

# ENVE 424

## Anaerobic Treatment

### Review Lecture

2012-2013 Fall

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# Basics of Microbiology

Principles of microbiology is applied to the solution of environmental problems

- Treatment of municipal and industrial ww
- Enhancement of the quality of drinking water
- Restoration of sites contaminated with hazardous materials
- Protection or restoration of rivers, lakes etc.
- Prevention of spread of pathogens
- Production of environmentally benign chemicals

# The Cell

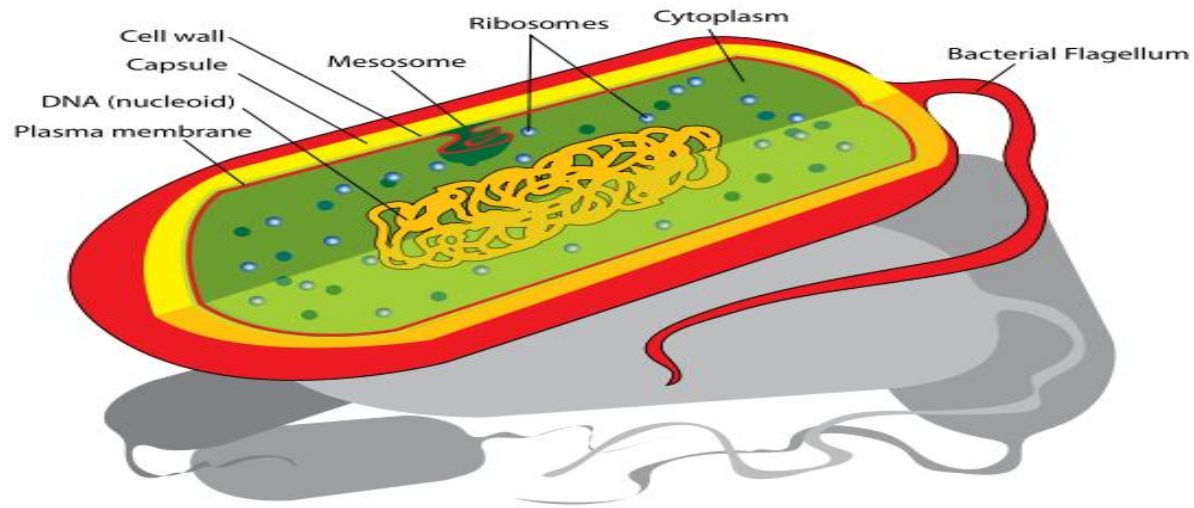
- The cell is the fundamental building block of life
- Cells are capable of growth and reproduction
- Cells are highly organized and selectively restrict what crosses their boundaries
- Cells are composed of C, N, O, S in particular and are chemically reduced
- Cells are self feeding

# The Cell

- *Cell Membrane*: a barrier between the cell and its environment. It restricts what crosses its boundaries.
- *Cell Wall*: gives rigidity to the cell and protects the membrane
- *Cytoplasm*: Comprises most of the inside of the cell. It contains water and the macromolecules
- *Chromosome*: Store's the genetic code
- *Ribosomes*: Convert the genetic code into working catalysyst
- *Enzymes*: catalysts that carry out the desired biochemical reaction

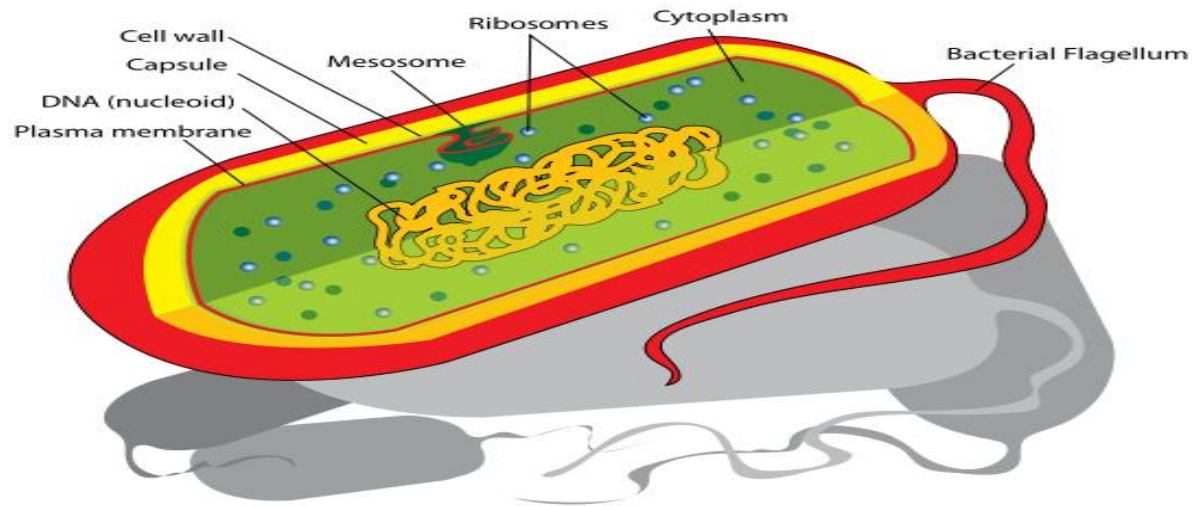
# Cell Membrane

- Cell membrane is simple in most bacteris
- more complex in *autotrophic bacteria*, which synthesize essentially all cellular components from inorganic materials
- even more complex in the *phototrophic bacteria*, which obtain energy from sunlight



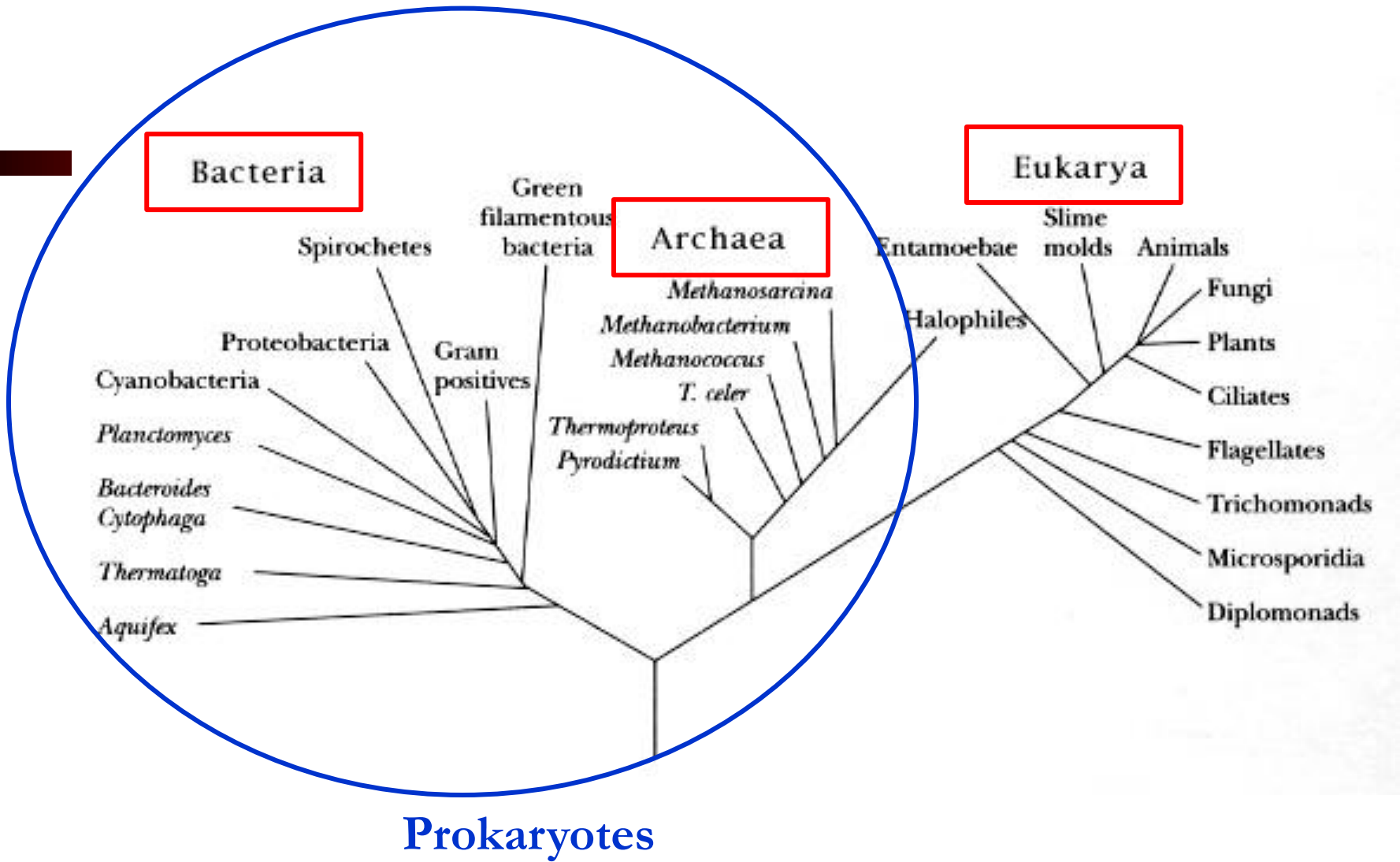
## FLAGELLA

- Protein hairlike structures that extend from the cytoplasm
- Provide mobility by rotating at high speeds



## FIMBRIAE and PILI

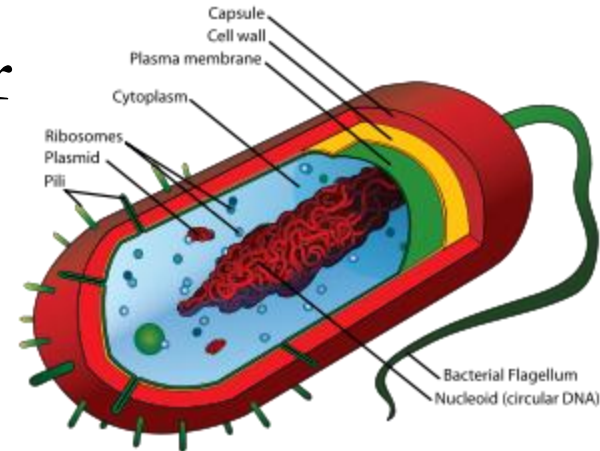
- Short protein hairlike structures
- Enable bacteria to stick to surfaces
- Pili enable bacteria to attach to each other





# Basic Microbiology

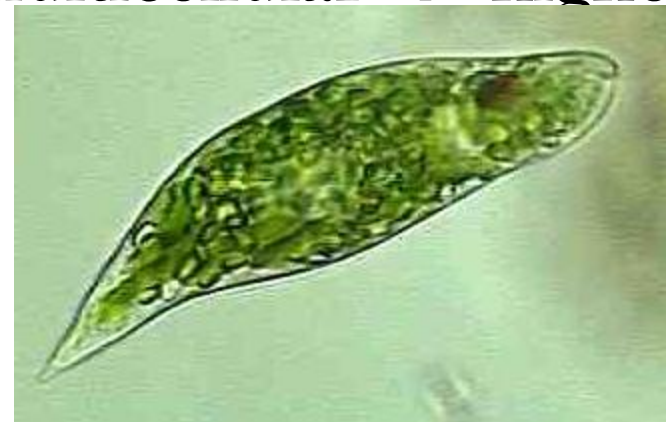
- *Prokaryotes* are single cellular



Ref: <http://en.wikipedia.org/wiki/Prokaryote>

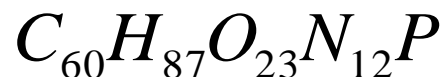
- *Eukarya* is the other major domain, can be either single (algae, protozoa) or multicellular → higher plants and animals

Ref: <http://blogs.scientificamerican.com/a-blog-around-the-clock/2012/03/13/biological-clocks-in-protista/>



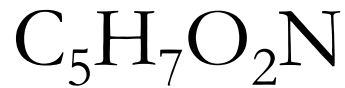
# Bacteria

- Principal microorganism used in biological wastewater treatment
- single cell, prokaryotic organism
- their usual mode of reproduction binary fission
- are composed of
  - 80 % water
  - 20 % dry material (90% organic, 10 % inorganic)
- approximated empirical formula for the organic fraction (may vary with time and species)



# Typical Composition of Bacteria

Empirical formula for bacteria



Empirical molecular weight is

113 g

12.4 % N (w)

53% C (w)



Constituent	Percentage
Water	75
Dry Matter	25
Organics	90
C	45 – 55
O	22 – 28
H	5 – 7
N	8 – 13
Inorganic	10
P <sub>2</sub> O <sub>5</sub>	50
K <sub>2</sub> O	6.5
Na <sub>2</sub> O	10
MgO	8.5
CaO	10
SO <sub>3</sub>	15

To continue to reproduce and function properly bacteria must have sources of :

- Carbon
- Energy
- Inorganic elements (nutrients)

# Classification of Bacteria Based on Nutrition

Group	Energy Source	Carbon Source
<b>Autotrophic</b> - chemolithotrophic - photolithotrophic	inorganic redox radiant energy	CO <sub>2</sub> CO <sub>2</sub>
<b>Heterotrophic</b> - chemoorganotrophic - photoorganotrophic	organic redox radiant energy	organics organics
<b>Mixotrophic</b>	Inorganic redox	organics

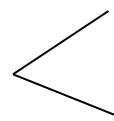
- Heterotrophs derive cell carbon from organic carbon sources
- Autotrophs derive cell carbon from CO<sub>2</sub>
- Autotrophs spend more energy for synthesis compared to that of heterotrophs

The energy producing reactions by chemotrophs;  
oxidation-reduction reactions

electron transfer from electron donor to electron acceptor  
electron donor  $\rightarrow$  is oxidized electron acceptor  $\rightarrow$  is reduced

- in respiratory metabolism  $\rightarrow$  external electron acceptor is used
- in fermentative metabolism  $\rightarrow$  internal electron acceptor is used
  - less sufficient energy yielding process than respiration

Growth rate and cell yield of strictly fermentative heterotrophic organisms



Growth rate and cell yield of respiratory heterotrophs

# Classification of Bacteria Based on Oxygen

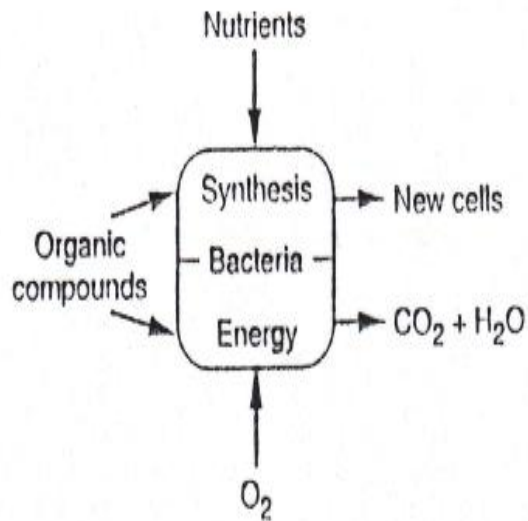
Group	O <sub>2</sub> Effect
<b>Aerobes</b>	
- Obligate	Required
- Facultative	Not required but grow better with O <sub>2</sub>
- Microaerophilic	Required, but at levels lower than atmospheric
<b>Anaerobes</b>	
- Aerotolerant	Not required; grow no better when O <sub>2</sub> present
- Obligate (strict)	Harmful or lethal

# Energy- Producing Chemical Reactions

- Aerobic:
  - Electron acceptor  $\rightarrow$   $O_2$
- Anoxic
  - Possible electron acceptors;  $NO_3^-$ ,  $NO_2^-$  etc.
- Anaerobic
  - Electron acceptors  $\rightarrow$  Organics, Fe(III)

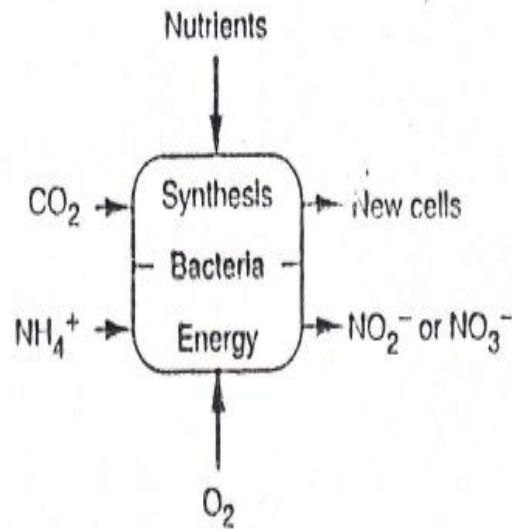


### AEROBIC HETEROTROPHIC



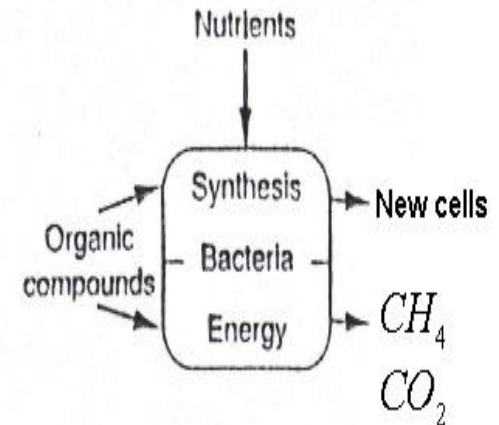
(a)

### AEROBIC AUTOTROPHIC



(b)

### ANAEROBIC HETEROTROPHIC



(c)

## Figure 7-9

Examples of bacteria metabolism: (a) aerobic, heterotrophic, (b) aerobic, autotrophic, (c) anaerobic, heterotrophic

Ref: Metcalf & Eddy, Inc. (2003). *Wastewater Engineering-Treatment and Reuse*, 4<sup>th</sup> ed., McGraw-Hill, New York, NY.

# Classification of microorganisms by electron, electron acceptor, sources of cell carbon and end products

	Common reaction name	Carbon Source	Electron Donor	Electron acceptor	End Products
<b>Aerobic Heterotrophic</b>	Aerobic oxidation	Organic compounds	Organic compounds	O <sub>2</sub>	CO <sub>2</sub> , H <sub>2</sub> O
<b>Aerobic Autotrophic</b>	Nitrification	CO <sub>2</sub>	NH <sub>3</sub> <sup>-</sup> NO <sub>2</sub> <sup>-</sup>	O <sub>2</sub>	NO <sub>2</sub> <sup>-</sup> NO <sub>3</sub> <sup>-</sup>
	Iron oxidation	CO <sub>2</sub>	Fe(II)	O <sub>2</sub>	Ferric Iron, Fe(III)
	Sulfur oxidation	CO <sub>2</sub>	H <sub>2</sub> S, S, S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	O <sub>2</sub>	SO <sub>4</sub> <sup>2-</sup>
<b>Facultative Heterotrophic</b>	Denitrification anoxic reaction	Organic compounds	Organic compounds	NO <sub>2</sub> <sup>-</sup> NO <sub>3</sub> <sup>-</sup>	N <sub>2</sub> , CO <sub>2</sub> , H <sub>2</sub> O
<b>Anaerobic Heterotrophic</b>	Acid fermentation	Organic compounds	Organic compounds	Organic compounds	Volatile fatty acids (VFAs) (acetate, propionate)
	Iron reduction	Organic compounds	Organic compounds	Fe(III)	Fe(II), CO <sub>2</sub> , H <sub>2</sub> O
	Sulfate reduction	Organic compounds	Organic compounds	SO <sub>4</sub>	H <sub>2</sub> S, CO <sub>2</sub> , H <sub>2</sub> O
	Methanogenesis	Organic compounds	Volatile fatty acids (VFAs)	CO <sub>2</sub>	Methane

•Metcalf & Eddy, Inc. (2003). *Wastewater Engineering-Treatment and Reuse*, 4<sup>th</sup> ed., McGraw-Hill, New York, NY.



# Classification of Bacteria Based on Temperature

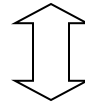
<b>Temperature Class</b>	<b>Normal Temperature Range for Growth (°C)</b>
<b>Psycrophile</b>	-5 – 20
<b>Mesophile</b>	8 – 45
<b>Thermophile</b>	40 – 70
<b>Hyperthermophile</b>	65 - 110

Growth rate double with approximately every 10°C increase in temperature until the optimum temperature is reached

Most bacteria can not tolerate pH levels above 9.5 or below 4.

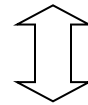
# BACTERIAL GROWTH

•Reproduction of bacteria → by BINARY FISSION



•Original cell become two new organisms

Time required for each division → GENERATION TIME

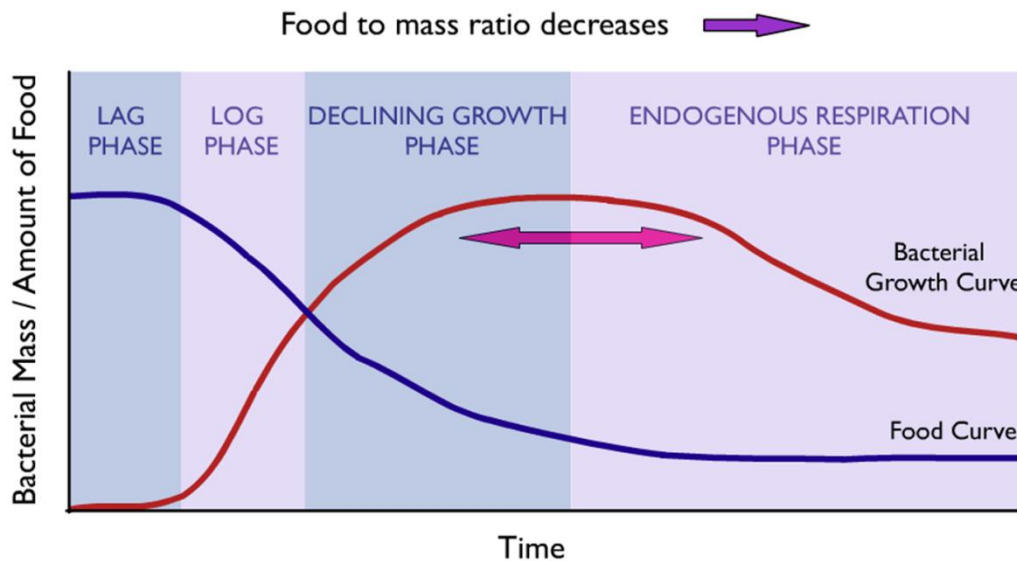


- can vary from days to less than 20 min
- depending on species and environmental condition

# BACTERIAL GROWTH

## •BACTERIAL GROWTH PATTERNS IN A BATCH REACTOR

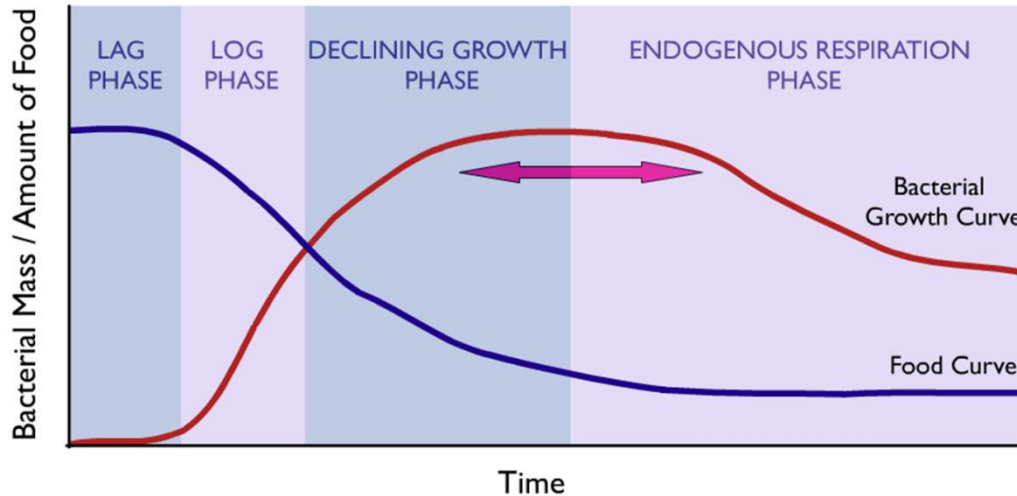
- Consider the case of a single species of bacteria inoculated in (that is, added to) a medium containig substrate and all nutrients required for growth.



<http://www.ebsbiowizard.com/2010/11/24/biological-growth-curve-in-aerated-stabilization-basins/>

# BACTERIAL GROWTH

Food to mass ratio decreases →



<http://www.ebsbiowizard.com/2010/11/24/biological-growth-curve-in-aerated-stabilization-basins/>

## LAG PHASE

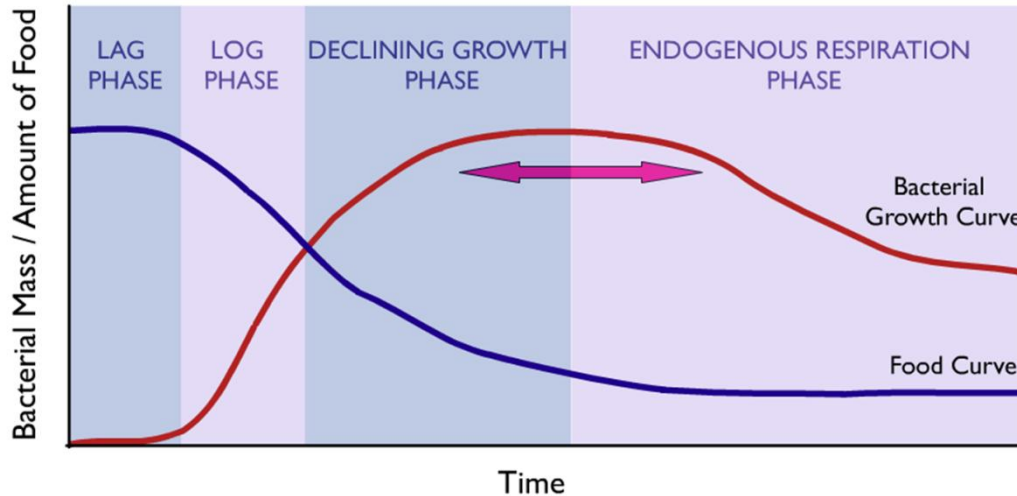
- Time required for microorganisms to acclimate to their environment before significant cell division and biomass production occur
- No increase in the number of cells
- Duration of lag phase is greatly dependent upon the age of inoculum culture and the amount of inoculum

If the parent culture is young and biologically active

} the lag phase will be extremely short

# BACTERIAL GROWTH

Food to mass ratio decreases →

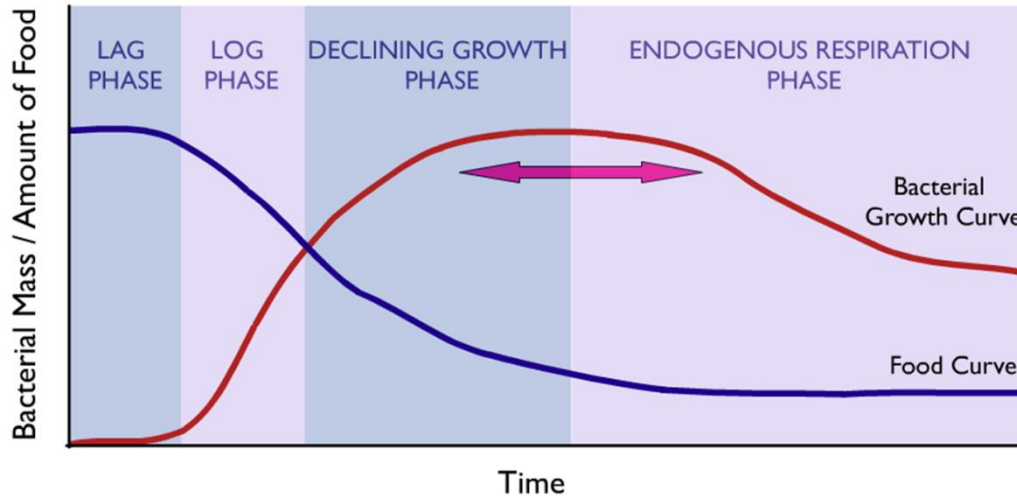


<http://www.ebsbiowizard.com/2010/11/24/biological-growth-curve-in-aerated-stabilization-basins/>

- **EXPONENTIAL GROWTH PHASE (log-growth phase)**
- The rate of fission is the maximum possible as there is no limitation due to substrate or nutrients
- Towards to the end of this phase
  - depletion of the substrate or an essential nutrient
  - accumulation of toxic end products

# BACTERIAL GROWTH

Food to mass ratio decreases →



<http://www.ebsbiowizard.com/2010/11/24/biological-growth-curve-in-aerated-stabilization-basins/>

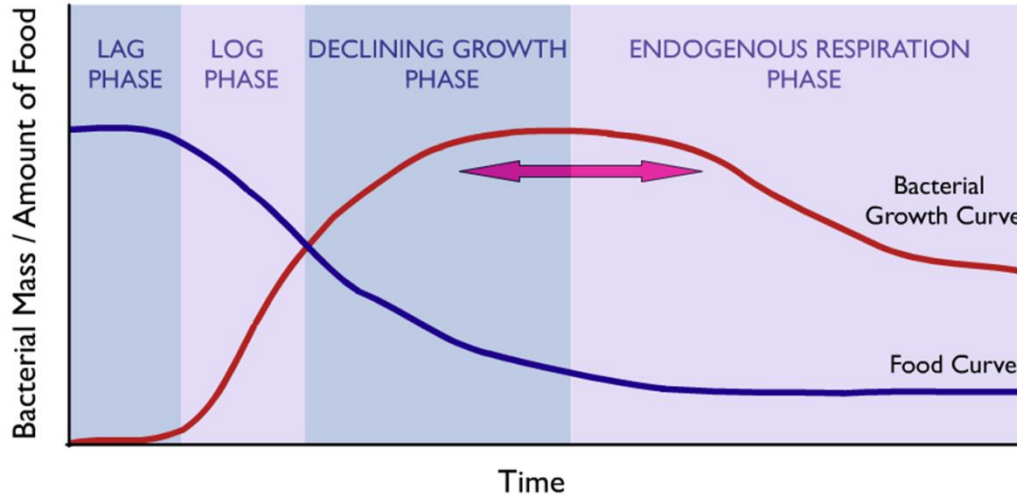
## STATIONARY PHASE

- number of cells dying = number of cells being produced
- bacterial population remains relatively constant with respect to time



# BACTERIAL GROWTH

Food to mass ratio decreases →



<http://www.ebsbiowizard.com/2010/11/24/biological-growth-curve-in-aerated-stabilization-basins/>

## DEATH PHASE

As the environment becomes more and more adverse to microbial growth (e.g. depletion of substrate)

Cells reproduce more slowly

Cell die rate exceeds cell growth rate

- Microorganisms start to utilize their own stored food materials and protoplasm in addition to a part of dead cells in the environment as food
- An exponential decline in the biomass concentration

$$\text{BIOMASS YIELD, } Y = \frac{\text{g biomass produced}}{\text{g substrate utilized (i.e consumed)}}$$

for aerobic heterotrophic rxns  
w/ organic substrates

$$\longrightarrow Y = \frac{\text{g biomass}}{\text{g organic substrate}}$$

for nitrification

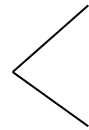
$$\longrightarrow Y = \frac{\text{g biomass}}{\text{g NH}_4\text{-N oxidized}}$$

for anaerobic rxns

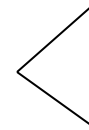
$$\longrightarrow Y = \frac{\text{g biomass}}{\text{g VFAs used}}$$

Growth Condition	Electron Donor	Electron Acceptor	Synthesis Yield
Aerobic	Organic Compound	Oxygen	0.40 g VSS/g COD
Aerobic	Ammonia	Oxygen	0.12 g VSS/g NH <sub>4</sub> -N
Anoxic	Organic Compound	Nitrate	0.3 g VSS/g COD
Anaerobic	Organic Compound	Organic Compound	0.06 g VSS/g COD
Anaerobic	Acetate	CO <sub>2</sub>	0.05 g VSS/g COD

Cell yield of anaerobic bacteria degrading organics



Cell yield of nitrifiers



Cell yield of aerobic heterotrophs degrading organic substrate

Anaerobic bacteria degrading organics use internal electron acceptor (fermentative metabolism)

Nitrifiers & aerobic heterotrophs use external electron acceptor (respiratory metabolism)