    - Either way get same final product, but parameters are different

### Example

* Enzyme + Substrate 🡨(k-1), (k­1)🡪 ES (k2)🡪 Enzyme + Product
  + Assumptions
    - k­1 and k-1 >> k2
    - dES/dt = 0 (steady state)
      * nonzero value
    - batch reactor (in and out are zero)
  + Steady-state assumption
* Mathematical trick: need because we don’t have molecular counter
  + Don’t know how much is E vs. ES
  + We can only write as a total amount of enzyme!
  + Want to know dP/dt, which is in terms of ES, but we don’t know what ES is, only Etotal
  + Substitute into expression to replace E
  + Skip algebra to final product:
  + ES = some function, substitute into dP/dt
  + KM = (K­-1 + K2)/K1
  + Vmax = k2Etotal
  + **If you change how much enzyme you have, vmax changes!!! It’s not intrinsic to the enzyme**
    - **Only the k2 value is intrinsic**

# February 22, 2019

# February 25, 2019

## Non-Linear Regression

* Couples statistics (least squares) with modeling (computer simulation)
* Ordinary Least Squares
* Weighted Least Squares

### Elements of Non-Linear Regression

* A model
  + Hypothesis of mechanism of reaction
  + Mathematic representation that relates parameters and measured values
    - Enzyme parameters
    - Substrate, product
    - Rate of reaction
  + Choice of model is statement of mechanism
  + Use to test hypotheses
* Initial “guesses” for parameters
  + Supply initial values for all parameters
* Experimental data
  + Use to derive statistics
  + Compute error/mismatching
* A “fitness function”: min{SSE}
  + Driven by error
  + Function describing state of model with respect to experimental data
  + Least Squares: sum of squares of errors (residuals)
  + Similar to linear regression: want to minimize the sum of the squares of the error
* From an engineering standpoint, you will get a “good” answer, but is in the most accurate answer? No.

### GRG Non-Linear Solver in Excel

* Generalized Reduced Gradient Algorithm
* Good at finding local minimum, not global minimum
* Converts non-linear system to reduced linear system, follows the downward slope, then iterates
  + Uses eigenvectors
* Try a lot of different guesses
  + Does the solver consistently find the same answer as the minimum?
  + Determines global minimum

## Classical Inhibition: Assumptions

* Irreversible reaction
  + Consume ATP? Probably irreversible
* Simplified effect of inhibitor
  + Competitive: blocks substrate binding
  + Non-competitive: blocks catalysis without affecting substrate binding

### Where Assumptions Break Down

* Inhibitor affects binding kinetics/equilibrium without blocking binding or inhibiting catalysis
* Inhibitor affects catalysis kinetics without affecting binding of substrate
* Inhibitor affects both substrate binding and catalytic rate
* More than one substrate

#### Multiple Substrates:

* Must both substrates be bound for reaction to occur?
* Does binding of A affect binding of B (and vice-versa)?
* Does reaction proceed via intermediate step

#### A Question of Mechanism

* Bi = two substrates, one product
* Bi-Bi = two substrates, two products
* Ping-Pong = retaining mechanism
  + Order matters
  + A piece of one substrate that is left behind
  + Second substrate binds and that substrate takes up the second piece of substrate

## King-Altman Method

* Method for deriving Michaelis-Menten-type rate expressions
* Application of Euler circuit

### Leonhard Euler

* 1707 – 1783
* Natural logarithm (e – Euler’s number)
* Euler’s method of integration
* Graph theory and topology

# February 27, 2019

# March 1, 2019

# March 4, 2019

## Immobilized vs. Homogeneous Enzymes

* Homogeneous
  + In solution with substrate/product
  + Small mass transfer limitations
    - May not have a homogeneous substrate (i.e. laundry stains
* Immobilized
  + “fixed” to solid surface
  + Ionic, hydrophobic, antibody-linked, covalent
  + Mass transfer limitations
  + Better retention in reactions
    - No longer a homogeneous solution!!!
    - Pay attention to mass transfer

### Methods of Immobilization

* Carrier-bound enzyme
  + Polymer or plastic, silica, etc.
* Cross-linked enzyme
  + Not as common anymore
  + Enzymes have chemically active side chains (amines, carboxylic acids)
  + React polymer/monomers to them, incorporate them into a chain
  + Danger: chemistry directed at enzyme may end up with an inactive enzyme
  + Poor activity, denatured backbone
* Enzyme inclusion
  + Similar to capsule
  + Do not fix to surface, surround with polymer matrix through which enzyme cannot diffuse
  + Substrate and product can diffuse in/out (180-200 Da), but enzyme is too large (25,000 – 250,000 Da)
* Microcapsule
  + See inclusion

### Effect of Immobilization

* Wang et al., Fermentation and Enzyme Technology
* Support (solid material on which immobilized)
* Method of immobilization
  + Inclusion and ionic binding are best, only lose about half
* Observed activity
  + Not Kcat because it’s hard to directly measure
* Percent of free enzyme activity
  + Percent of typical K­cat
  + Normalized to amount of protein
  + Vs. free solution

### Diffusion and Immobilized Enzymes

* Need to think about mass transfer
  + Film thickness – concentration decreases as enters film
    - Kmt = mass transfer coefficient, m/s
    - Am = surface area / unit volume of support, m­-1
    - V’s = rate of substrate disappearance
    - Amount of enzyme per unit of surface area per volume of support
  + At steady state, J’S = -v’S
* V’ vs SS, as substrate increases, rate increases

#### Damkohler Number

* Da = maximum rate of reaction / maximum rate of diffusion = Vmax / kmtam[Sb] = V’max/kmt[Sb]
  + >> 1, reaction is diffusion limited
  + << 1, reaction is kinetic limited

# March 18, 2019

## Damkohler Number

* Da >> 1, reaction is diffusion limited
* Da << 1, kinetic limited

## Porous Beads with Enzyme

* Steady state, no external mass transfer limitations (Ss = Sb­)
* Fickian (or other) Diffusion = Rate of Reaction
  + - Where dS/dr = 0 at r = 0 and S = Ss at r = R

### Overall Substrate Consumption at Steady State

* = rate of diffusion of substrate through outer area of particle

### Porous Bead with Enzyme

### Define Thiele Modulus

* Physically (Thiele Modulus)2 similar to Damkohler number

#### Convenient to use Effectiveness Factor (η)

#### Plot V vs [S]

* + At low [S], V acts first order
  + At high [S], V acts zero order

### Effectiveness Factor (η)

* + Beta approaches 0, η = 1, for 0th order reactions for a wide range of ϕ
  + Beta approaches ∞, η = 3/ϕ \* [1/tanh(ϕ) – 1/ ϕ], for first order reactions

#### Plot Effectiveness Factor vs Thiele Modulus

* Similar to Bode plots from 460
* Adding more enzyme gets you worse performance

## Determining Vmax and Km

* Eliminate diffusion resistances
  + Small particle size
  + High fluid velocity (even turbulence)
  + High substrate concentration

### Immobilized Enzyme Bioreactor Design Considerations

* V”max and R are variables
* K­m and Deff are fixed (intrinsic to system)
* Minimize R
  + Pressure drop constraints
  + Structural integrity constraints
* Optimize V”max­
  + More is not necessarily better

#### Pressure Drop in Packed Bed

* Ergun Equation
  + ΔP = pressure drop across the bed
  + L = length of the bed
  + Dp = spherical diameter of bead
  + Ρ = density of fluid
  + µ = dynamic viscosity of the fluid
  + v = the superficial velocity (i.e. the velocity that the fluid through empty tube at the same volumetric flow rate)
  + ε = the void fraction of the bed (external porosity); between 0.350 – 0.475
* square packing vs. hexagonal packing of spheres
* plot delta P / L vs. Dp
  + as Dp increases, delta P/L drops QUICKLY for constant v and ε