



Aalto University  
School of Engineering

# Biological treatment processes of water and waste

## Lecture 6

# WAT - E2180

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# Lecture outline

## **Biological growth**

Kinetics

## **Advanced process design**

## **BACTERIA GAME**

Dynamic nitrogen removal process

## **Storage processes**

Storage polymers

Applications

## **Biological phosphorus removal**

Removal mechanism

Existing process configurations

## **Anaerobic processes**

Anaerobic digestion

Fermentation

# Biological processes - growth

# Cell growth

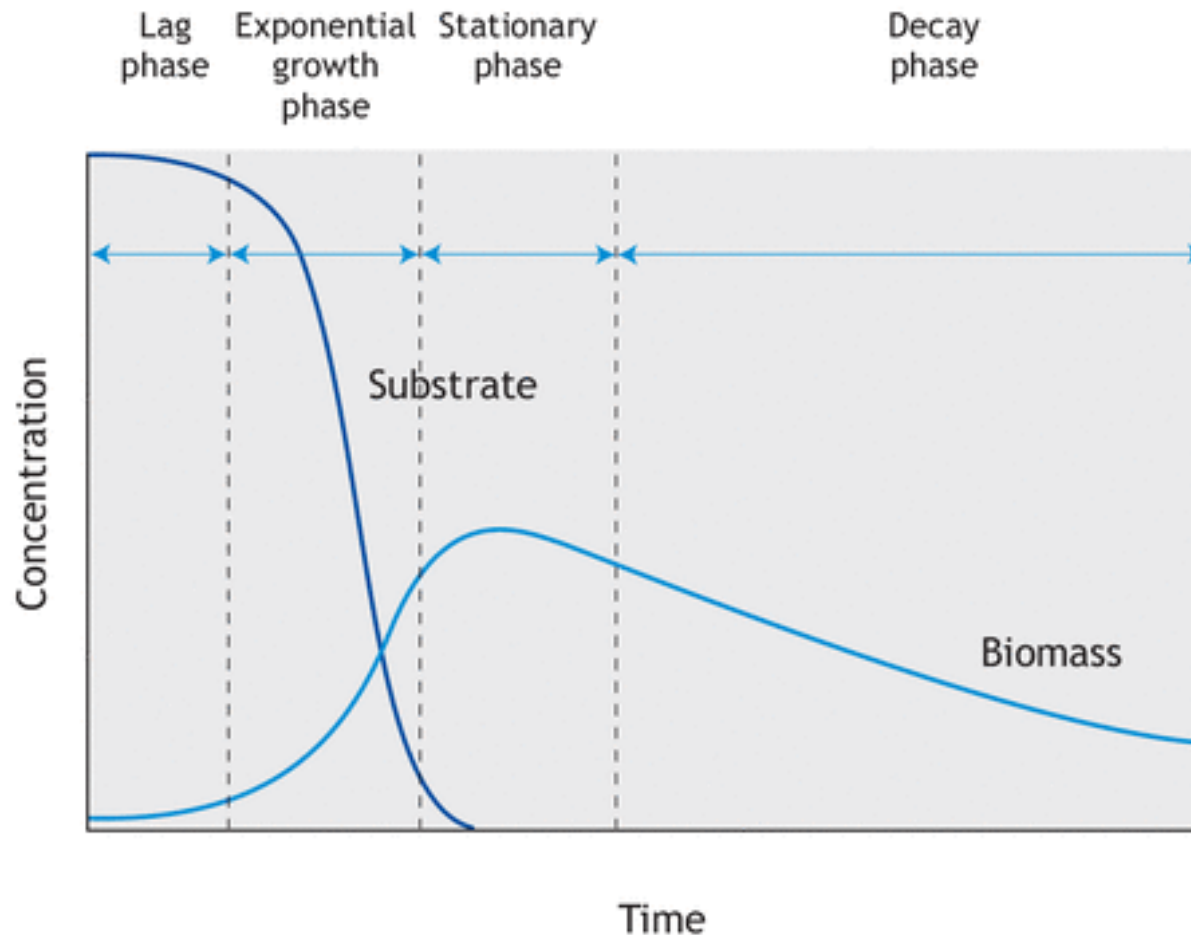


Figure 2.16 Biomass growth in batch mode (adapted from Metcalf & Eddy, 2003)

# Microbial growth

Growth can be described with the equation:

$$r_{V,XB} = \mu_{\max} f(S) X_B$$

Where

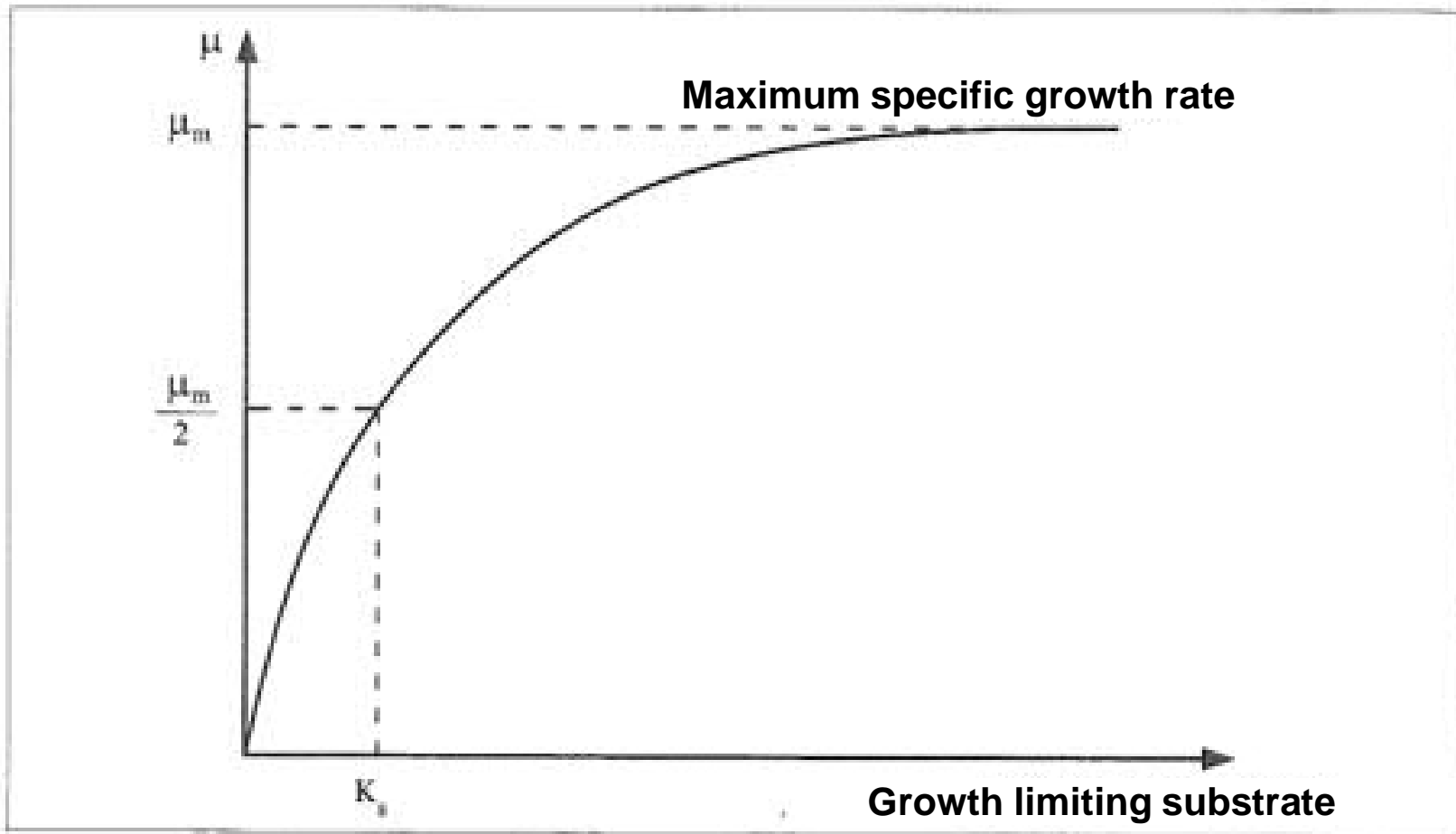
$r_{V,XB}$  = growth per unit of volume and time (e.g. kgCOD/m<sup>3</sup>d)

$\mu_{\max}$  = max specific growth rate (1/h or 1/d)

$f(S)$  = growth kinetic function (depending on substrate), typically Monod

$X_B$  = biomass concentration (kgCOD/m<sup>3</sup> or kgVSS/m<sup>3</sup>)

# Monod's kinetics



# Bacterial growth

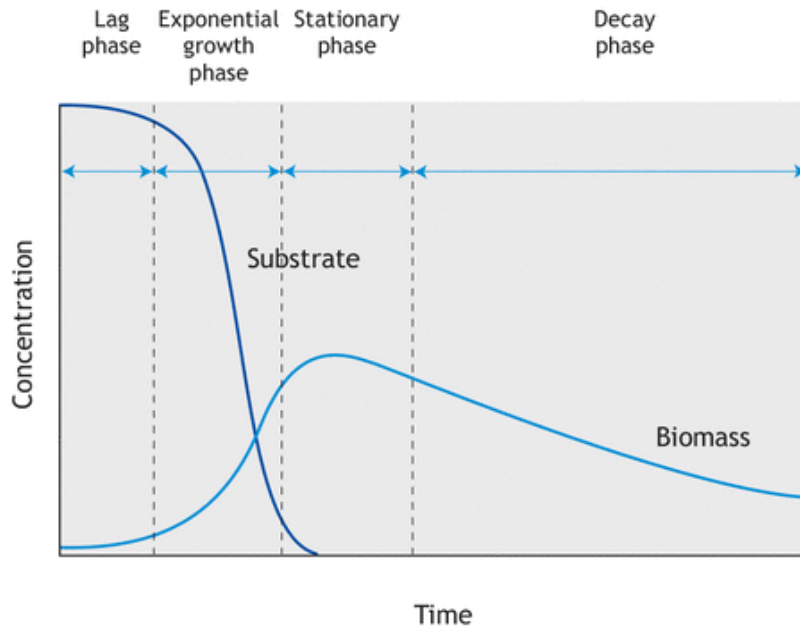


Figure 2.16 Biomass growth in batch mode (adapted from Metcalf & Eddy, 2003)

$$r_{V, XB} = \mu_{\max} \cdot \frac{S}{S + K_S} \cdot X_B$$

$$r_{V, S} = \frac{\mu_{\max}}{Y_{\max}} \cdot \frac{S}{S + K_S} \cdot X_B$$

The growth of biomass depends on the substrate consumption (with a yield) and on the decay rate  $b$

$$r_g = Y r_s - b X$$

# Substrate consumption

$$r_{V,S} = r_{V,B} / Y_{\max}$$

$Y_{\max}$  = maximum yield  
(kgCOD(B)/kgCOD(S) or  
kgVSS(B)/kgCOD(S))

Yield shows how much of the consumed substrate is transformed into new biomass in the reaction.

Note also  $Y_{\text{obs}}$  which is smaller than  $Y_{\max}$



# Monod kinetics

$$r_{V,AB} = \mu_{\max} \cdot \frac{S}{S + K_S} \cdot X_B$$

$$r_{V,S} = \frac{\mu_{\max}}{Y_{\max}} \cdot \frac{S}{S + K_S} \cdot X_B$$

Monod kinetics are typically used for microbial growth

For biomass growth  
(g/m<sup>3</sup>d)

For substrate consumption  
(g/m<sup>3</sup>d)

$$\mu_{\text{obs}} = \mu_{\max} \frac{S}{S + K_S} \quad [1/d]$$

Observed specific growth rate

# Taking into account the growth conditions

Oxygen:

$$\mu_{obs} = \mu_{max} \cdot \frac{S_{O_2,2}}{S_{O_2,2} + K_{S,O_2}}$$

$$\mu_{obs} = \mu_{max} \cdot \frac{S_2}{S_2 + K_S} \cdot \frac{S_{O_2,2}}{S_{O_2,2} + K_{S,O_2}}$$

Temperature:

$$\mu_{max(T)} = \mu_{max(20^\circ C)} \cdot e^{K(T-20)}$$

# Typical values for stoichiometric and kinetic parameters

**Table 2.9** Typical values of stoichiometric ( $f_s^0$ ,  $Y$ ) and kinetic ( $q_{\max}$ ,  $\mu_{\max}$ ) parameters for various bacterial groups, (adapted from Rittmann and McCarty 2001)

Electron donor		Electron acceptor	$f_s^0$	$Y$	$\mu_{\max}$	$K$
Microbial group	$e^-$ donor					
<b>Chemotrophic organotrophs</b>						
Aerobic heterotrophs	Sugar	$O_2$	0.70	0.49 gVSS/gbCOD	13.2	27.0 g bCOD/gVSS.d
Aerobic heterotrophs	No sugar	$O_2$	0.60	0.42 gVSS/gbCOD	8.4	17.0 g bCOD/gVSS.d
Denitrifiers	Organic	$NO_3^-$ , $NO_2^-$	0.50	0.25 gVSS/gbCOD	4.0	16.0 g bCOD/gVSS.d
Fermenting organisms	Sugar	Organic	0.18	0.18 gVSS/gbCOD	1.2	10.0 g bCOD/gVSS.d
Sulphate reducers	Acetate	$SO_4^{2-}$	0.08	0.057 gVSS/gbCOD	0.5	8.7 g bCOD/gVSS.d
Methanogens (acetoclastic)	Acetate	Acetate	0.05	0.035 gVSS/gbCOD	0.3	8.4 g bCOD/gVSS.d
<b>Chemotrophic lithotrophs</b>						
Nitrifiers :AOB	$NH_4^+$	$O_2$	0.14	0.34 gVSS/g $NH_4$ -N	0.9	2.7 g $NH_4$ -N /gVSS.d
Nitrifiers :NOB	$NO_2^-$	$O_2$	0.10	0.08 gVSS/g $NO_2$ -N	0.5	1.1 g $NO_2$ -N/gVSS.d
Methanogens (hydrogenotrophic)	$H_2$	$CO_2$	0.08	0.45 gVSS/g $H_2$	0.3	1.1 g $H_2$ /gVSS.d

bCOD: biodegradable COD

$\mu_{\max}$  in gVSS /gVSS d

$k = \mu_{\max}/Y =$  specific  $r_{\max}$  (per unit biomass)

# Denitrification rate

$$r_{V,NO} = \left( \frac{1 - Y_H}{2.86 Y_H} \right) \mu_{\max,H} \left( \frac{S_{BOD}}{K_{COD} + S_{COD}} \right) \left( \frac{S_{NO}}{K_{NO} + S_{NO}} \right) \eta_g X_{b,h}$$

where

- $r_{V,NO}$  = reaction rate per unit volume nitrate- and nitrite-nitrogen,
- $Y_H$  = biomass yield coefficient,
- $\mu_{\max,H}$  = maximum specific growth rate of heterotrophs,
- $S_{COD}$  = soluble material concentration organic substrate,
- $K_{COD}$  = half-saturation coefficient organic substrate,
- $S_{NO}$  = soluble material concentration nitrate- and nitrite-nitrogen,
- $K_{NO}$  = half-saturation coefficient nitrate-nitrite,
- $\eta_g$  = correction factor for  $\mu_H$  under anoxic conditions, and
- $X_{b,h}$  = particulate material concentrations.

# Design of biological processes

# DEMO 1

Average daily flow rate	37 850 m <sup>3</sup> /d
Influent water:	
BOD <sub>7</sub>	140 mg/l
Ammonium-N	35 mg/l
Suspended solids	90 mg/l
Of which unbiodegradable	30 mg/l
Effluent water:	
BOD <sub>7</sub>	10 mg/l
Ammonium-N	0,5 mg/l
Total N	10 mg/l
Suspended solids	15 mg/l
Temperature	12 C
MLVSS/MLSS	0,8
MLVSS	2,4 g/l
Y (heterotrophs)	0,6 kg VSS/kg BOD
b (12 C)	0,044 d <sup>-1</sup>
Y (nitrifiers)	0,12 kg VSS/kg NH <sub>4</sub> -N
b <sub>N</sub> (12 C)	0,06 d <sup>-1</sup>

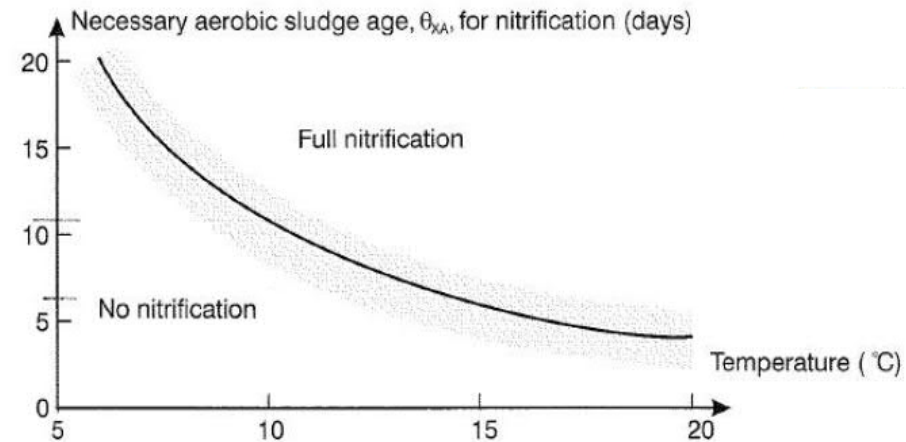
Dimension an activated sludge process where full nitrification and 70% denitrification is achieved.

Use the denitrification rate (12° C, raw WW) of 1,5 gN/kgVSSh

Assumption 1: No nitrate in the influent water

Assumption 2: No denitrification in the secondary clarifiers.

# DEMO 1



## Dimensioning of nitrifying process:

- Choose SRT → nitrification

12 ° C → 10 d

- Calculate the needed biomass per day:

$$\text{Biomass } XV = \frac{YQ(S_o - S_e)}{1 + b\theta c} + \frac{Y_n Q(S_{NH_4} - S_{e_{NH_4}})}{1 + b_n \theta c} =$$

$$2031,2 + 97,0 = 2128 \text{ kgVSS/d}$$

- Biomass (VSS → SS) = 2660 kgSS/d

- Inert particulate influent 1136 kgSS/d → Total sludge amount SS = 3796 kgSS/d, sludge concentration 3 g/l

- Reactor volume needed = (sludge amount x SRT) / X = 12653 m<sup>3</sup>

**NOTE! This calculation is simplified → inert SS produced not considered**

# DEMO 1

The volume needed for denitrification is calculated based on the denitrification rate.

Total N 70 % → to be denitrified  
927 kg/d = 38,6 kgN/h = 38 600 gN/h

Denitrification rate (12C, raw WW) → 1,5 gN/kgVSS/h  
MLVSS 2,4 g/l → needed volume 10 722 m<sup>3</sup>



# Removal in biological processes

Removal	Conditions	When
<b>Organic matter</b>	Aerobic, short SRT	Focus on removal
<b>Organic matter</b>	Anaerobic, long HRT&SRT	for high strength waters, focus on energy recovery
<b>Ammonium</b> (nitrification)	Aerobic, long SRT	Well known process
<b>Total nitrogen</b> Nitrification + denitrification	Aerobic + anoxic zones, Short/long SRT	Well known process
<b>Total nitrogen</b> Short-cuts	Low DO (nitritation + denitritation) Deammonification	Focus on energy savings High strength waters
<b>Phosphorus</b>	Aerobic + anaerobic zones	P reuse, sludge production, carbon source
NOM, iron, manganese, organic micropollutants	Aerobic, mainly long SRT	Often cost effective solution

# Design approaches for biological processes

Sludge age (days) → Sludge age selected on the basis of process goals (e.g. nitrification) → Basin volume

Sludge loading (kgBOD/kgMLSSd) → As above but does not take into account differences in sludge yield (e.g. process design when water is very typical)

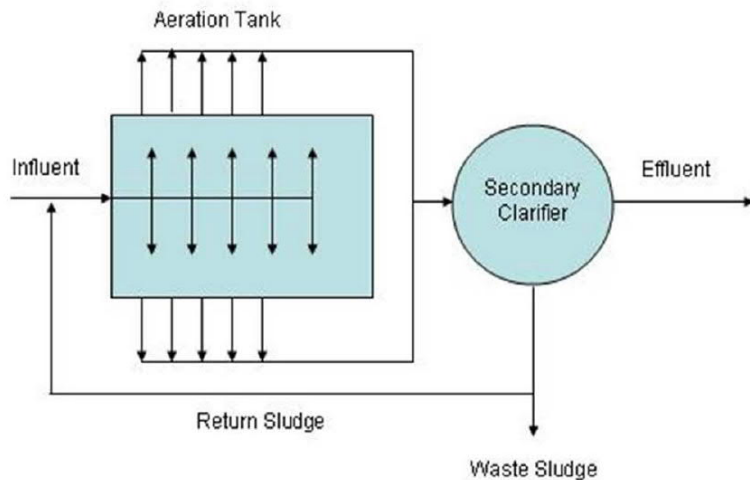
Volumetric loading (kgBOD/m<sup>3</sup>d) e.g. rough estimations for budgeting

Surface loading (kgBOD/m<sup>2</sup>d or kgN/m<sup>2</sup>d) MBBR and biological filters

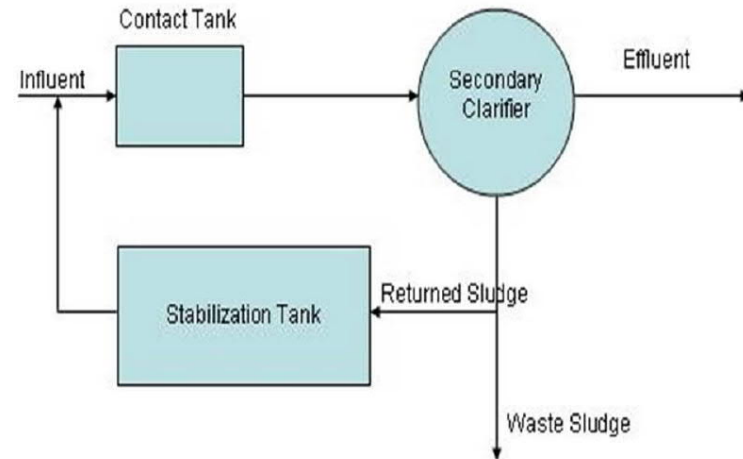
Reaction rate (gN/gMLSSh) e.g. anoxic zones

Hydraulic retention time (hours) e.g. anaerobic and anoxic zones

# Important design aspects

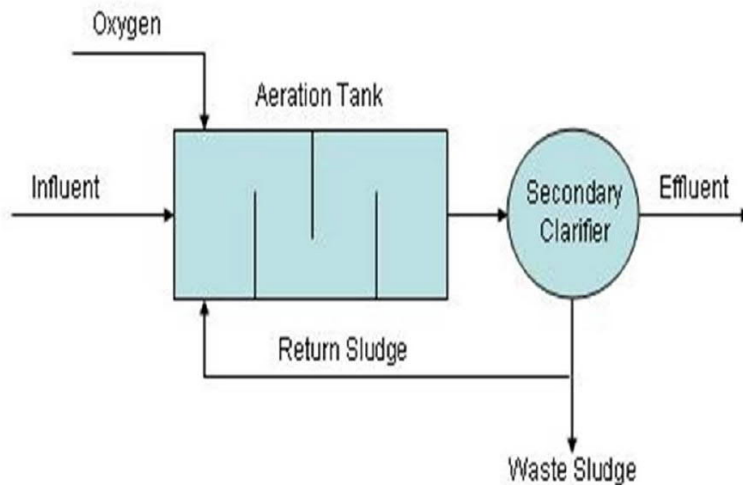


Complete Mix Activated Sludge Process

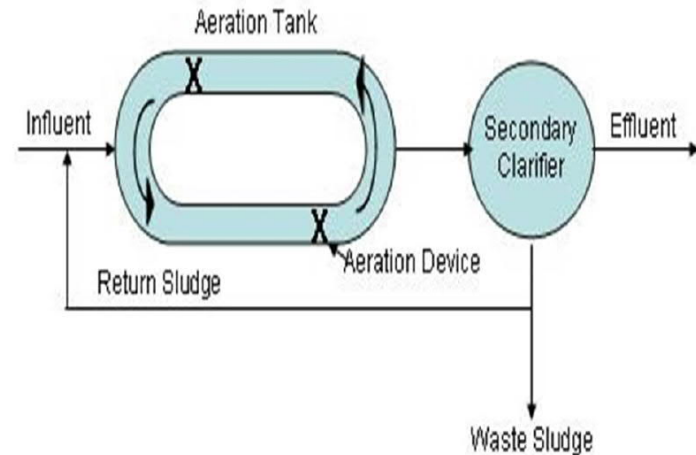


Contact Stabilization Activated Sludge

# Intensive or extended aeration

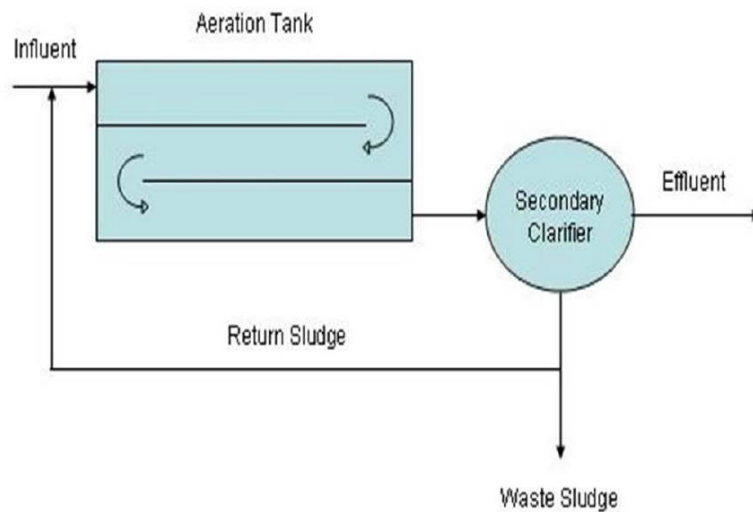


High Purity Oxygen Activated Sludge

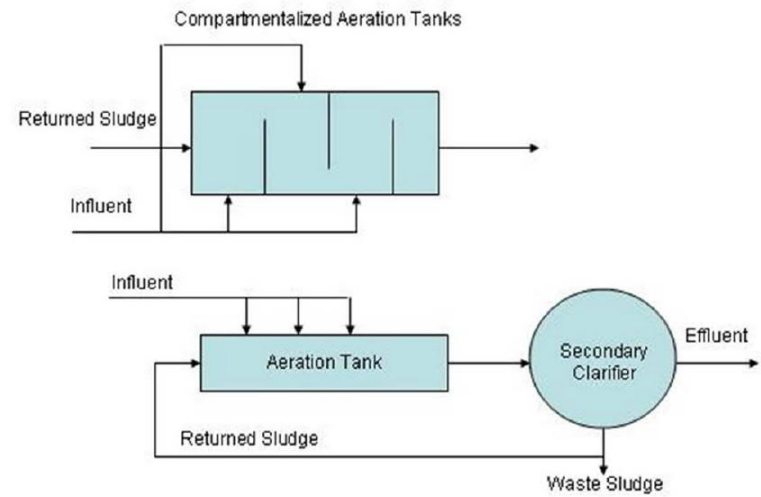


Oxidation Ditch Activated Sludge Process

# Process configurations

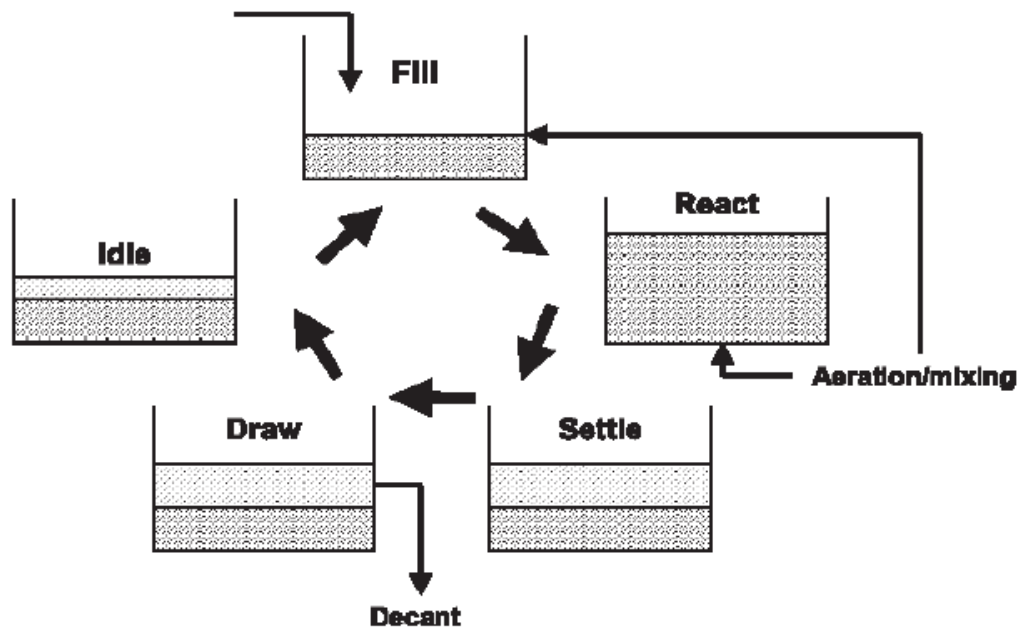


Plug Flow Activated Sludge Process



Step Feed Activated Sludge Process

# Process configuration



**Sequencing batch reactor**

**All reactions happen in the same volume**

**Typically 2 or 3 parallel SBRs**

# Process type

Activated sludge

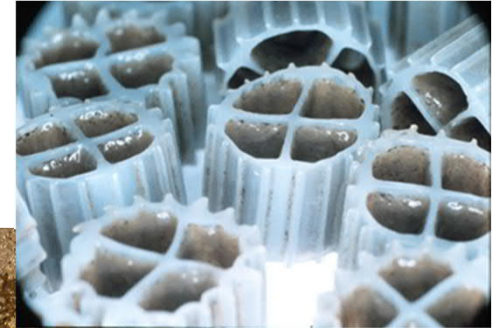
Moving bed bioreactor (MBBR)

Membrane bioreactor (MBR)

Biological filters

Aerobic granular sludge (AGS)

Selection depends on wastewater characteristics, climate conditions, energy aspects, effluent requirements, size etc.



# Nitrogen and phosphorus removal

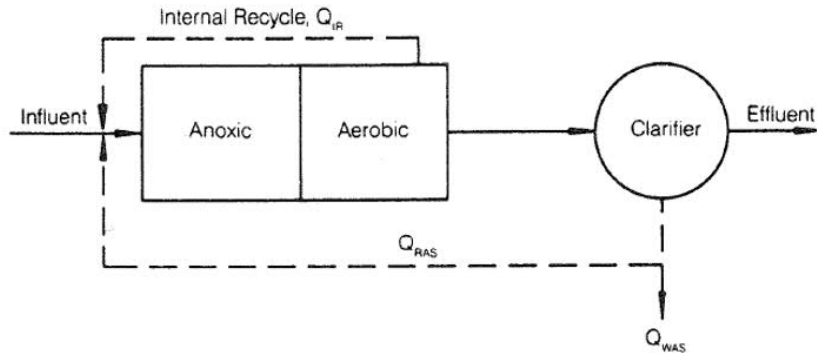


Figure 8.9 Modified Ludzack–Ettinger process for nitrogen removal (WAS = waste activated sludge).

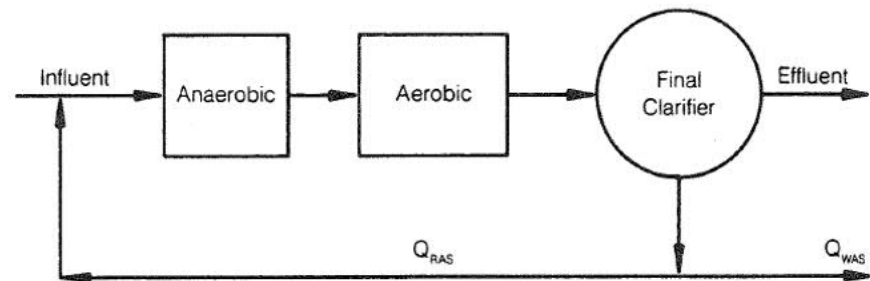


Figure 8.3 The A/O process (RAS = return activated sludge, WAS = waste activated sludge).



# Alternative configurations

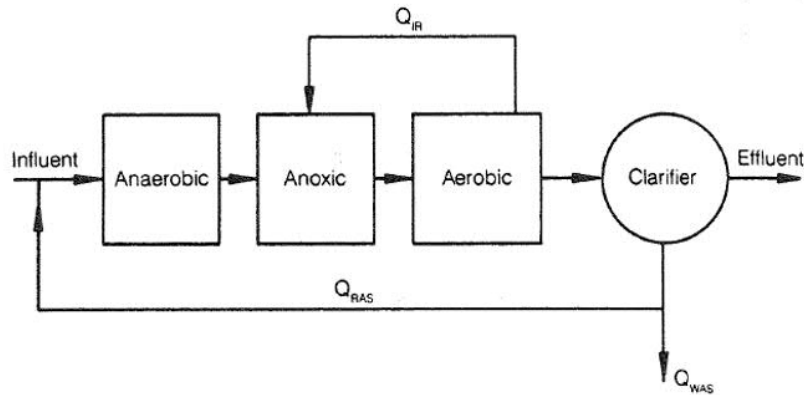


Figure 8.14 A<sup>2</sup>/O process for phosphorus removal (WAS = waste activated sludge).

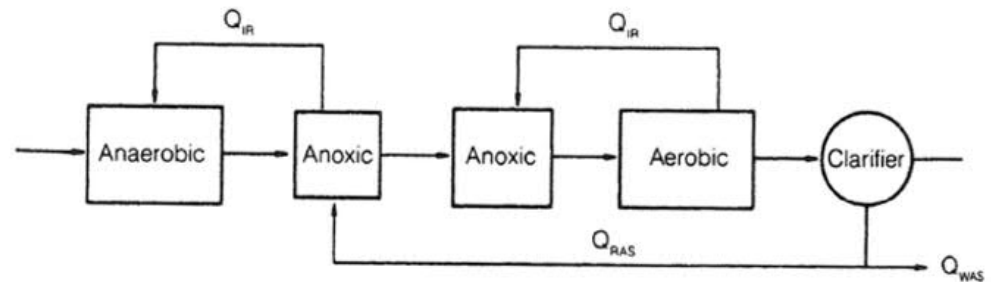


Figure 8.15 Modified University of Cape Town process for phosphorus and nitrogen removal (WAS = waste activated sludge).

# How to calculate the recycle ratio of RAS?

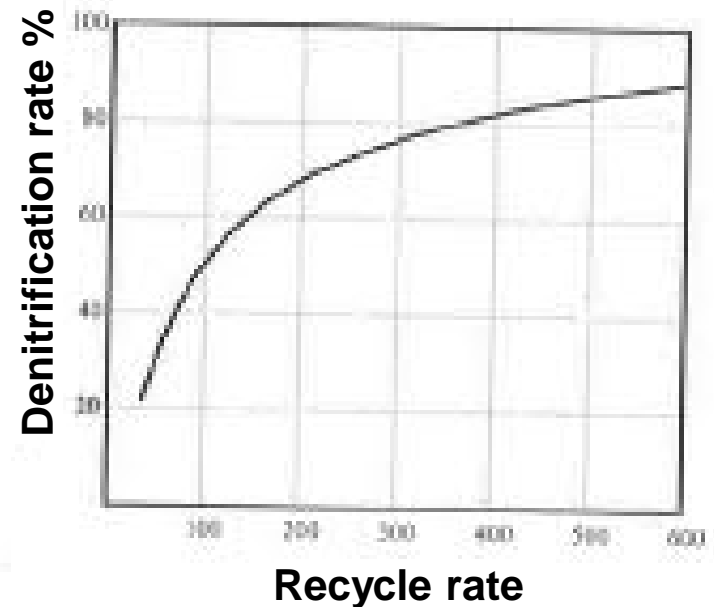
Recycle ratio =  $100X / ((1200/SVI) - X)$

Where  $X$  = MLSS (g/l)

And SVI = sludge volume index (ml/g)

Recycle must be sufficient for denitrification

Typical recycle ratio 100 - 200 %



# BREAK

# BACTERIA GAME: Advanced version 2

## Dynamic nitrogen removal process 😊

- **First test the process with ND configuration**
- **Each player picks a type of microbe and places it in suitable conditions in the process**
- **ZONE 1:**
  - Each player picks two wastewater constituent. This is the influent wastewater that enters the first zone.
  - Try to form suitable sets for your microbe's reaction using influent wastewater and zone conditions.
  - When a reaction occurs, select the correct end-products
  - Check if possible reactions exist with the end-products
- **ZONE 2:**
  - Move the wastewater constituents and the end-products that have not yet reacted to zone 2.
  - Repeat the steps from zone 1.
- **RAS flow**
  - Move the wastewater constituents and the end-products that have not yet reacted to zone 1 via RAS flow.
  - Repeat the steps from above.
- **GAME ENDS WHEN ALL THE REACTIONS HAVE OCCURRED.**

# BACTERIA GAME: Advanced version 2

## Dynamic nitrogen removal process 😊

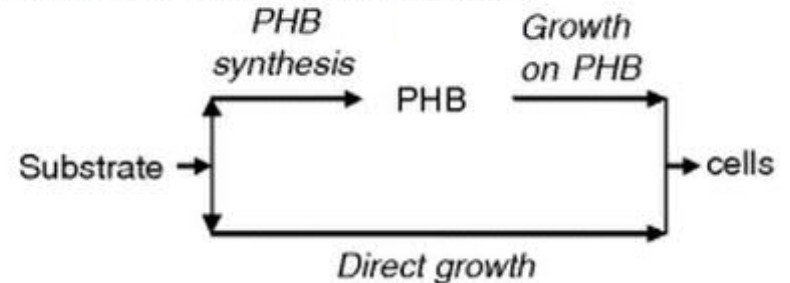
- **Now test the process with DN configuration**
  - **Each player picks a type of microbe and places it in suitable conditions in the process**
  - **ZONE 1:**
    - Each player picks two wastewater constituent.
    - Try to form suitable sets for your microbe's reaction using influent wastewater and zone conditions.
    - When a reaction occurs, select the correct end-products
    - Check if possible reactions exist with the end-products
  - **ZONE 2:**
    - Move the wastewater constituents and the end-products that have not yet reacted to zone 2.
    - Repeat the steps from zone 1.
  - **RAS flow**
    - Move the wastewater constituents and the end-products that have not yet reacted to zone 1 via RAS flow.
    - Repeat the steps from above.
  - **GAME ENDS WHEN ALL THE REACTIONS HAVE OCCURRED.**
-

# Storage processes

# Role of storage processes in growth

- Substrate can be converted and stored within bacterial cells as energy storage.
- Bacterial growth can be based on direct growth on the substrate or on growth on these storage polymers.
- Growth on storage has a bit lower yield (energetically less efficient) – 4 - 10% less sludge production.
- Common storage polymers  
Polyhydroxyalkanoate PHA and polyhydroxybutyrate PHB
- Storage polymers are a benefit in bacterial competition.

Two ways to use a substrate for growth.



# PHA & PHB

## PHA

- Up to 90% cell dry weight
- Similar characteristics to plastics
- Biodegradable
- Example: Mars, Attero Venlo (PHA from biowaste)

## PHB: example Mirel (USA)

Caproates: animal feed,

## PHA&PHB production

### Important things to consider

- **Production yield**
- **Volumetric productivity**
- **PHA&PHB concentration**
- **PHA&PHB composition**



**PHA**

PHA extraction  
and processing



Bioplastic



Chemical conversion  
with methanol



Biofuel



Direct Chemical  
conversion:



Biochemical



**Table 15.1** Some Companies Involved in PHA Production.

Company	Products
Berlin Packaging Corp. (U.S.)	Zeneca/ICI Biopol
Bioscience Ltd. (Finland)	Medical applications of PHAs
Bioventures Alberta, Inc. (Canada)	PHA produced by recombinant <i>Escherichia coli</i>
Metabolix, Inc. (U.S.)	PHB, P(HB : HV) (Mirel)
Metabolix/ADM	Transgenic plant PHAs
Monsanto (U.S.)	Transgenic plant PHAs
Polyferm, Inc. (Canada)	PHAs from hemicellulose; use of <i>Burkholderia cepacia</i> on xylose
Monsanto-Metabolix (U.S.)	Biopol from <i>Cupriavidus necator</i>
Nodax Procter and Gamble (U.S.)	PHBHx, PHBO, PHBOd (Nodax)
Tianan Biologic Material Co (China)	PHB and P(HB : HV) (Enmat)
Tianjin GreenBio Materials Co., Ltd. (GreenBio) (China)	Sogreen
Biocycle Copersucar (Brasil)	PHB and P(HB : HV) (Biocycle)
Biomer (Germany)	PHB and P(HB : HV) (Biomer L)
BIO-ON (Italy)	Minerv-PHA (from sugar beets)
NatureWorks LLC (U.S.)	Ingeo biopolymer
Micromidas	Constructed microbial population able to adapt to a variety of materials, including waste

# How Mirel is Made

## Biodegradable\*

Mirel is biodegradable in natural soil and water environments, home and industrial composting facilities, where available.



## Biobased

Starting with corn.

## Corn Sugar

One of many products made from each kernel of corn, used as feedstock for Mirel.

## Fermentation

A patented process, transforms the sugar into Mirel biopolymers.

## Applications

Mirel can be processed on conventional equipment and used in everyday products.

## Formulation

Mirel is compounded into resin pellets.

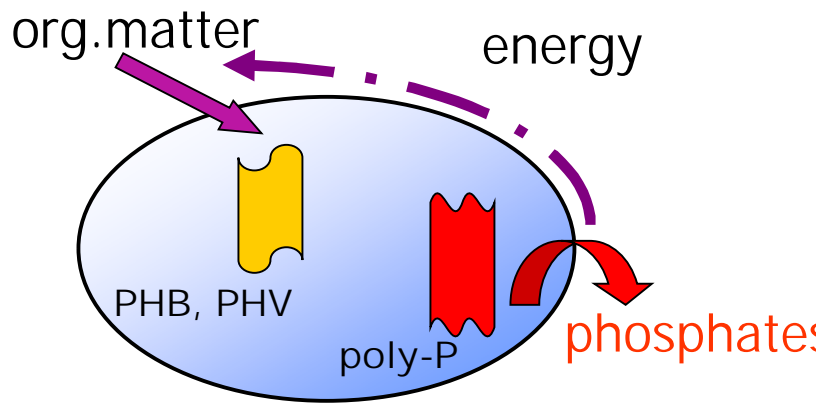
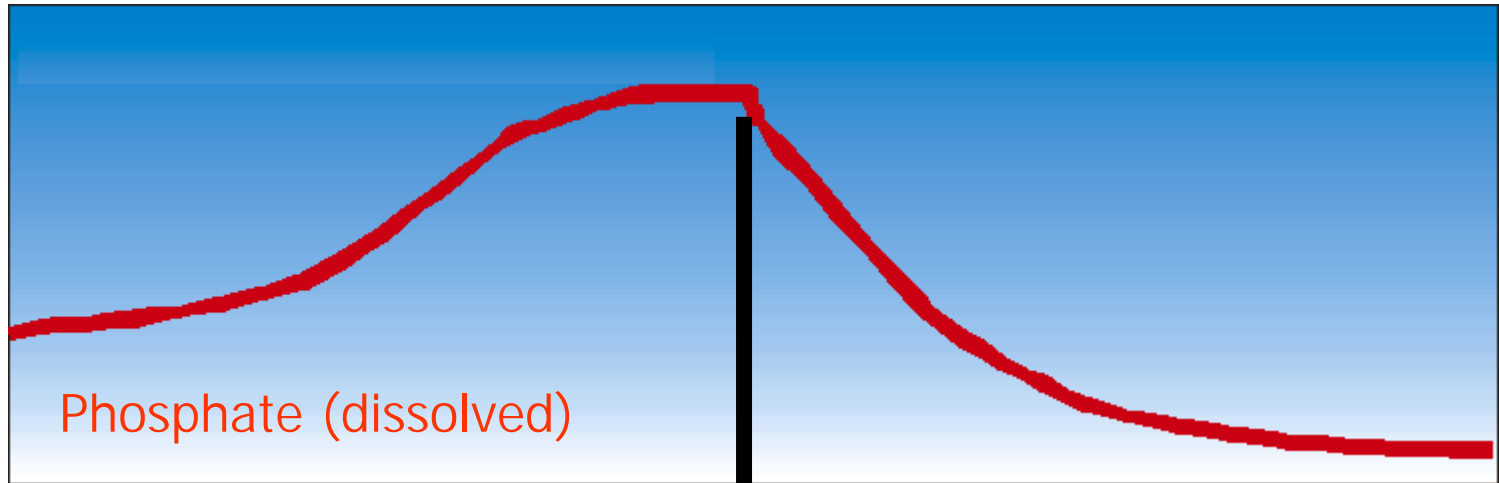
# BREAK

# Biological phosphorus removal

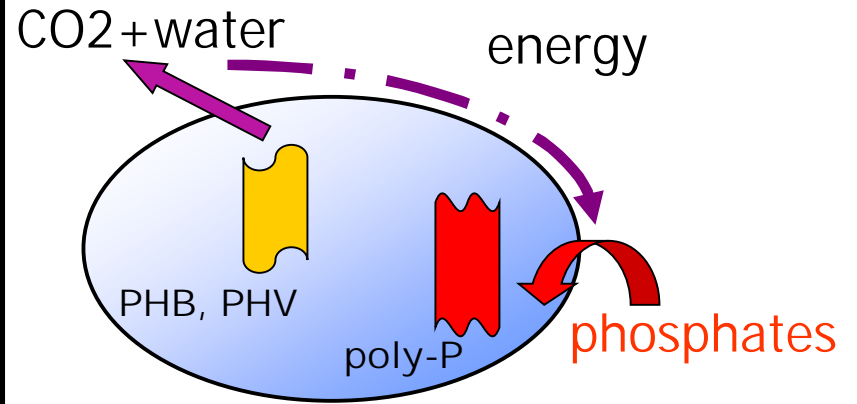
# Biological phosphorus removal

- Phenomenon was discovered by accident in India 1959
- Observed in full-scale plant in South Africa in the 70s also by accident
- Based on microbes capable of storing polyphosphates
- Require alternating anaerobic (not even nitrates) and aerobic conditions and carbon source in the anaerobic phase.
- PAOs phosphorus accumulating organisms
- Competition with GAOs (Glycogen accumulating organisms) especially in warm temperatures

# BioP



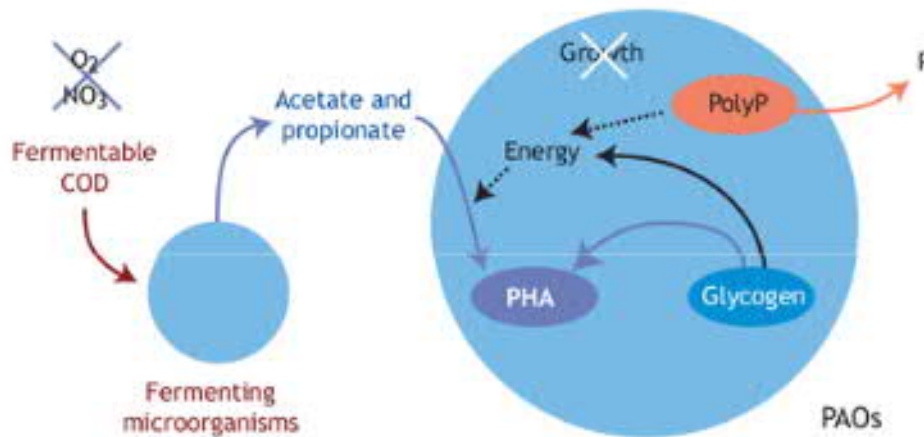
ANAEROBIC



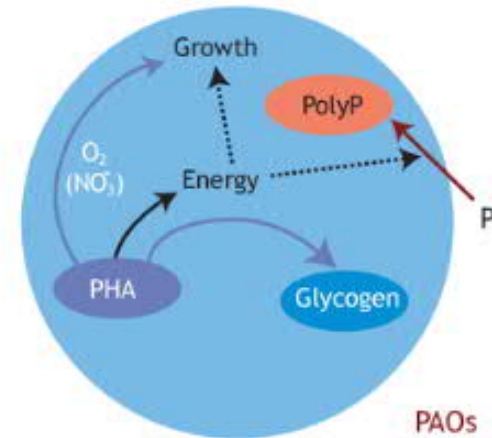
AEROBIC

# BioP

## ANAEROBIC CONDITIONS



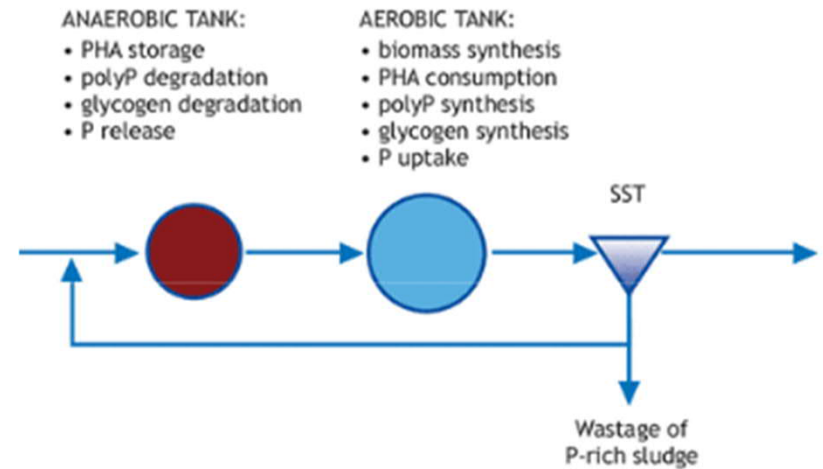
## AEROBIC CONDITIONS





# Principles of bioP

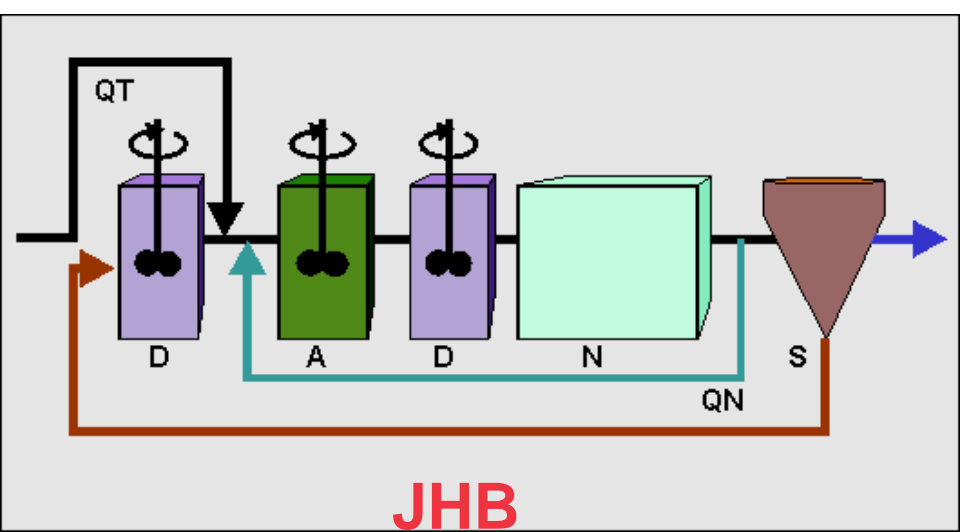
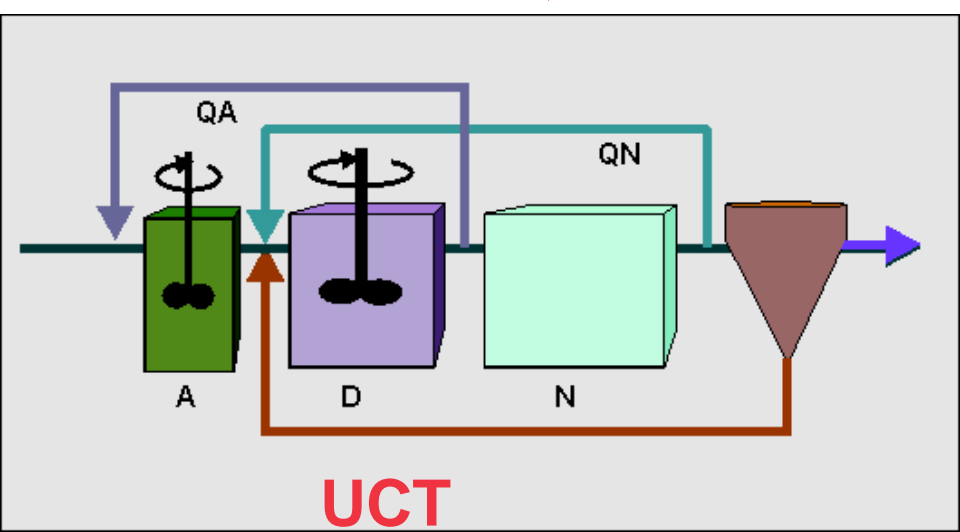
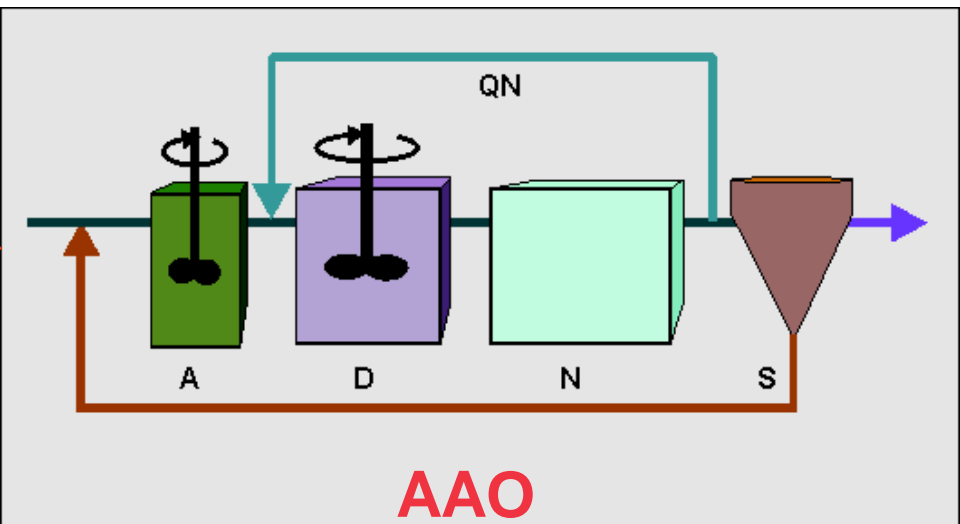
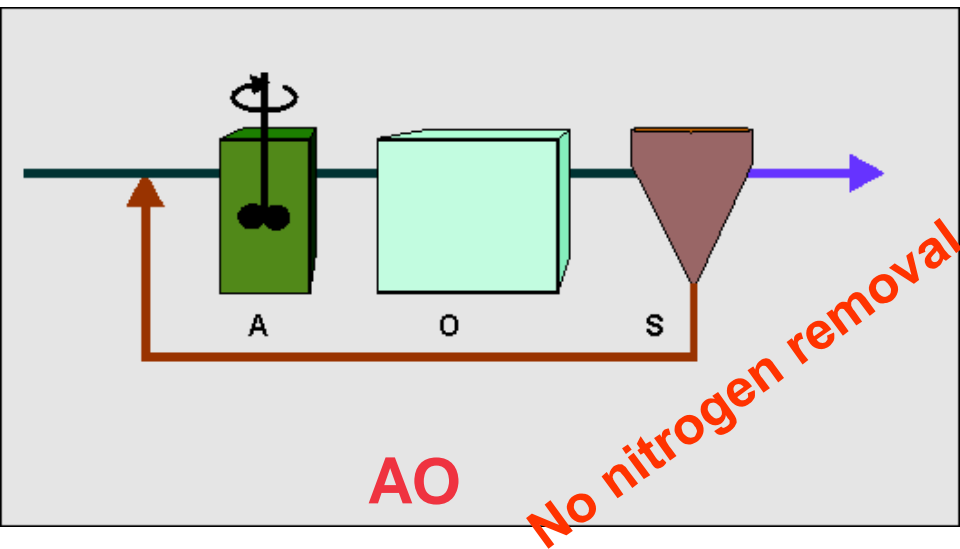
- Phosphorus accumulating organisms (PAOs) store organic matter as polyhydroxyalcanoates (PHA) in anaerobic conditions using energy from poly-P inside the cell
- In aerobic conditions PAOs store more poly-P than needed for the normal metabolism using stored PHA
- Phosphorus is removed with the sludge (3-8 % of P)



# Important aspects in bioP processes

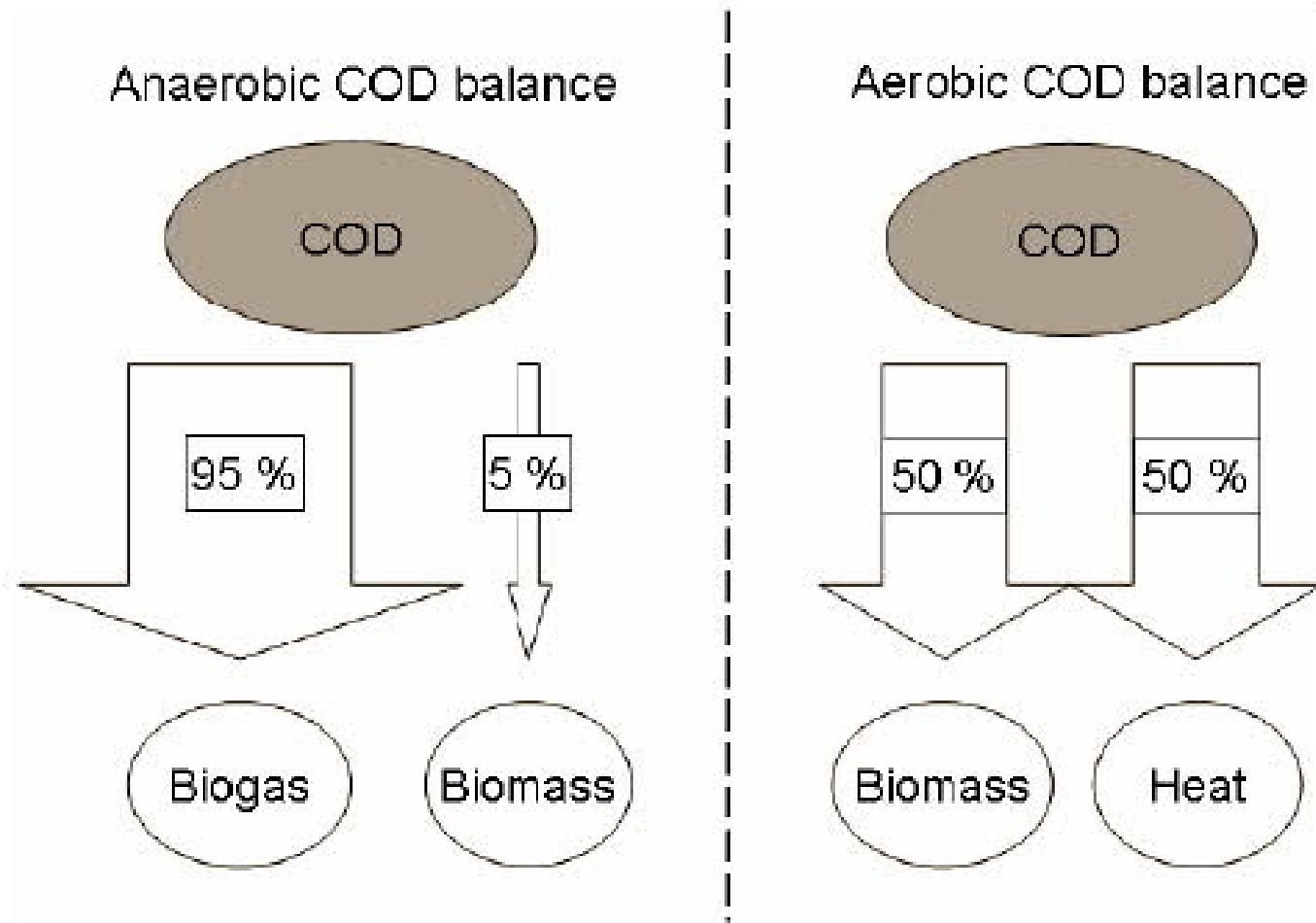
- **Minimizing oxygen in the anaerobic zone**
- **Minimizing nitrates and nitrites in the anaerobic zone.**
- **Increase volatile fatty acids (VFA) concentration in the anaerobic zone. (VFA is taken up and forms PHA)**
- **Minimizing solids in the effluent (high P content)**
- **Maximizing phosphorus uptake = short SRT and good oxygen concentration pattern**

# Biological P removal processes



# Anaerobic processes

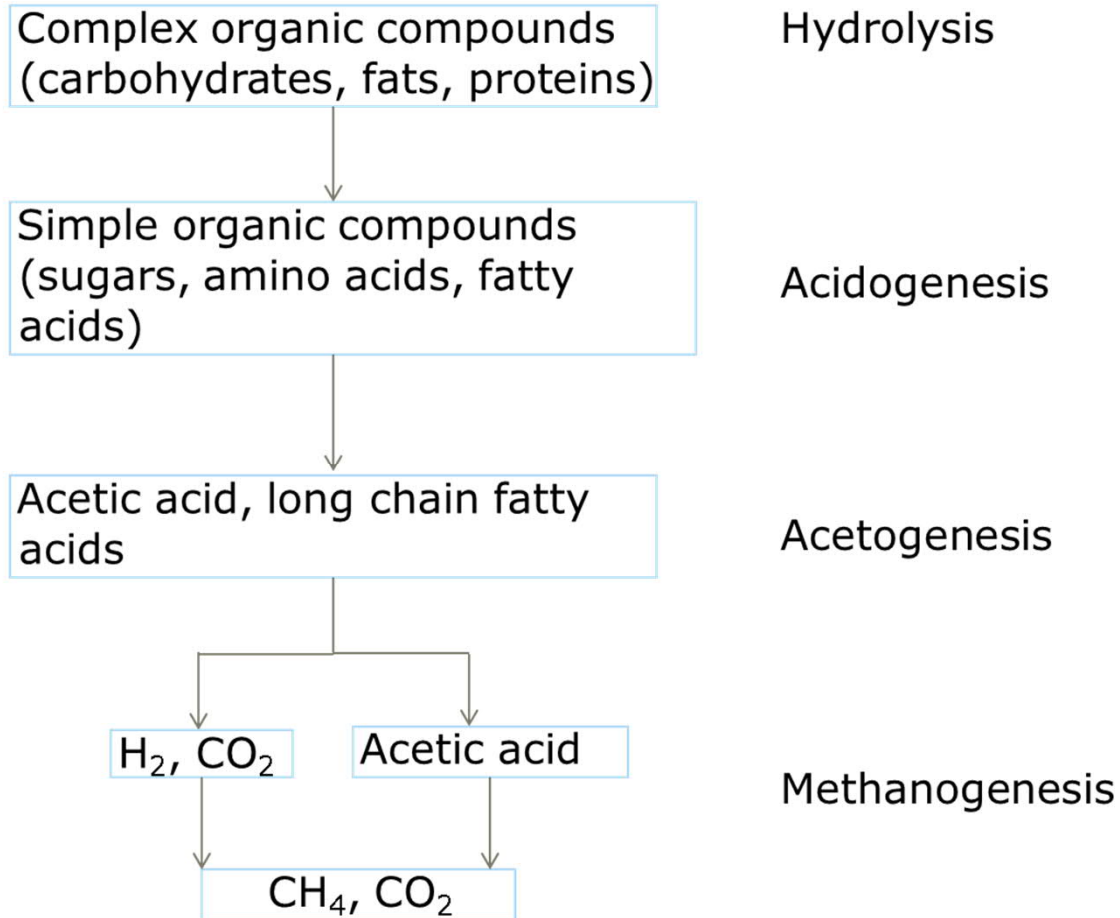
# Why anaerobic treatment?



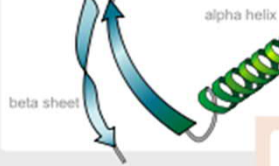
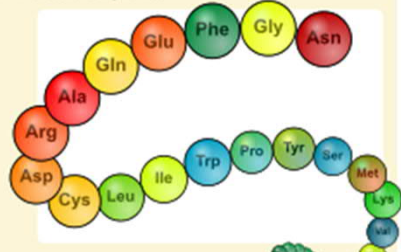
# Pros and cons of anaerobic digestion

- +  $\text{CO}_2$  as electron acceptor
- + no need for aeration
- + Low sludge yield
- + Produces methane, 90% can be used as energy (9000 kcal/m<sup>3</sup>)
- + high loading → less space
- + Works with certain organic compounds that can not be degraded in aerobic conditions
- Slow process (HRT about 30 d)
- Sensitive to toxic substances
- Long start-up
- Requires high substrate concentrations

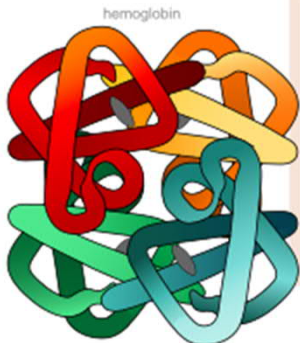
# Anaerobic digestion



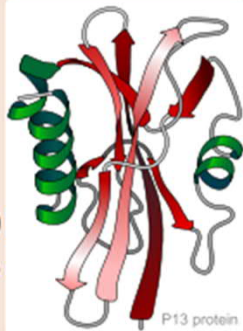
Primary structure  
amino acid sequence



Secondary structure  
regular sub-structures

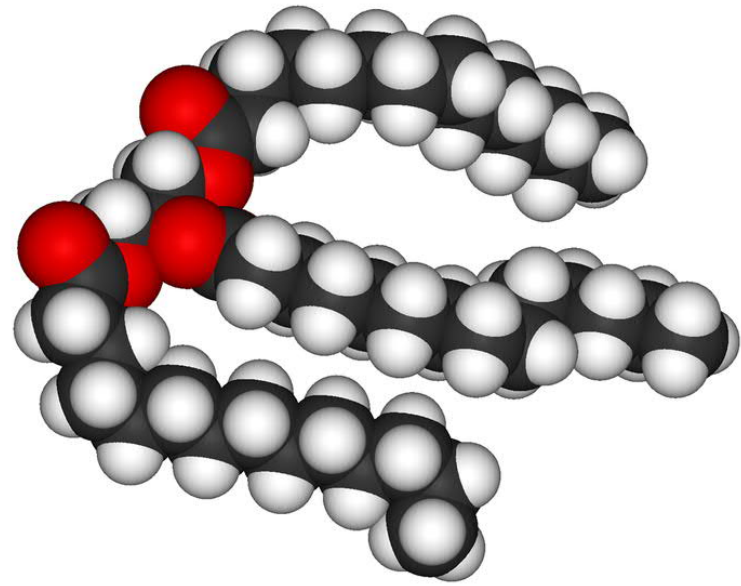


Quaternary structure  
complex of protein molecules

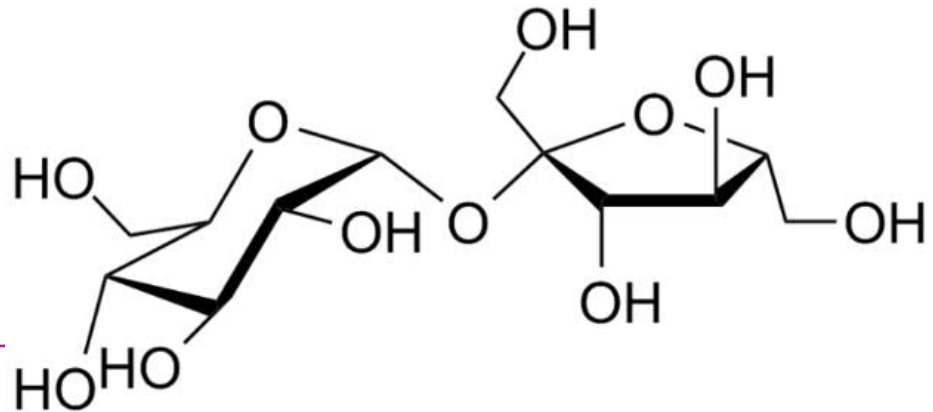


Tertiary structure  
three-dimensional structure

## Proteins



Fats (triglyceride molecule)



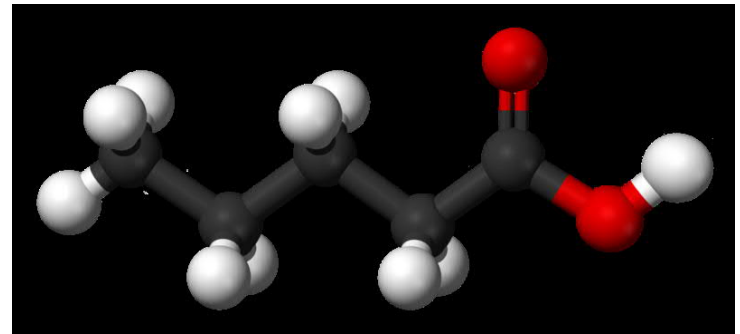
Sugar



# Hydrolysis

- **First step of the anaerobic digestion**
- **Different groups of bacteria produce extracellular enzymes to cut the larger organic molecules into smaller ones**
- **Larger molecules = proteins, fats, carbohydrates**
- **Smaller molecules = small molecule sugars, amino acids, short chain fatty acids**

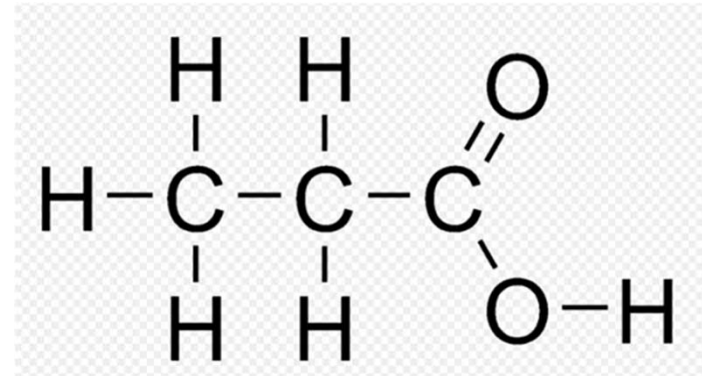
**Hydrolysis products, example valeric acid**



# Acidogenesis

- **Second step of the anaerobic digestion**
- **Acidogenesis**
- **Bacteria degrades the organic molecules further to short-chain fatty acids and alcohols**
- **Ammonium, hydrogen and CO<sub>2</sub> also produced**

End product in this step, for example propanoic acid



# Acetogenesis and methanogenesis

- **Third step of the anaerobic digestion**
- **Acetogenic bacteria degrades the short chain fatty acids to acetic acid (and hydrogen and CO<sub>2</sub>)**

**Last step of the anaerobic digestion**

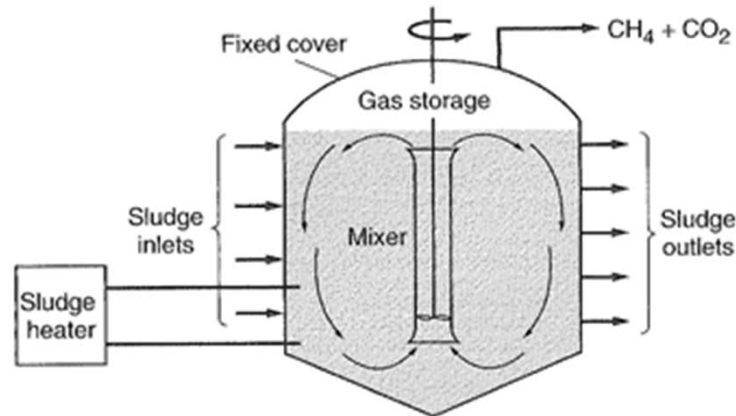
**Methanogenic bacteria use acetic acid, CO<sub>2</sub> and hydrogen to produce biogas (=methane)**

# Pre-fermentation

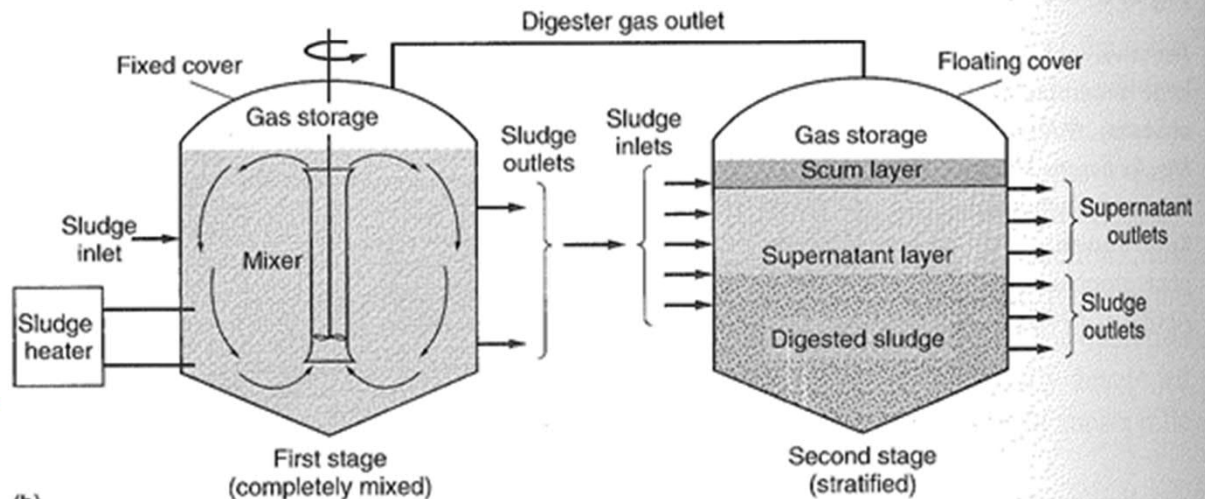
- In order to produce VFA = volatile fatty acids
- VFAs are enhancing **denitrification and biological phosphorus removal**
- Can be done with influent waste water, raw sludge, waste activated sludge or a industrial influent



# Anaerobic processes for sludge digestion (biogas plants)



(a)



(b)

# Digestion processes

**Mesophilic**

**33 – 37 °C**

**Retention time about 21 days**

**Thermophilic**

**54 – 55 °C**

**Retention time about 14 days**

**Requires more energy**

# Anaerobic processes in wastewater treatment

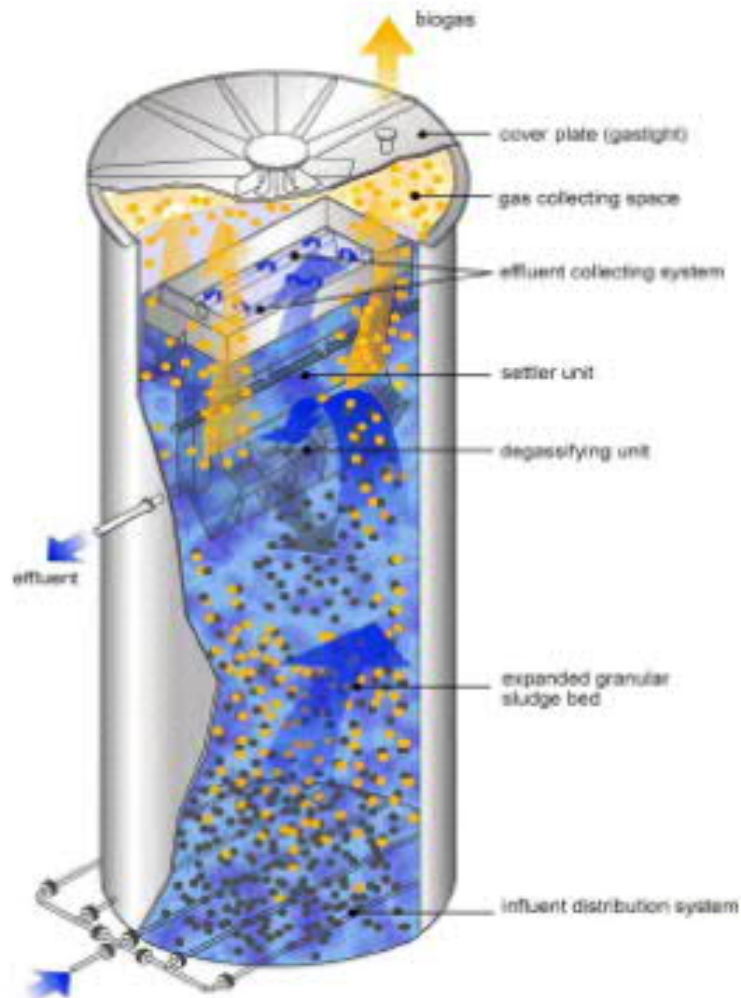


Figure 1: Typical Biobed® EGSB reactor

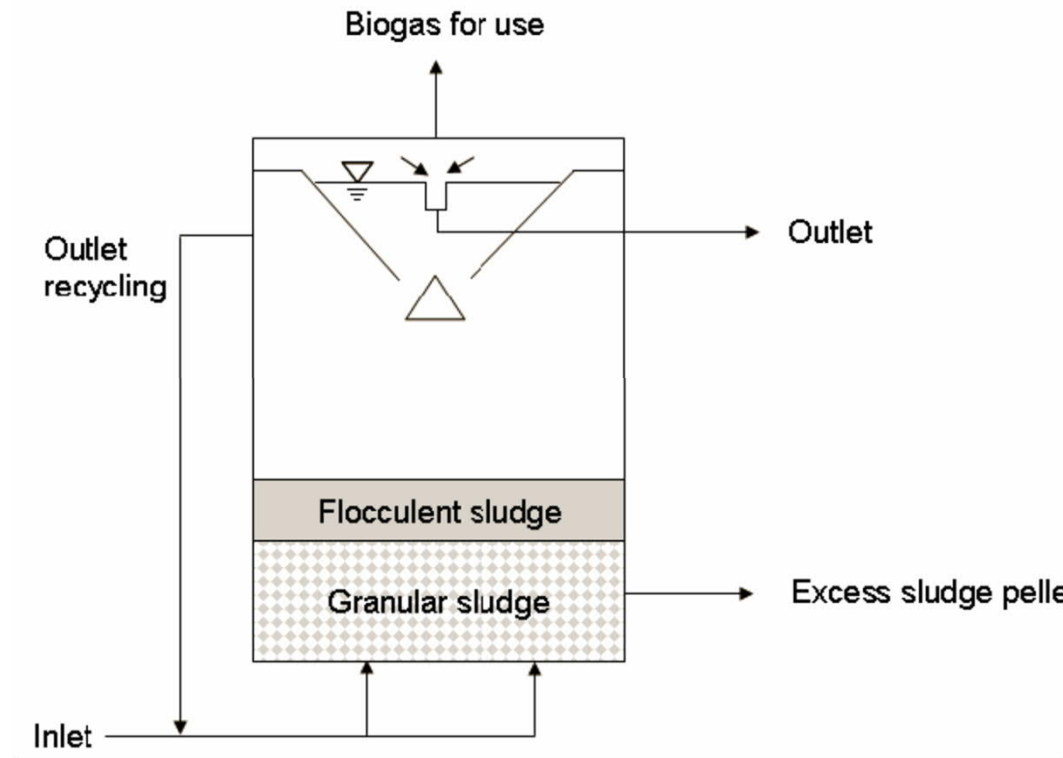


Typical Biobed® EGSB plant at Lapin Kulta, Haparanda (Finland)

# UASB reactor (Upflow anaerobic sludge blanket)

Granular biomass is created in the reactor

Biomass is kept in suspension by the gravity of the granules and the upflow of wastewater





# Reading material

**Biological wastewater  
treatment (Course book):**

**Chapters**

**2.1**

**2.2.7 – 2.2.8**

**2.3**

**2.4**

**7.1 – 7.4**