Systembiologie 551-1174-00L

ODE Modeling of Enzyme Kinetics

2 March, 2017

Uwe Sauer & Jörg Stelling

Content:

- Finding the appropriate modeling approach (US)
- Steady state vs dynamics (US)
- From reaction kinetics to pathway dynamics (JS)
- Example: Substrate inhibition (JS)

Teaching Goals Lecture 2:
ODE Modeling of Enzyme Kinetics

- **Overview of metabolic modeling approaches and how to chose the appropriate one.**
- **Understand general differences of steady state vs dynamics in both cellular growth and computational modeling.**
- **Translate pathway maps to dynamic models.**
- **Analyze dynamics and steady states of two coupled reactions.**

Exercise 3

translate reaction sets into a system of ODEs - learn to go from biochemical reactions to dynamic models.

understand steady state in enzyme kinetics and describe effect of competitive inhibition on reaction rate.

Exercise 4

Moving to dynamic analyses, you will be able to:

- find the steady state of a two-coupled-reactions system, analytically.
- analyze dynamic system behavior and understand its dependency on different parameters and initial conditions.

The Question of Model Scale

- Modeling conceptually **simplifies** complicated phenomena by dissecting them into manageable modules whose interaction with the rest of the world can be specified.
- Often the goal is to find out how such modules - at a given scale - respond to changes in an understandable fashion.
- Multiscale modeling (e.g. across time scales from sec to hours, or from cells to tissues to organs) is one of the frontiers of SB. Typically the focus is on a single scale.

Flow Chart of Modeling Process

Everything starts with: What is the question?

From literature or fitted from data?

Goal, inputs	Scope/scale of model, objectives, data availability
Model selection	Type of model: explanatory (mechanistic) vs correlative, static/dynamics, and others
Model Design	Interactions (structure), variables, **parameter** values
Model analysis & diagnosis	Consistency, robustness, exploration of possible behaviors, steady state achievable?
Model applications	Hypothesis testing & generation, simulation, discovery, explanation and optimization

Differentiating mechanisms vs hypothesis generation

Types of Biological Networks

Systems biology seeks to understand complex **interactions** between (molecular) components in bio systems.

Model purposes range from mechanistic understanding of a process to discovery of something unknown.

The SB challenges change with the system

The more complex and the less pre-knowledge, the more it is in discovery mode

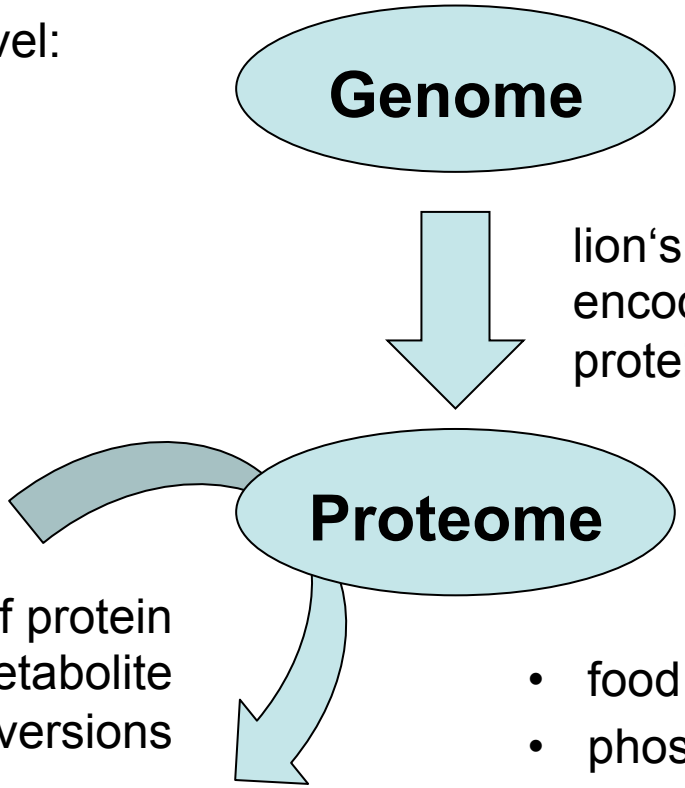
….

	Components	Interactions
- enzyme reactions	3-10	>>50
- signaling pathway	3-30	>100
- metabolic network	~1 000	~2 000
- protein network	>1 000	~50 000
- cell	>10 000	?
- organs	>100 000	?
- human	?	?

Why Start with Metabolism ?

„Omics“ techniques (attempt to) measure the complement of all species in one level:

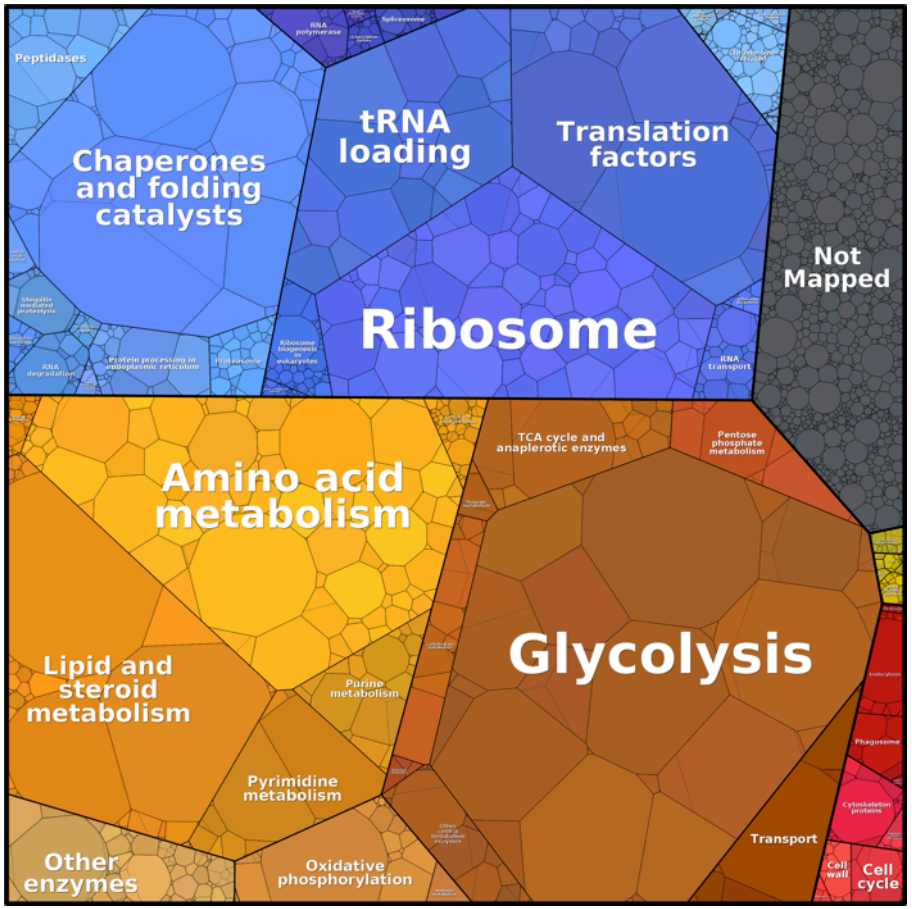
- Genome
- Transcriptome
- Proteome
- Metabolome
- Lipidome
- .....



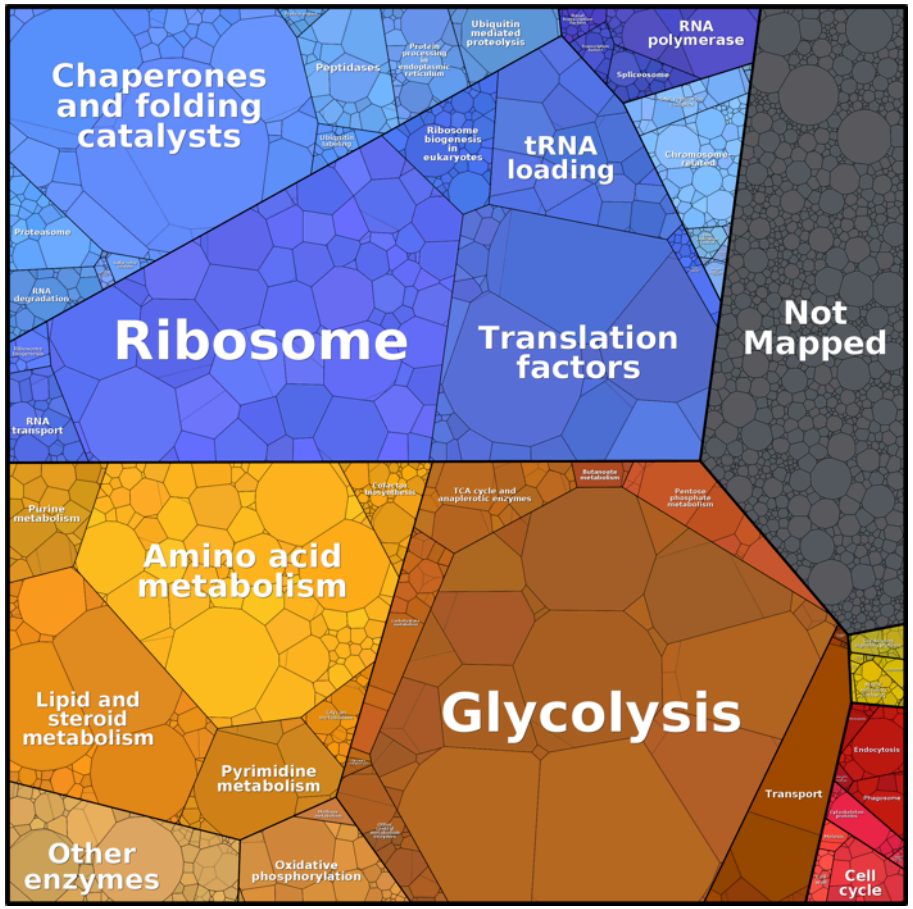
- food conversions
- phosphorylations, glycosylations …
- syntheses/degradation of signaling molecules
- ……

Yeast Proteome Distribution

Minimal medium

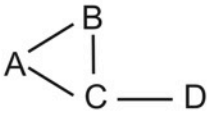
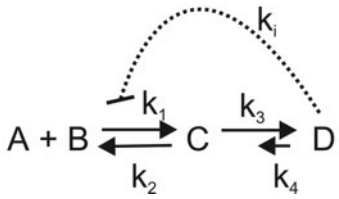


Complex medium



http://www.proteomaps.net/

Modeling Approaches: Requirements

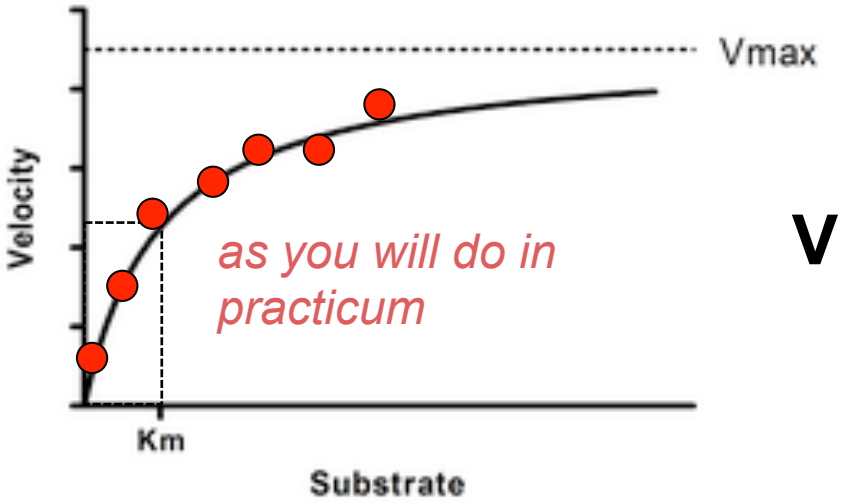
Model class	Level of abstraction	Required information	Example applications
Topological (steady state)	Interaction 	Components and unspecified connections must be known	- Genetic networks - Protein-protein interaction - Metabolite-protein interaction
Stoichiometric (steady state)	Reaction stoichiometry 	Mass and energy balances thermodynamics (directionality)	Metabolic networks - flux balance analysis - elementary flux modes - .....
Steady state			
Mechanistic (dynamic)	Enyzme mechanism and regulation 	Kinetic parameters	Kinetic models (including regulation)
Dynamic			

- **Constants** – a quantity with a fixed value
  (eg gas constant)
- **Parameters** – quantities with a given value
  (eg K_m of an enzyme, often at least in part unknown)
- **Variables** – quantities with a changeable value for which the model establishes relations (eg a reaction rate)
- **State Variables** – the subset of variables that is sufficient to fully describe a model's behavior

Estimating Unknown Parameter
Example:Michaelis-Menten Function

V_{max} – maximal speed (velocity) of conversion at high [S]

K_M – [S] at half max velocity of reaction



$$V = V_{max} \bullet S / (K_M + S)$$

The generally unknown parameters V_{max} and K_m are either to be determined from experimental data (red points) through fitting or from original literature. **Today databases such as BRENDA or ENZYME conveniently collect such data!**

- **System State** – a snapshot of a system at a given time. Is described by a set of variables that must be kept track of in a model.
  Each modeling framework defines what this considered for this state, eg:
  - for kinetic rate equations it is the state of molecule concentrations.
  - for a stochastic model it is probability distributions for numbers of molecules
- **Model Behavior** – output of system is determined by 2 factors: i) the **environment** (input) and ii) **internal processes**; ie relation between variables, constants, and parameters within a given model structure.
  Different model structures may lead to same/similar model output – ***What could that mean?***

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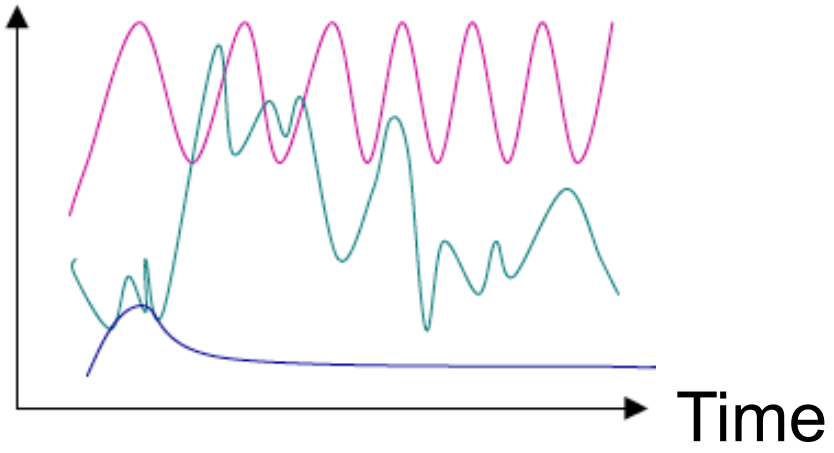
Steady State Concept

Reaction systems are often analyzed in steady (or stationary) state, when all state variables remain constant in time. To focus on relevant aspects of a system.

→ Study the asymptotic behavior of a dynamic system (e.g. after a sufficiently long time). This is of course an abstraction that depends on the considered time-scale (e.g. enzyme rate < genetic regulation < cell division < evolution)

Asymptotic behavior can be:
- **oscillatory**
- **chaotic**
- but approaches in many cases a **steady state**

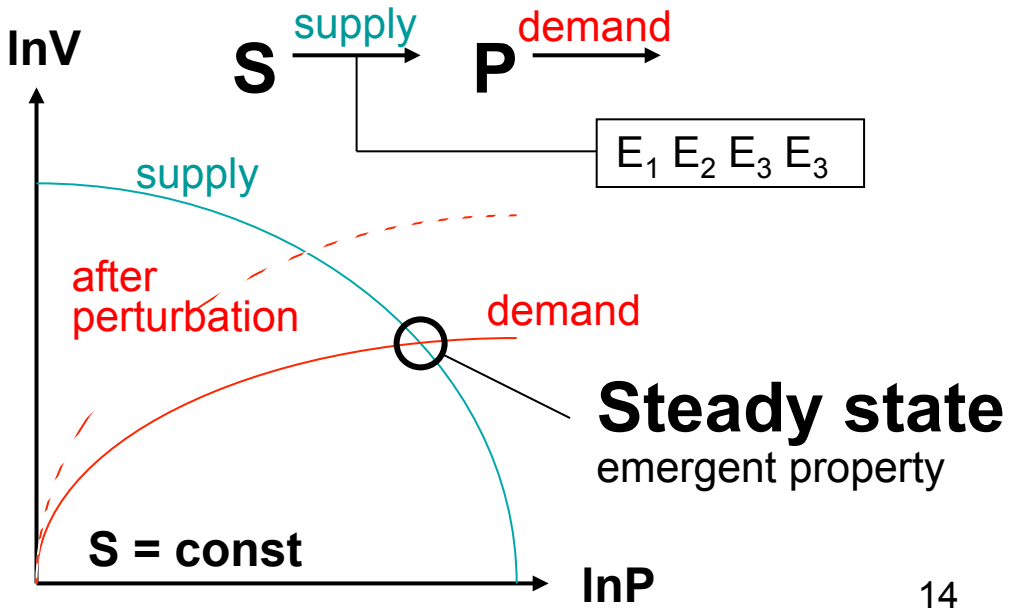


Guess a few examples!

Steady State: Mathematically

Obviously, in nature everything flows always. The abstraction of steady state in modeling is achieved by separation in time scales; ie the process considered is in quasi steady state, embedded in a larger non-stationary cellular environment. Can you give an example of time-scale separation?

Benefit: simplifies the mathematical problem; ie differential equations that describe time depend behavior are simplified to algebraic equations! In modeling, often the goal is to identify whether (and which!) steady state exits for a given data set/model.



Steady State: Biologically

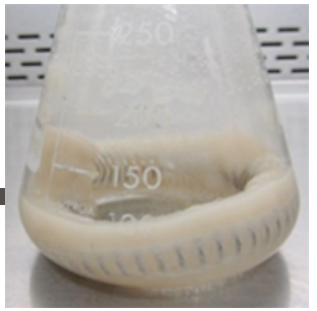
Akin to mathematical representation, environment acts as a source/sink for some molecules, but [c] of all intracellular molecules (e.g. metabolites, proteins) are constant because net rates of formation/degradation are balanced

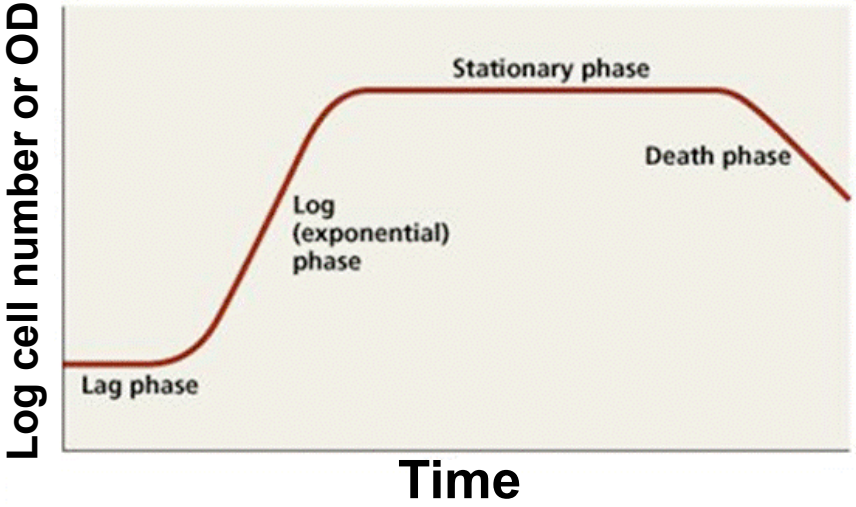
How long does it take for a biological system to be in steady state?

Consider a few examples, eg enzyme kinetics, gene regulation, embryo development ……

Some experimental systems are designed for steady state analysis, e.g. continuous cultures. How about exponential growth in cell cultures?

Microbial Growth Kinetics: Batch Culture





Unlimited growth is exponential - but not for long

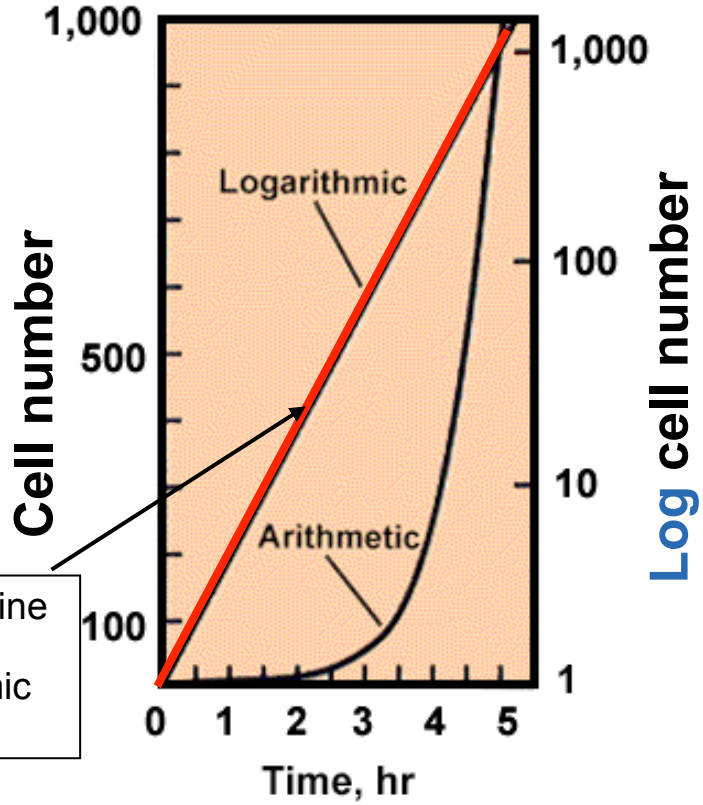
$$\frac{dX}{dT} = \mu_{max}\frac{S\,X}{K_S+S}$$

$$\mu = \mu_{max}\frac{S}{K_S+S}$$

Monod model of bacterial growth

μ = specific growth rate
t_d = doubling time
S = substrate conc.
X = biomass conc.
K_s = substrate conc. at which μ is reduced to 50% of μ_{max}

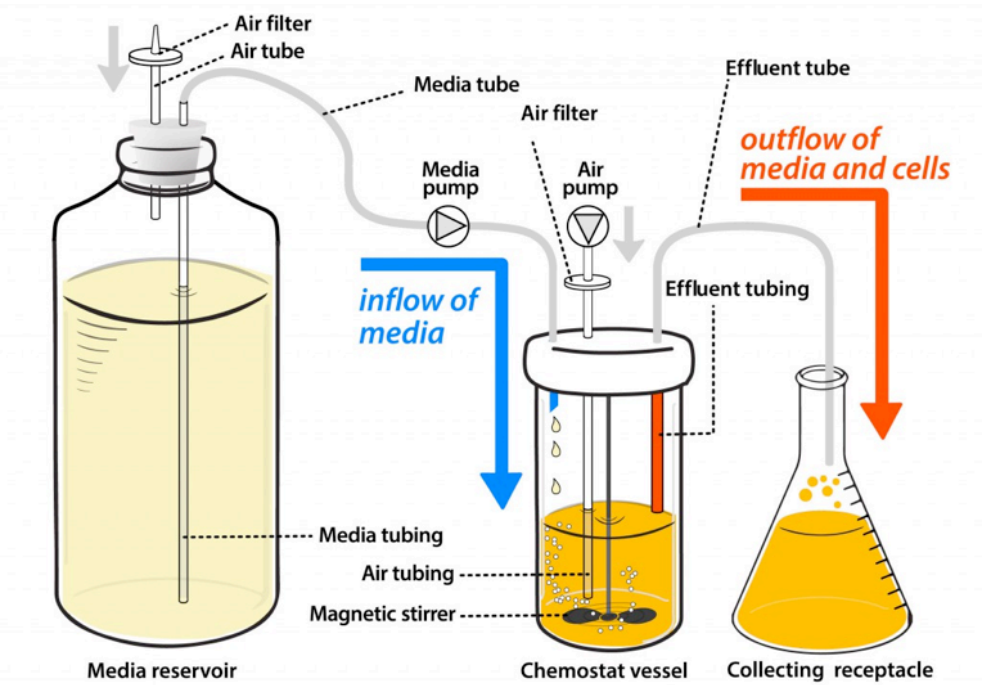
Semilogarithmic plot



Cells are in quasi steady state

Straight line indicates logarithmic growth

Microbial Growth: Continuous Culture



Schematic diagram of a chemostat

A device for continuous culture of bacteria. The chemostat relieves the environmental conditions that restrict growth by continuously supplying nutrients to cells and removing waste substances and spent cells from culture medium.

A self regulating system with constant flow rate, where the concentration of a nutrient (‚chemo') maintains a stable state (‚stat').

$$D\ [h^{-1}] = \frac{F}{V} = \mu$$

F = flow rate
V = volume
D = dilution rate

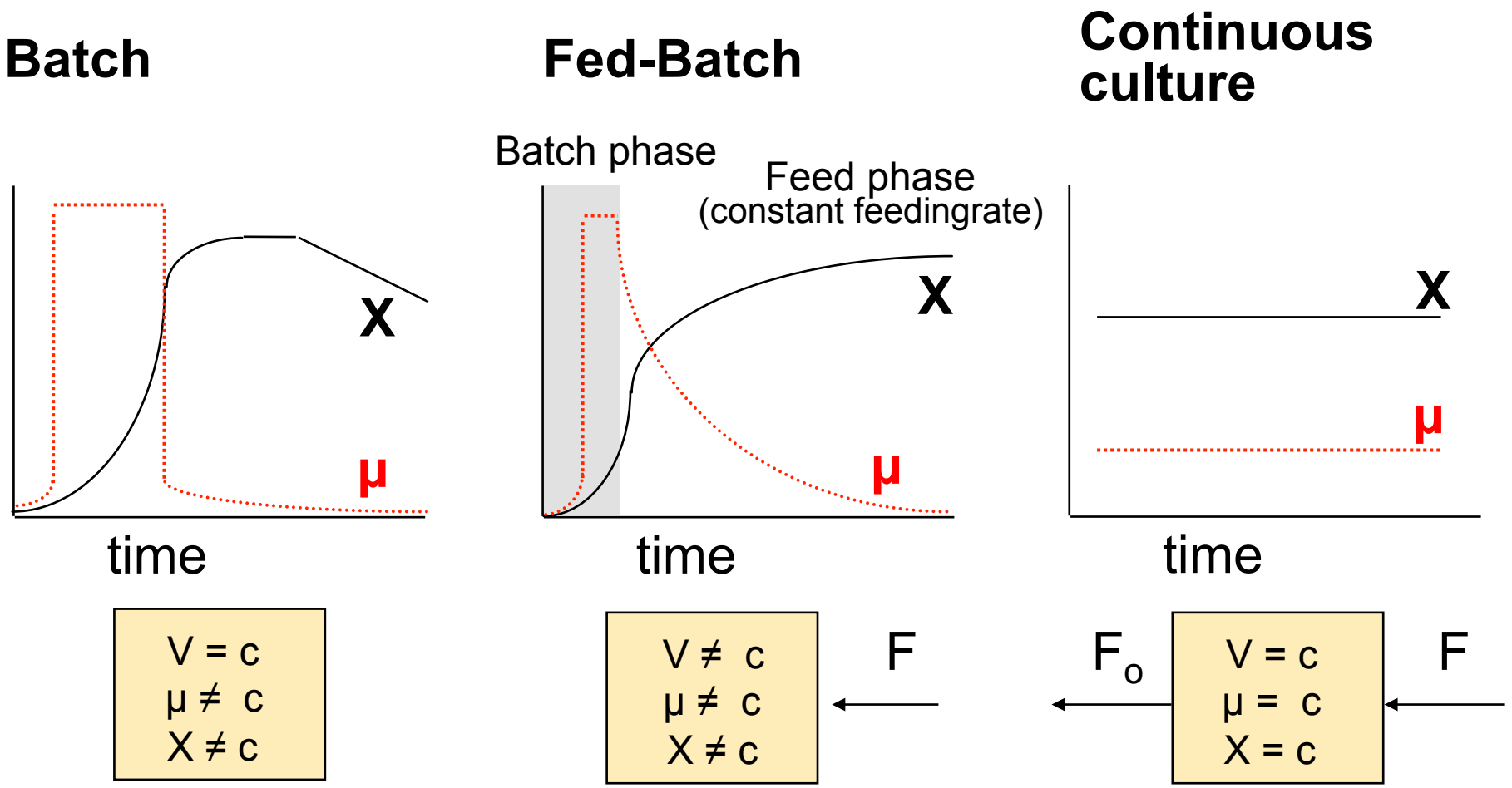
Where would similar situations occur in nature?

Which 2 factors influence the amount of cells in the chemostat?

- In batch culture growth rate (μ) is either max or zero ....
- Chemostat allows to modulate growth rate ..... by changing feed rate and/or volume

How could one make use of chemostats if we wanted to characterize cellular growth or biotechnological production behavior?

Steady State in Main Microbial Cultivations



C = constant; V = volume; F = feed; X = biomass; μ = growth rate

Dynamic Reaction Analysis with Kinetic Models

Uses chemical kinetic theory, differential equations, mainly applied in **metabolism** and **information processing**.

$$A \xrightarrow{k_A} B$$

Elemental reaction

In simplest case just uncatalyzed decay reaction such as radioactive decay or diffusion; ie the change in time is proportional to amount of time.

$$\frac{[A]}{[B]} = k_A\,(T,\, p,\, \ldots)$$

Law of mass action

- Concentrations of reacting molecules in thermodynamic equilibrium
- At const temp, pressure without a catalyst: chemical reaction rates are proportional to products of their substrate concentrations (ie they equal a constant)

The proportionality constants k are also called kinetic or **rate constants**. They exist for forward and backward reaction.

$$\frac{d[A]}{dt} = -\,k_A \bullet [A] \qquad \frac{d[B]}{dt} = k_A\,[A]$$

Differential equations for kinetic models

Modeling Metabolism Kinetically

- Biochemical kinetics are based on the mass action law.
- In mass action law the **reaction rate is proportional to the probability of collision of reactants**, which, in turn, depends on reactant concentrations.
- Hence kinetic modeling of metabolism works under two assumptions (see lecture 1 Stelling):
  - **large numbers of molecules**
  - **well-mixed system** with freely moving metabolites (no spatial heterogeneity) *Can you imagine biological situations where spatial homogeneity is not a safe assumption?*
- Michaelis-Menten kinetics is a specialized case of mass action for enzyme catalyzed reactions with rate constants for each subreaction (see lecture 1 Stelling)

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Topological, stoichiometric and mechanistic (dynamic) models.

Steady state greatly simplifies mathematical treatment. Experimentally pseudo steady states can be achieved (eg. maximum exponential growth).