Chapter 4

Influence of Environmental Factors

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Environmental Factors

1. Nutrients
2. Temperature
3. pH-Buffer capacity
4. Mixing
5. Presence of alternative electron acceptors (SO_4^{2-}, NO_3^-, etc.)

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Environmental factors affect ...

- Specific growth rate
- Decay rate
- Gas production rate
- Substrate utilization rate
- Start-up
- Response to change in input

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1. Nutrients

- Nutrients supply the basic *cellular building blocks* for growth and ensure the cell is able to *synthesize the enzymes and cofactors* that drive the biochemical and metabolic reactions.
- According to the relative quantities required by the cell, nutrients can be divided into two groups;
  - Macro-nutrients
  - Micro-nutrients

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Macronutrients

- Both macro- and micronutrients have to be present in an available form in the growth environment to allow effective uptake.
- Ideally, nutrient levels should be in excess of the optimum concentrations required.
- Because anaerobic bacteria can be severely inhibited by even slight nutrient deficiencies.
- However, many essential nutrients can become toxic when present in high concentrations.

Macronutrients: Nitrogen

- A rough estimate of the theoretical amount of macro-nutrients (N, P and S) can be derived from elemental composition of bacterial cells within anaerobic sludge.
- Emprical formula of biomass: $C_5H_7O_2N$ then;

  3 - 6 kg N /1000 kg of COD consumed or

  0.5 -10 kg N /60 m^3 of CH_4 produced
- COD:N ratio ~ 350:7 (High organic loading)
- COD:N ratio ~ 1000:7 (Low organic loading)
- COD:N ratio ~ 200:5 (Recommended)

Macronutrients: Nitrogen

- Most common nitrogen forms; ammonia (NH_3), nitrate (NO_3^-), nitrite (NO_2^-), nitrogen gas (N_2).
- NH_3 is the most readily utilized inorganic forms of nitrogen, existing in the *reduced state* that is required for anabolic metabolism and an uncharged state that facilitates *cellular uptake*.

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Macronutrients: Phosphorus

- N:P ratio ~ 7:1 (Recommended)
- Then COD/N/P ratio ~ 300:7:1
- The usual forms of 'P' in aqueous solution include orthophosphate, polyphosphate & organic phosphate.
- The orthophosphates are immediately available for biological metabolism without further modification.
- Organic phosphates must generally be hydrolysed by the cell to release inorganic phosphate before use.

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Macronutrients: Sulfur

- In addition to N and P, the sulfur (S) requirement of anaerobic bacteria should also be satisfied and this can be supplied as sulfur, sulfide, sulfite, thiosulfate, sulfate or amino acids (cysteine and methionine).
- Optimum anaerobic digester concentrations of S have been reported between 0.001 and 1.0 mg/l.

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Composition of methanogens

Typical elemental composition (mg/l) of methanogens

C	370 000	–	440 000
H	55 000	–	65 000
N	95 000	–	128 000
Na	3000	–	40 000
K	1300	–	50 000
S	5600	–	12 000
P	5000	–	28 000
Ca	85	–	4500
Mg	900	–	5300
Fe	700	–	2800
Ni	65	–	180
Co	10	–	120
Mo	10	–	70
Zn	50	–	630
Cu	<10	–	160
Mn	<5	–	25

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Micronutrients

Nutrient	Concentration required (mg/l)	Effects on digestion
Ca	100-200	Granulation and increase in activity
Mg	75-150	Granulation and increase in activity
Na	100-200	Increase in activity
Fe	20-100	Increase in activity and precipitation of sulphide
K	200-400	Increase in activity
Ba	0.01-0.1	Divalent cation effect hence good granulation
Co	20	Vitamin B12 dependent
W	-	Formate dehydrogenase
Se	0.8	Formate dehydrogenase, glycine reductase, hydroxylase, and dehydrogenase dependent
SO_4^{2-}	0.1-10	Sulfur source of cell synthesis

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2. Temperature

- The most influential environmental factors as it controls the activity of all microorganisms.
- A *rise* in *temperature* leads to an increase in the rate of biochemical and enzymatic reactions within cells, causing *increased growth rates*.
- *Above* a *specific temperature*, which is characteristic of each species, this phenomenon gives way to *inhibition* and then *mortality*, as the *proteins* and *structural components* of the cell become *irreversibly denatured*.

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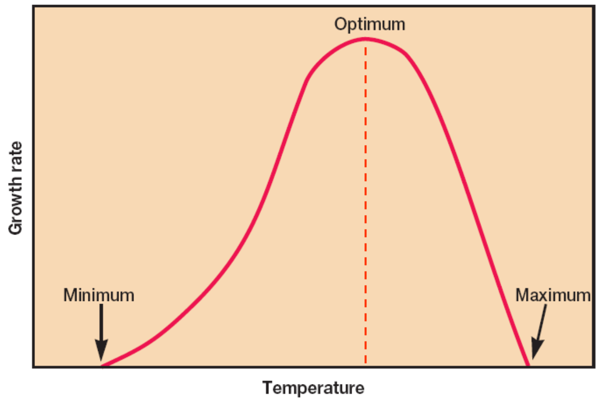
Temperature and Growth



The effect of temperature on growth rate

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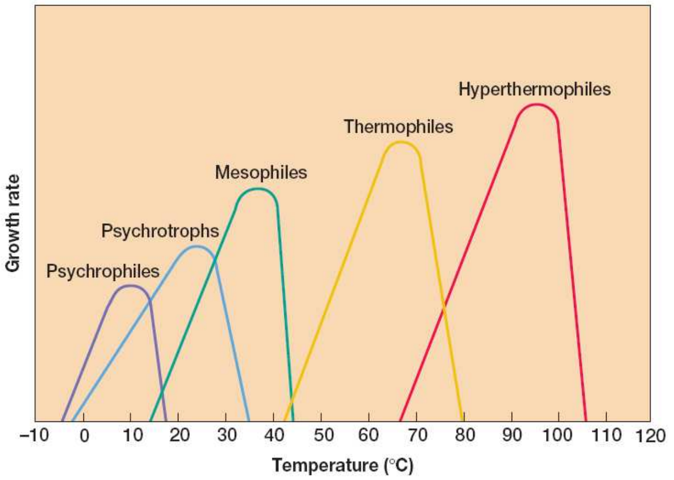
Temperature ranges for growth



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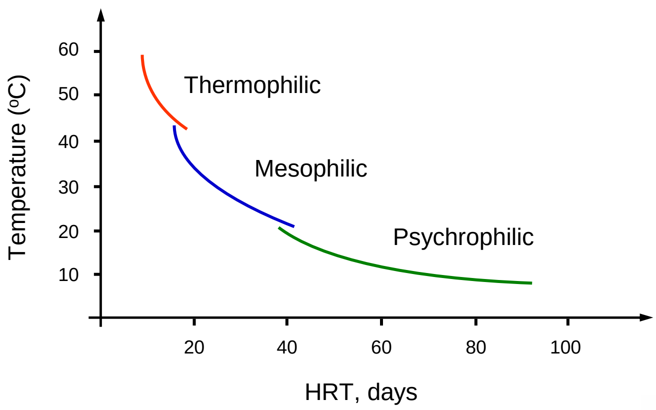
Temperature ranges for AD

- Psychrophilic AD is favoured by organisms having a temperature optimum at 15-20ºC. Although it is not as efficient as mesophilic and thermophilic AD, it has desirable economic trade-offs in temperate climates.
- Mesophilic AD with a temperature optimum at 30-37ºC is the most commonly employed in engineered processes of anaerobic treatment.
- Thermophilic AD with an optimum at 55-60ºC is more efficient than mesophilic AD.

Mesophilic vs Thermophilic

- Thermophilic reactors can accept higher organic loading rates and produce lower quantities of sludge.
- Mesophilic reactors are often more stable.
- Thermophilic reactors require more energy to heat the reactor
- Thermophilic reactors produce high concentrations of VFA in their effluent.
- Thermophilic AD is an attractive option for treating warm industrial effluents and slurries of relatively constant composition.

Anaerobic Sludge Digestion



Influence of temperature

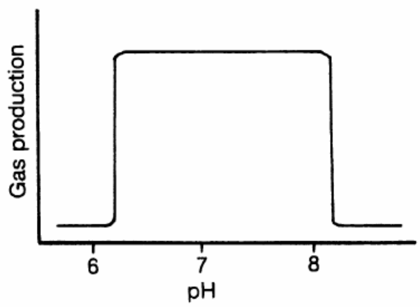
- Compared to aerobic processes which are relatively robust to temp. variations, AD is sensitive to sudden temperature fluctuations
- Temp. changes as small as 1-2ºC have significant adverse effects on process performance particularly when changes occur rapidly (<2 hrs).
- If bacteria become adversely affected by temperature variations, several days or even weeks may be required to restore a healthy population once again.

3. pH-Buffer capacity

- For an efficient methanogenic digestion a suitable and stable pH has to be maintained within the digester.
- pH has critical influences on;
  - Microorganisms (esp. Methanogens) responsible for AD
  - Biochemistry of AD process
  - Alkalinity buffering and
  - Chemical reactions affecting the solubility and availability of dissolved ions.

Effect of pH

- Best pH range appears to be around neutrality, while 6.5-8.0 is generally believed to be optimal.



The effect of pH on biogas production.

Reactions affecting pH

- Four major chemical and biochemical reactions that influence the pH of an AD are:
  - Ammonia (NH_3) consumption and release
  - Volatile fatty acid production and consumption
  - Sulfide (S^{2-}) release by dissimilatory reduction of sulfate (SO_4^{2-}) or sulfite (SO_3^{2-})
  - Conversion of neutral carbonaceous organic carbon to methane (CH_4) and carbon dioxide (CO_2)

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Controlling the pH

- In AD, pH reduction can be countered by formation of bicarbonate alkalinity and consumption of VFAs by methanogens.
- Consumption of VFAs is dependent on the equilibrium between rapid growing acidogens and slow growing methanogens.
- This equilibrium can be easily upset by changes in the operational or environmental conditions.

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Controlling the pH

- If the balance is disturbed and therefore pH starts to drop because of VFAs accumulation.
  - Stop feeding the reactor to give the methanogens sufficient time to consume excess VFAs and raise the pH value to an acceptable level. OR
  - Dose the reactor with alkali, i.e. NaOH, Na_2CO_3 or $NaHCO_3$ in order to raise the pH or provide additional buffering capacity.
- In some cases both options are used simultaneously.

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Controlling the pH

- The amount of alkalinity required to accommodate VFA increases in AD depends on many factors.
- Well- established anaerobic reactors treating typical organic loads are likely to contain alkalinity in the range 2000 to 3000 mg/l as $CaCO_3$.
- This level of alkalinity will impart an improved resistance to acidification caused by short-term fluctuations in feed composition.

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4. Mixing

- AD comprises an inherent degree of mixing from the continuous rise of biogas bubbles within the reactor,
- This mixing is not sufficient for efficient mass transfer.
- Mixing enhances the AD process by distributing microorganisms, substrate, and nutrients throughout the digester as well as equalizing temperature.
- Mixing also provides for rapid hydrolysis of wastes by allowing the hydrolytic bacteria to attack a much larger surface area.

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Types of Mixing

- The level and type of mixing also affects;
  - Growth rate and distribution of bacteria within the sludge
  - Substrate availability and utilization rates
  - Granule formation and
  - Biogas production.
- Mixing can be enhanced using;
  - Mechanical devices (paddles, turbines and propellers)
  - Hydraulic shear force (feed recycle)
  - Biogas recirculation

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Advantages of Mixing

- Eliminating or reducing scum build-up
- Eliminating thermal stratification or localized pockets of depressed temperature
- Maintaining digester sludge chemical and physical uniformity throughout the tank
- Rapid dispersion of metabolic wastes (products) produced during substrate digestion
- Rapid dispersion of any toxic materials entering the tank (minimizing toxicity)
- Prevent deposition of grit

Excessive Mixing

- Excessive mixing may lead to a reduction in reactor performance by disrupting the flocs and granules containing active anaerobic microorganisms.
- Result in short-circuiting of the reactor, leading to unconverted substrate appearing in the reactor effluent.
- Lead to the formation of smaller flocs, which have poor settling characteristics.
- The kind of mixing equipment and amount of mixing varies with the type of reactor and the solids content in the digester.

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5. Presence of alternative electron acceptors

- Under anaerobic conditions sulfate (SO_4^{2-}) and sulfite (SO_3^{2-}) is reduced to sulfide (S^{2-}) by SRB.
- The SRB utilize electron-donating substrates present in wastewater for the reduction of sulfate.
- The substrates are either partially oxidized to acetate or fully oxidized to CO_2.
- Sulfate behaves as an alternative electron acceptor to support anaerobic respiration.
- Sulfate redution lower the CH_4 yield per kg organic waste
- Biogas treatment is required to remove corrosive H_2S

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5. Presence of alternative electron acceptors

- Denitrification is an anoxic process in which either an organic or inorganic electron-donating substrates are oxidized at the expense of reducing nitrate (NO_3^-) or nitrite (NO_2^-) to dinitrogen gas (N_2).
- Denitrifiers have the ability to utilize a variety of fermentative/methanogenic substrates therefore these microorganisms compete for the same substrate(s) such as glucose, VFAs and H_2.
- Propionate is the most preferred VFAs as carbon source by denitrifiers.

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