

# *Microbial Nutrition & Growth*

A. Nutrient Requirements

B. Nutrient Transport Processes

C. Culture Media

- Growth in Batch Culture
- Mean Generation Time and Growth Rate
- Measurement of Microbial Growth
- Continuous Culture
- Factors Influencing Growth
- Quorum Sensing

# *Nutrient Requirements*

- Energy Source
  - Phototroph
    - Uses light as an energy source
  - Chemotroph
    - Uses energy from the oxidation of reduced chemical compounds

# *Nutrient Requirements*

- Electron (Reduction potential) Source
  - Organotroph
    - Uses reduced organic compounds as a source for reduction potential
  - Lithotroph
    - Uses reduced inorganic compounds as a source for reduction potential

# *Nutrient Requirements*

- Carbon source
  - Autotroph
    - Can use  $\text{CO}_2$  as a sole carbon source  
(Carbon fixation)
  - Heterotroph
    - Requires an organic carbon source; cannot use  $\text{CO}_2$  as a carbon source

# *Nutrient Requirements*

- Nitrogen source
  - Organic nitrogen
    - Primarily from the catabolism of amino acids
  - Oxidized forms of inorganic nitrogen
    - Nitrate ( $\text{NO}_3^{2-}$ ) and nitrite ( $\text{NO}_2^-$ )
  - Reduced inorganic nitrogen
    - Ammonium ( $\text{NH}_4^+$ )
  - Dissolved nitrogen gas ( $\text{N}_2$ ) (Nitrogen fixation)

# *Nutrient Requirements*

- Phosphate source
  - Organic phosphate
  - Inorganic phosphate ( $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$ )

# *Nutrient Requirements*

- Sulfur source
  - Organic sulfur
  - Oxidized inorganic sulfur
    - Sulfate ( $\text{SO}_4^{2-}$ )
  - Reduced inorganic sulfur
    - Sulfide ( $\text{S}^{2-}$  or  $\text{H}_2\text{S}$ )
  - Elemental sulfur ( $\text{S}_0$ )

# *Nutrient Requirements*

- Special requirements
  - Amino acids
  - Nucleotide bases
  - Enzymatic cofactors or “vitamins”



# *Nutrient Requirements*

- Prototrophs vs. Auxotrophs
  - Prototroph
    - A species or genetic strain of microbe capable of growing on a minimal medium consisting a simple carbohydrate or CO<sub>2</sub> carbon source, with inorganic sources of all other nutrient requirements
  - Auxotroph
    - A species or genetic strain requiring one or more complex organic nutrients (such as amino acids, nucleotide bases, or enzymatic cofactors) for growth

# *Nutrient Transport Processes*

- Simple Diffusion
  - Movement of substances directly across a phospholipid bilayer, with no need for a transport protein
  - Movement from high → low concentration
  - No energy expenditure (e.g. ATP) from cell
  - Small uncharged molecules may be transported via this process, e.g. H<sub>2</sub>O, O<sub>2</sub>, CO<sub>2</sub>

# *Nutrient Transport Processes*

- Facilitated Diffusion
  - Movement of substances across a membrane with the assistance of a transport protein
  - Movement from high → low concentration
  - No energy expenditure (e.g. ATP) from cell
  - Two mechanisms: Channel & Carrier Proteins

# *Nutrient Transport Processes*

- Active Transport
  - Movement of substances across a membrane with the assistance of a transport protein
  - Movement from low → high concentration
  - Energy expenditure (e.g. ATP or ion gradients) from cell
  - Active transport pumps are usually carrier proteins

# *Nutrient Transport Processes*

- Active Transport (cont.)
  - Active transport systems in bacteria
    - **ATP-binding cassette transporters (ABC transporters):**  
The target binds to a soluble cassette protein (in periplasm of gram-negative bacterium, or located bound to outer leaflet of plasma membrane in gram-positive bacterium). The target-cassette complex then binds to an integral membrane ATPase pump that transports the target across the plasma membrane.

# *Nutrient Transport Processes*

- Active Transport (cont.)
  - Active transport systems in bacteria
    - **Cotransport systems:** Transport of one substance from a low → high concentration as another substance is simultaneously transported from high → low.

For example: lactose permease in *E. coli*:

As hydrogen ions are moved from a high concentration outside → low concentration inside, lactose is moved from a low concentration outside → high concentration inside

# *Nutrient Transport Processes*

- Active Transport (cont.)
  - Active transport systems in bacteria
    - **Group translocation system:** A molecule is transported while being chemically modified.

For example:

phosphoenolpyruvate: sugar phosphotransferase systems (PTS)

PEP + sugar (outside) → pyruvate + sugar-phosphate (inside)

# *Nutrient Transport Processes*

- Active Transport (cont.)
  - Active transport systems in bacteria
    - **Iron uptake by siderophores:**

Low molecular weight organic molecules that are secreted by bacteria to bind to ferric iron ( $\text{Fe}^{3+}$ ); necessary due to low solubility of iron;  $\text{Fe}^{3+}$ - siderophore complex is then transported via ABC transporter



# *Microbiological Media*

- Liquid (broth) vs. semisolid media
  - Liquid medium
    - Components are dissolved in water and sterilized
  - Semisolid medium
    - A medium to which has been added a gelling agent
    - Agar (most commonly used)
    - Gelatin
    - Silica gel (used when a non-organic gelling agent is required)

# *Microbiological Media*

- Chemically defined vs. complex media
  - Chemically defined media
    - The exact chemical composition is known
    - e.g. minimal media used in bacterial genetics experiments
  - Complex media
    - Exact chemical composition is not known
    - Often consist of plant or animal extracts, such as soybean meal, milk protein, etc.
    - Include most routine laboratory media, e.g., tryptic soy broth

# *Microbiological Media*

- Selective media
  - Contain agents that inhibit the growth of certain bacteria while permitting the growth of others
  - Frequently used to isolate specific organisms from a large population of contaminants
- Differential media
  - Contain indicators that react differently with different organisms (for example, producing colonies with different colors)
  - Used in identifying specific organisms

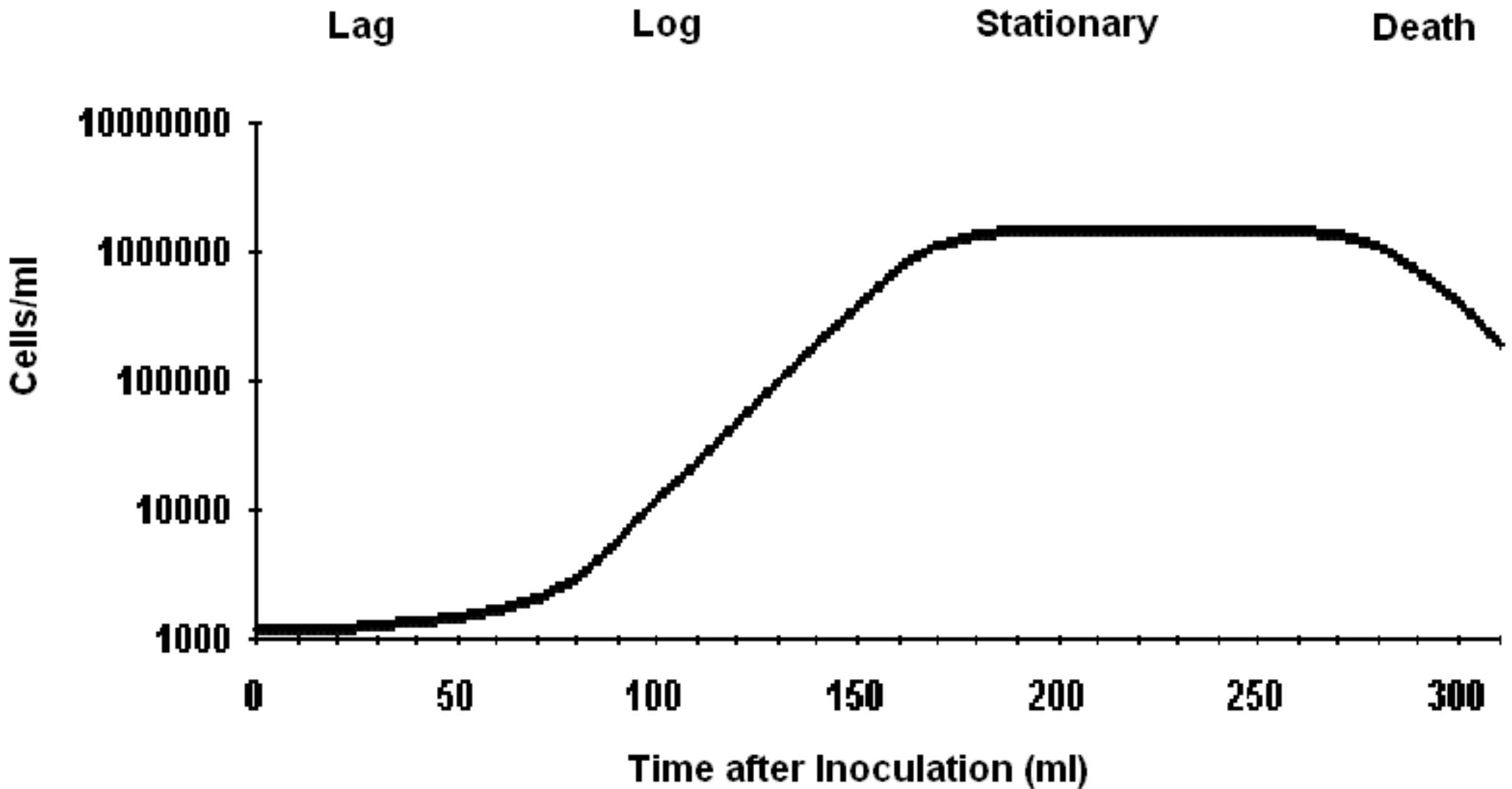
# *Growth in Batch Culture*

- “Growth” is generally used to refer to the acquisition of biomass leading to cell division, or reproduction
- A “batch culture” is a closed system in broth medium in which no additional nutrient is added after inoculation of the broth.

# *Growth in Batch Culture*

- Typically, a batch culture passes through four distinct stages:
  - Lag stage
  - Logarithmic (exponential) growth
  - Stationary stage
  - Death stage

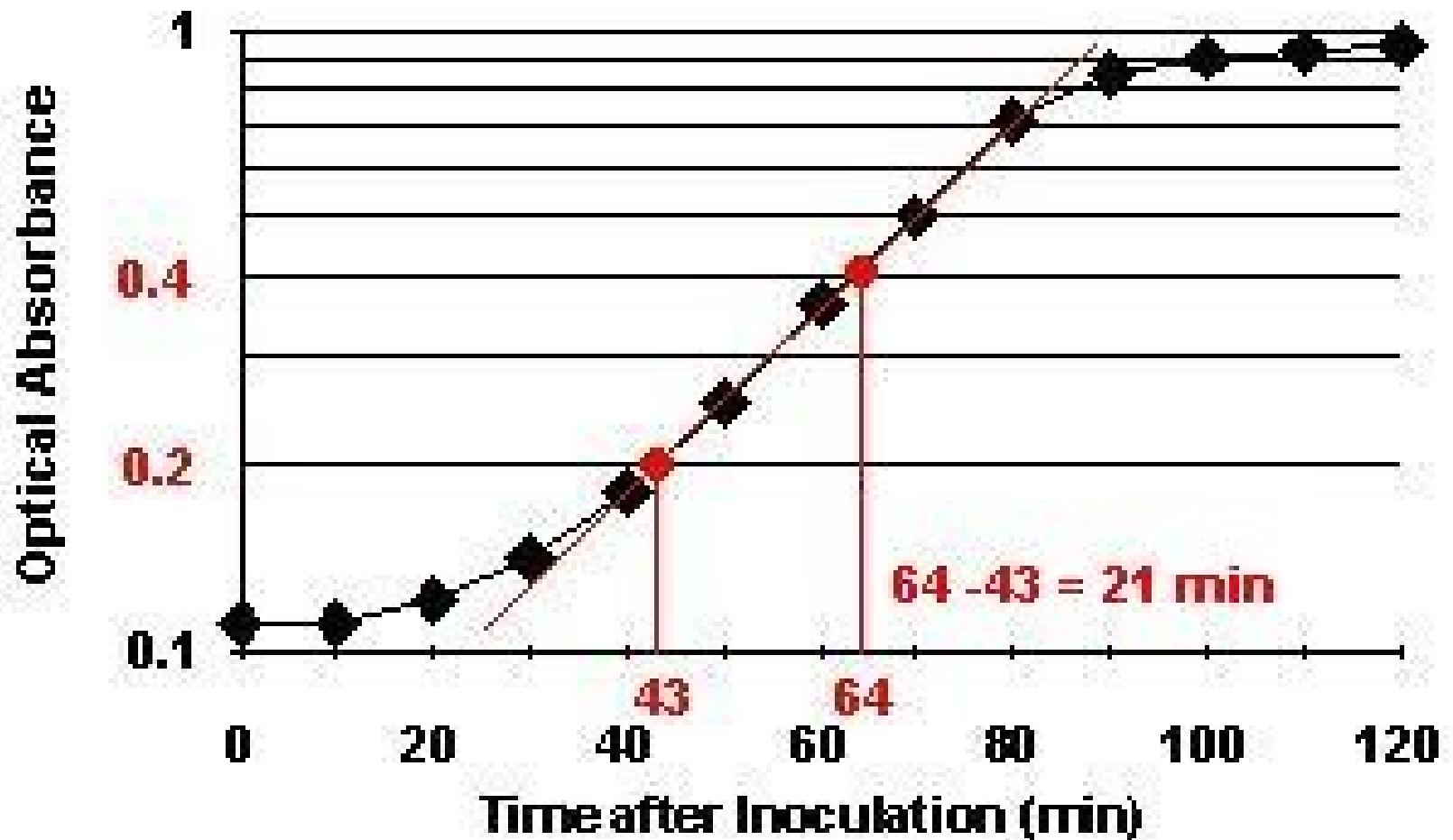
# *Growth in Batch Culture*



# *Mean Generation Time and Growth Rate*

- The mean generation time (doubling time) is the amount of time required for the concentration of cells to double during the log stage. It is expressed in units of minutes.
- Growth rate ( $\text{min}^{-1}$ ) = 
$$\frac{1}{\text{mean generation time}}$$
- Mean generation time can be determined directly from a semilog plot of bacterial concentration vs time after inoculation

# *Mean Generation Time and Growth Rate*

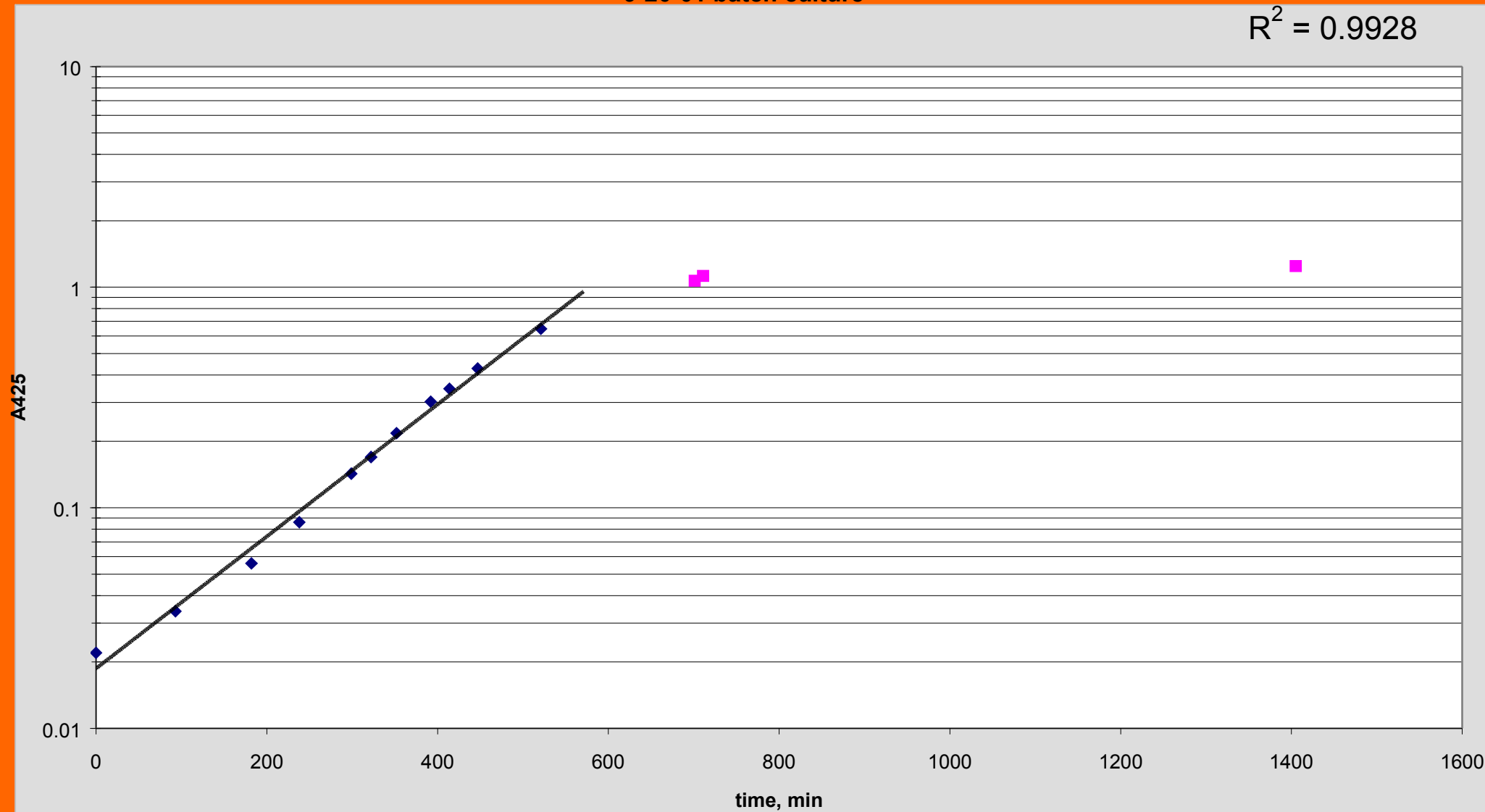




# Mean Generation Time and Growth Rate

Growth of *E. coli* 23716,  
9-20-01 batch culture

$$y = 0.0187e^{0.0069x}$$
$$R^2 = 0.9928$$

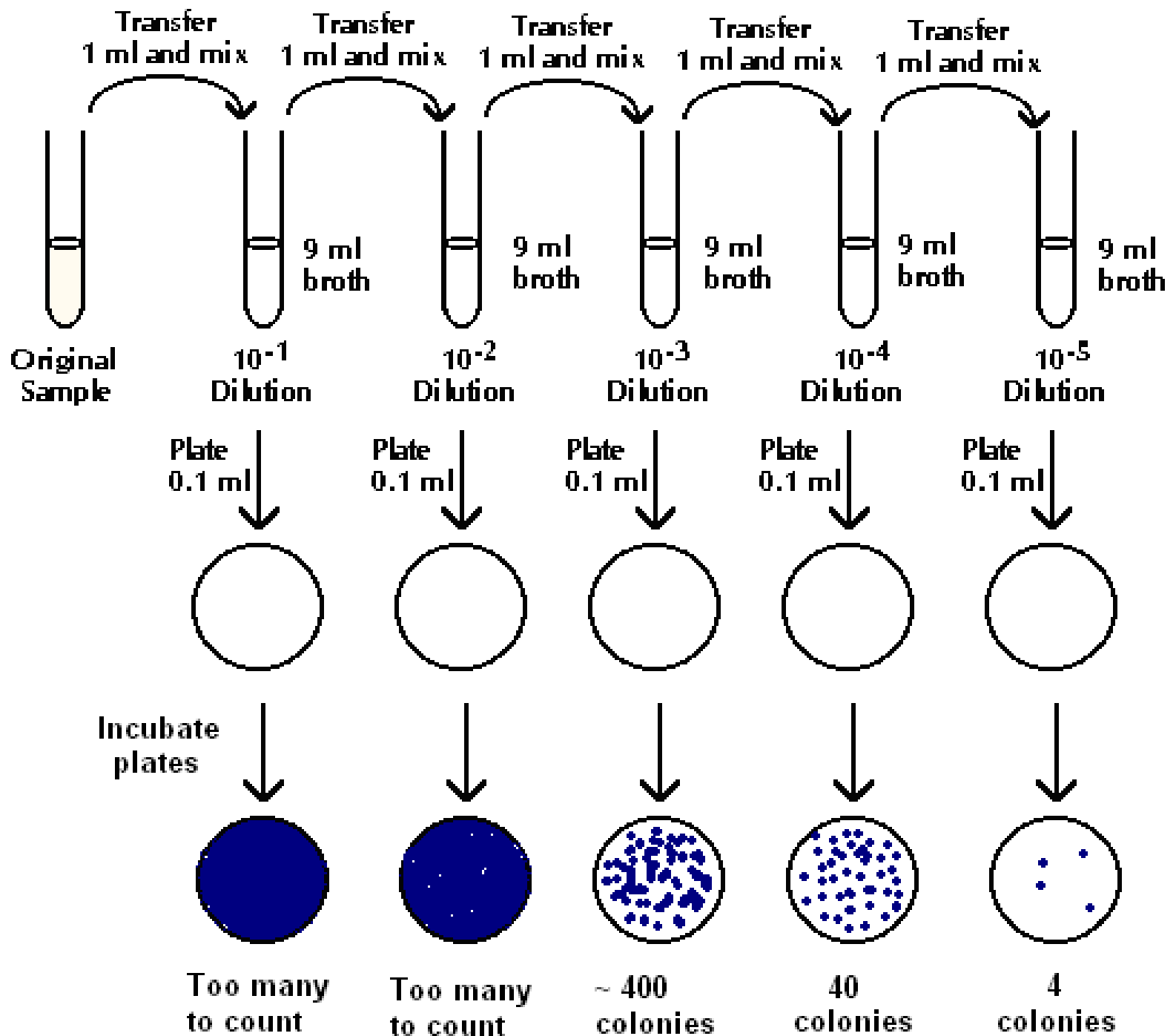


# *Measurement of Microbial Growth*

- Microscopic cell counts
  - Calibrated “Petroff-Hausser counting chamber,” similar to hemacytometer, can be used
  - Generally very difficult for bacteria since cells tend to move in and out of counting field
  - Can be useful for organisms that can’t be cultured
  - Special stains (e.g. serological stains or stains for viable cells) can be used for specific purposes
- Serial dilution and colony counting
  - Also know as “viable cell counts”
  - Concentrated samples are diluted by serial dilution

# *Measurement of Microbial Growth*

- Serial dilution and colony counting
  - Also know as “viable cell counts”
  - Concentrated samples are diluted by serial dilution
  - The diluted samples can be either plated by spread plating or by pour plating



# *Measurement of Microbial Growth*

- Serial dilution (cont.)
  - Diluted samples are spread onto media in petri dishes and incubated
  - Colonies are counted. The concentration of bacteria in the original sample is calculated (from plates with 25 – 250 colonies, from the *FDA Bacteriological Analytical Manual*).
  - A simple calculation, with a single plate falling into the statistically valid range, is given below:

$$\frac{\text{CFU}}{\text{ml}} \text{ in original sample} = \frac{\text{\# colonies counted}}{(\text{dilution factor})(\text{volume plated, in ml})}$$

# *Measurement of Microbial Growth*

- Serial dilution (cont.)
  - If there is **more than one plate** in the statistically valid range of 25 – 250 colonies, the viable cell count is determined by the following formula:

# *Measurement of Microbial Growth*

$$\frac{\text{CFU}}{\text{ml}} = \frac{\sum C}{[(1 * n_1) + (0.1 * n_2) + \dots] * d_1 * V}$$

- Where:

C = Sum of all colonies on all plates between 25 - 250

$n_1$  = number of plates counted at dilution 1  
(least diluted plate counted)

$n_2$  = number of plates counted at dilution 2  
(dilution 2 = 0.1 of dilution 1)

$d_1$  = dilution factor of dilution 1

V = Volume plated per plate

# *Measurement of Microbial Growth*

- Membrane filtration
  - Used for samples with low microbial concentration
  - A measured volume (usually 1 to 100 ml) of sample is filtered through a membrane filter (typically with a 0.45  $\mu\text{m}$  pore size)
  - The filter is placed on a nutrient agar medium and incubated
  - Colonies grow on the filter and can be counted



# *Measurement of Microbial Growth*

- Turbidity
  - Based on the diffraction or “scattering” of light by bacteria in a broth culture
  - Light scattering is measured as optical absorbance in a spectrophotometer
  - Optical absorbance is directly proportional to the concentration of bacteria in the suspension

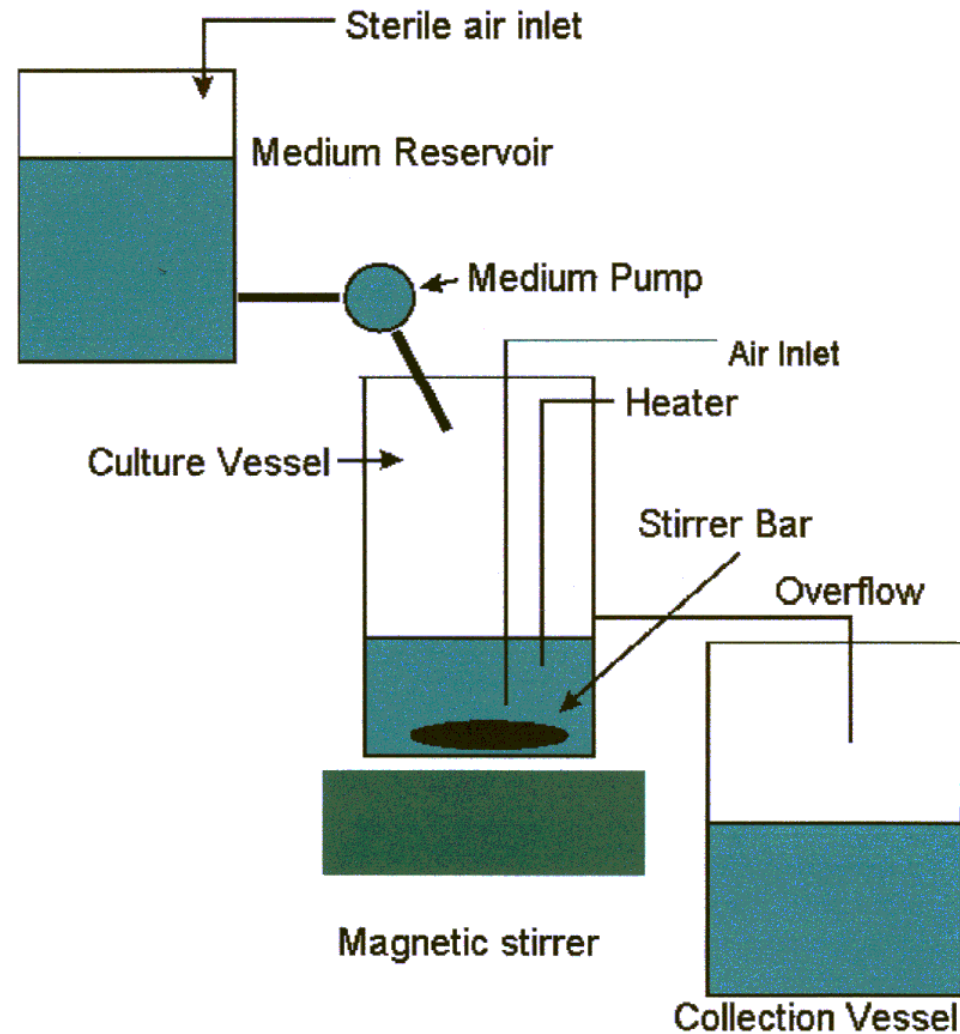
# *Measurement of Microbial Growth*

- Mass determination
  - Cells are removed from a broth culture by centrifugation and weighed to determine the “wet mass.”
  - The cells can be dried out and weighed to determine the “dry mass.”
- Measurement of enzymatic activity or other cell components

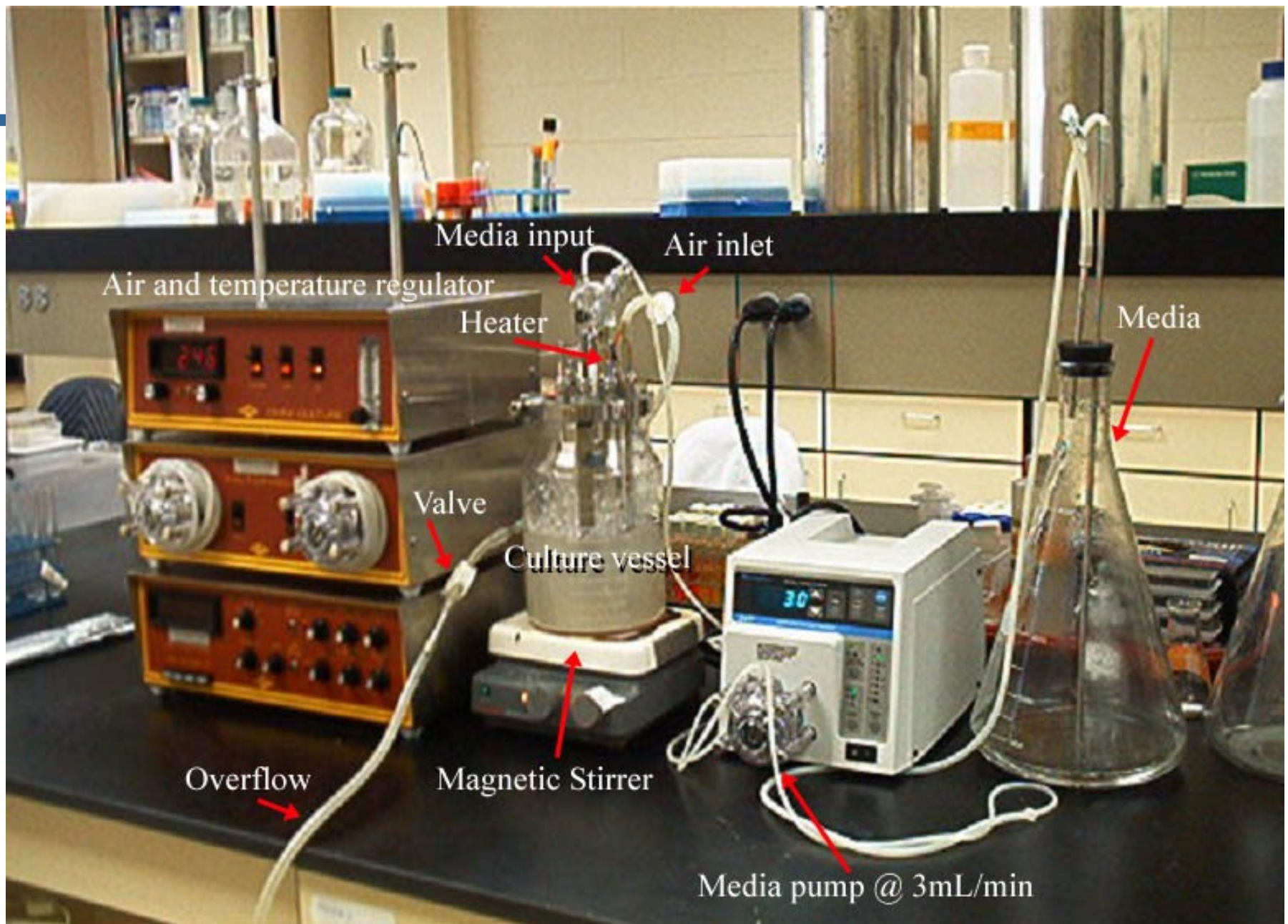
# *Growth in Continuous Culture*

- A “continuous culture” is an open system in which fresh media is continuously added to the culture at a constant rate, and old broth is removed at the same rate.
- This method is accomplished in a device called a chemostat.
- Typically, the concentration of cells will reach an equilibrium level that remains constant as long as the nutrient feed is maintained.

# Basic Chemostat System

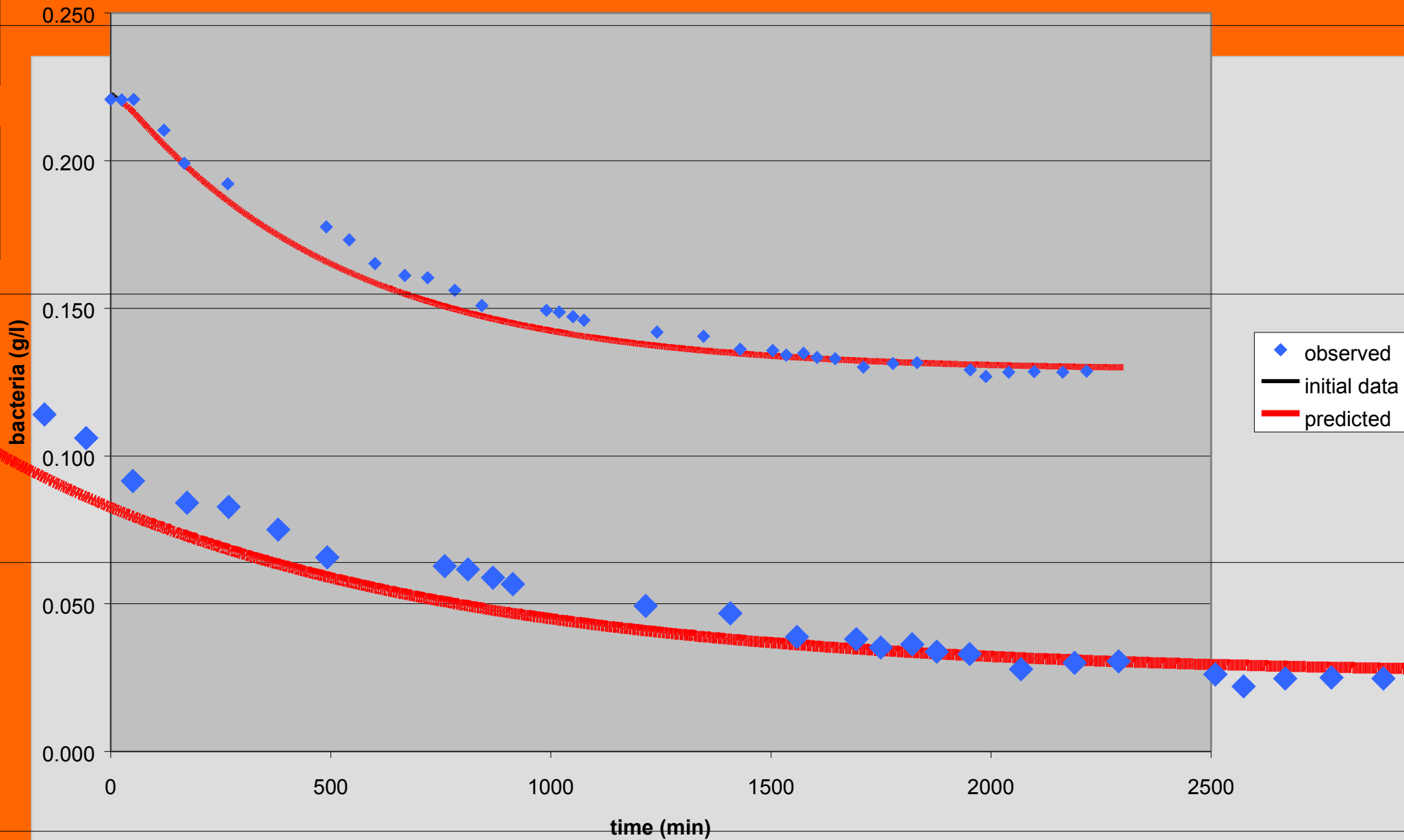


# Our Chemostat System



# 0.05% glucose run assuming delay of 20 min and 28% yield

0.05% glucose run assuming delay of 20 min and 28% yield





# *Factors that Influence Growth*

- Growth vs. Tolerance
  - “Growth” is generally used to refer to the acquisition of biomass leading to cell division, or reproduction
  - Many microbes can survive under conditions in which they cannot grow
  - The suffix “-phile” is often used to describe conditions permitting growth, whereas the term “tolerant” describes conditions in which the organisms survive, but don’t necessarily grow
  - For example, a “thermophilic bacterium” grows under conditions of elevated temperature, while a “thermotolerant bacterium” survives elevated temperature, but grows at a lower temperature

# *Factors that Influence Growth*

- Obligate (strict) vs. facultative
  - “Obligate” (or “strict”) means that a given condition is required for growth
  - “Facultative” means that the organism can grow under the condition, but doesn’t require it
  - The term “facultative” is often applied to sub-optimal conditions
  - For example, an obligate thermophile requires elevated temperatures for growth, while a facultative thermophile may grow in either elevated temperatures or lower temperatures



# *Factors that Influence Growth*

- Temperature
  - Most bacteria grow throughout a range of approximately 20 Celsius degrees, with the maximum growth rate at a certain “optimum temperature”
  - Psychrophiles: Grows well at 0°C; optimally between 0°C – 15°C
  - Psychrotrophs: Can grow at 0 – 10°C; optimum between 20 – 30°C and maximum around 35°C
  - Mesophiles: Optimum around 20 – 45°C
  - Moderate thermophiles: Optimum around 55 – 65 °C
  - Extreme thermophiles (Hyperthermophiles): Optimum around 80 – 113 °C

# *Factors that Influence Growth*

- pH
  - Acidophiles:
    - Grow optimally between ~pH 0 and 5.5
  - Neutrophiles
    - Grow optimally between pH 5.5 and 8
  - Alkalophiles
    - Grow optimally between pH 8 – 11.5

# *Factors that Influence Growth*

- Salt concentration
  - Halophiles require elevated salt concentrations to grow; often require 0.2 M ionic strength or greater and may some may grow at 1 M or greater; example, *Halobacterium*
  - Osmotolerant (halotolerant) organisms grow over a wide range of salt concentrations or ionic strengths; for example, *Staphylococcus aureus*

# *Factors that Influence Growth*

- Oxygen concentration
  - Strict aerobes: Require oxygen for growth (~20%)
  - Strict anaerobes: Grow in the absence of oxygen; cannot grow in the presence of oxygen
  - Facultative anaerobes: Grow best in the presence of oxygen, but are able to grow (at reduced rates) in the absence of oxygen
  - Aerotolerant anaerobes: Can grow equally well in the presence or absence of oxygen
  - Microaerophiles: Require reduced concentrations of oxygen (~2 – 10%) for growth

# *Quorum Sensing*

- A mechanism by which members of a bacterial population can behave cooperatively, altering their patterns of gene expression (transcription) in response to the density of the population
- In this way, the entire population can respond in a manner most strategically practical depending on how sparse or dense the population is.

# *Quorum Sensing*

- Mechanism:
  - As the bacteria in the population grow, they secrete a quorum signaling molecule into the environment (for example, in many gram-negative bacteria the signal is an acyl homoserine lactone, HSL)
  - When the quorum signal reaches a high enough concentration, it triggers specific receptor proteins that usually act as transcriptional inducers, turning on quorum-sensitive genes