

Biochemistry Fundamentals

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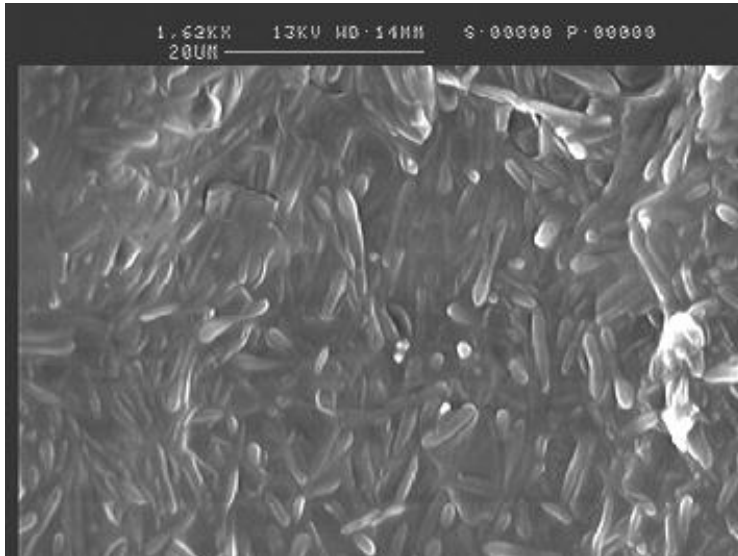
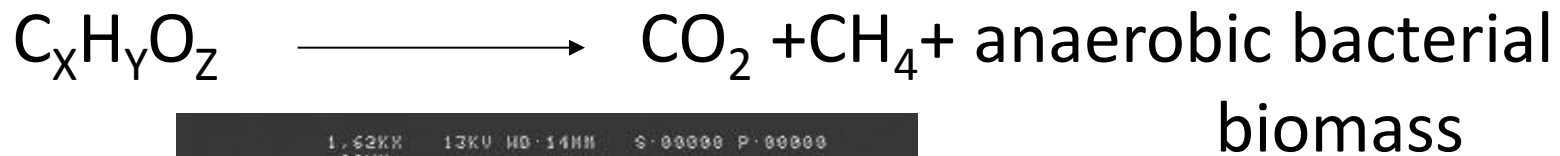


**Inaugural Bio-Methane Regions Event
Training the Trainers**

26-27th May 2011 - University of Glamorgan, South Wales

Overall Aim of the Anaerobic Digestion Process

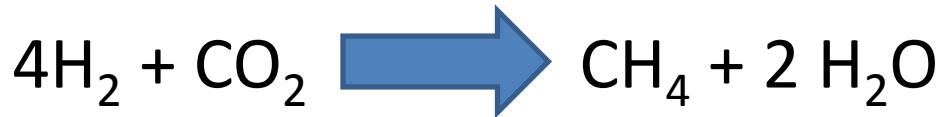
Anaerobic digestion is the conversion of organic material in the absence of oxygen to methane and carbon dioxide by a microbial consortia.



How Do We Get Methane?

Need Methanogens!

Either Hydrogen (Lithotrophic methanogens)



AND/OR

Acetate (acetoclastic methanogens)



BUT have a limited range of substrates

Can also include carbon monoxide, formate, methanol

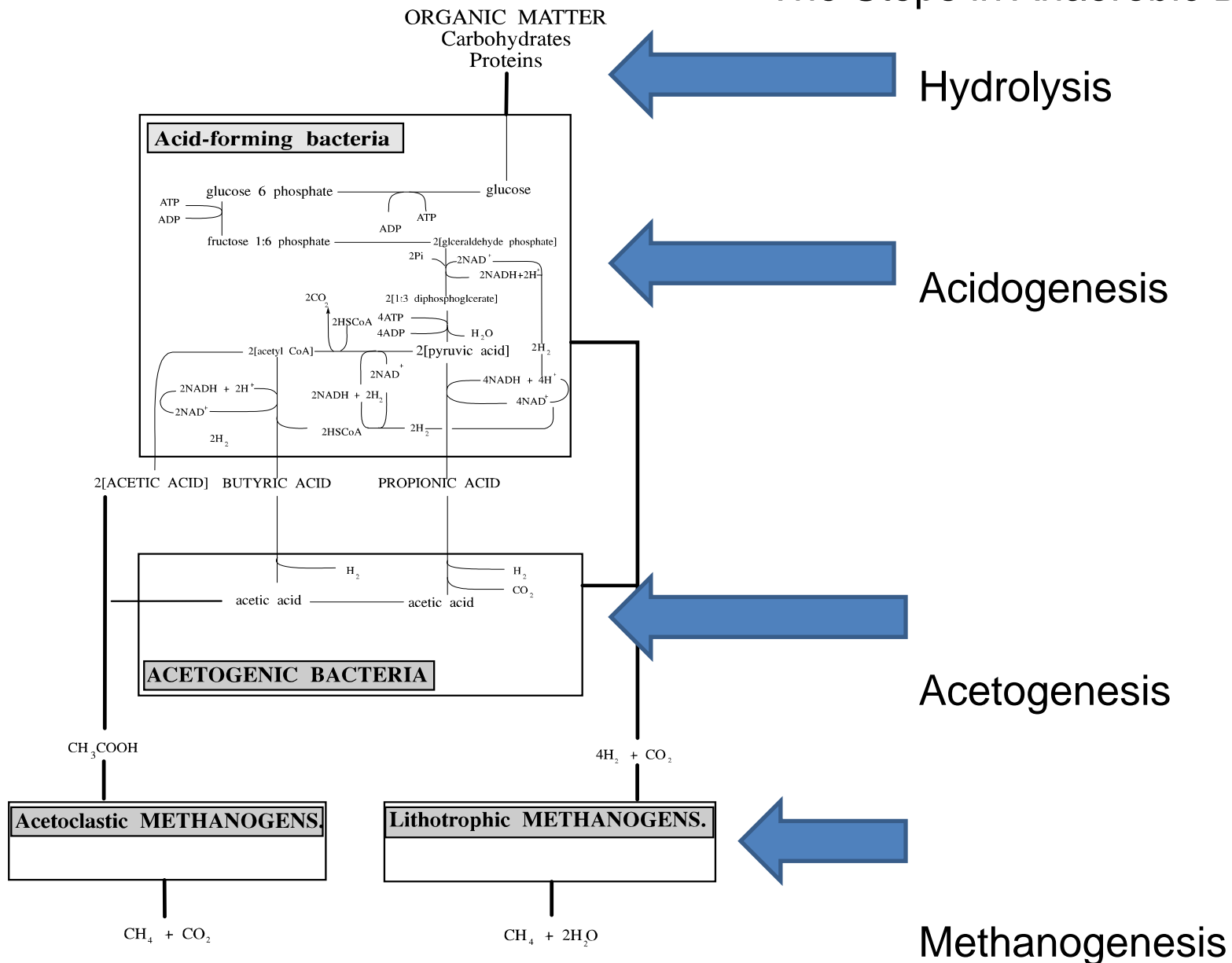
The Anaerobic Digestion Microbial Consortia

- The production of biogas is dependant on the successful interaction of interdependent microbial species.
- The production of methane is a property of a (relatively) limited number of micro-organism species, the “methanogens”.
- There is a limited number of substrates that the “methanogens” can use to produce methane.

The Stages in Anaerobic Digestion

- Hydrolysis
 - Long chain polymers broken down to smaller molecules
- Acidogenesis
 - Production of hydrogen and volatile fatty acids
- Acetogenesis
 - alcohols, >C₂ VFAs converted to acetate and hydrogen
- Methanogenesis
 - Hydrogen and acetate converted to methane

The Steps in Anaerobic Digestion



Taken from Guwy, (1996). Modified from Mosey, (1983)

Factors Effecting the Rate of Hydrolysis

- Particle size of the waste, smaller is better.
- Accessibility of the substrate i.e. how easy is it for the enzymes to attack the substrate
 - Problems with lipids
- Residence time in the reactor.
- Chemical structure of substrate
 - Negative impact of lignin and hydrocarbons
- Organic content of the substrate.

Acidogenesis

- The sugars, amino acids and longer chain fatty acids are then fermented to acetate, propionate, butyrate, valerate, ethanol, lactate, hydrogen, CO₂, ammonia and sulphide by the acidogenic bacteria.
- The proportion of the organic products of the acidogenic bacteria is determined by the H₂ concentration and pH.

Acetogenesis

- The acetogens (obligate hydrogen producing acetogenic bacteria) convert the fermentation products which the methanogens cannot use (alcohols, $>C_2$ VFAs, aromatic compounds) to the substrates which the methanogens can utilise.
- The Gibbs free energies for the conversion of ethanol, propionic and butyric to acetate and hydrogen are energetically unfavourable i.e. positive at standard free biochemical energy levels (pH 7.0, 1 atm.).

Why are Propionic and Butyric Acids bad?

- Don't smell very nice !
- Wasted energy as methanogens can't use them
- Very difficult to get rid of.



NOT beneficial for the microorganisms as

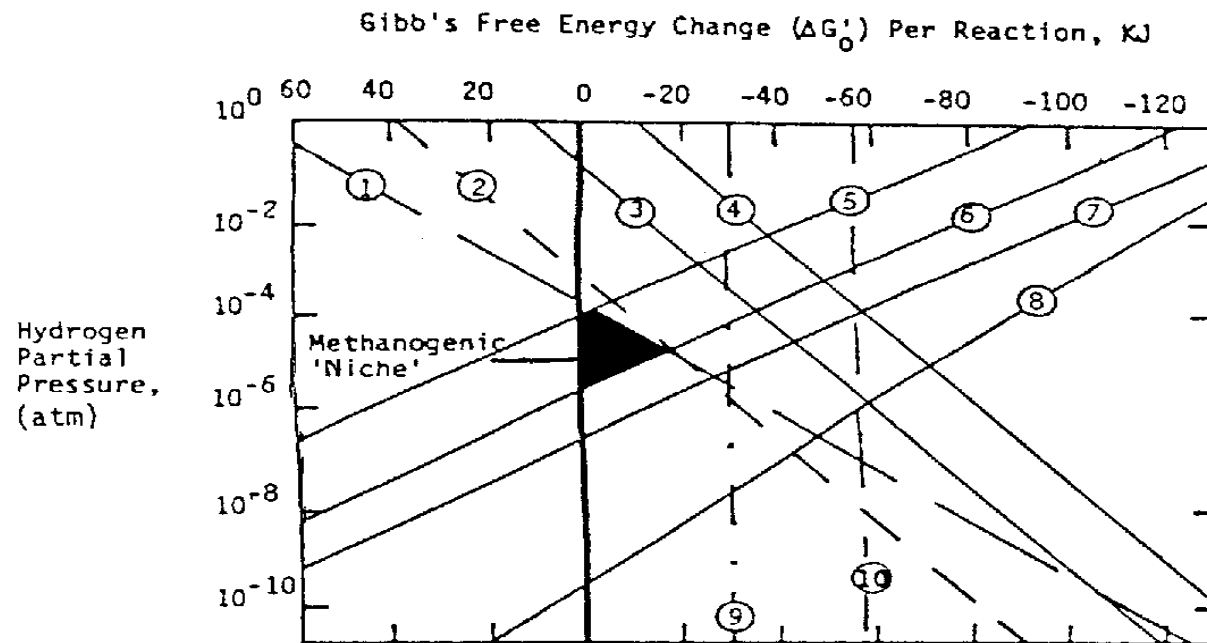
$$G_0 = + 76.1.9 \text{ KJ mol}^{-1}$$

Energy could be required to be put in to use the propionic acids.

Role of Interspecies Hydrogen Transfer

- The H_2 partial pressure must be maintained at between 10^{-6} - 10^{-4} atmospheres for sufficient energy for growth to be obtained from propionic and butyric acids.
- As H_2 is continually being generated it must be continually be removed for methanogenesis to occur.
- The removal of H_2 is achieved by conversion to methane by the hydrogen utilising methanogens.
- This symbiosis is known as interspecies hydrogen transfer and is crucial to the success of the anaerobic digestion process.

Methanogenic “Hydrogen” Niche

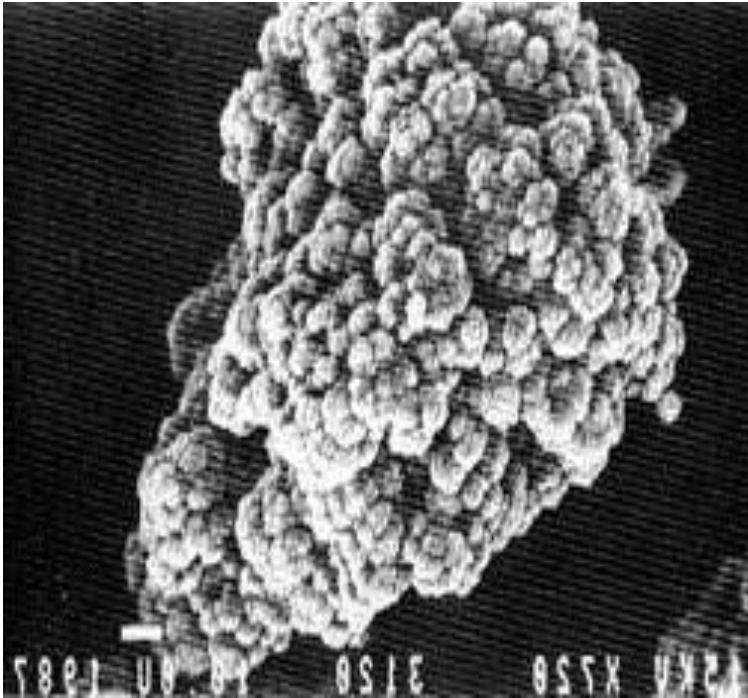


Graphical representation of the hydrogen-dependant thermodynamic favourability of acetogenic oxidations and inorganic respirations associated with the anaerobic degradation of waste organics. (1) Propionic oxidation to acetic acid, (2) butyric oxidation to acetic, (3) ethanol to acetic, (4) lactic to acetic, (5) acetogenic respiration of bicarbonate, (6) methanogenic respiration of bicarbonate, (7) respiration of sulphate to sulphide, (8) respiration of sulphite to sulphide, (9) methanogenic cleavage of acetic acid, (10) SRB-mediated cleavage of acetic acid. (From Harper and Pohland, 1985).

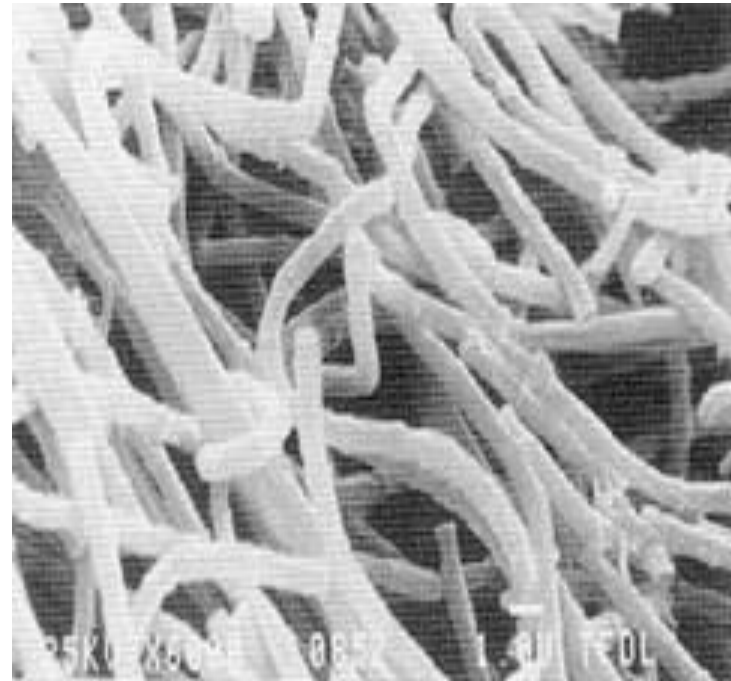
The Methanogens

- A group of gram –ve bacteria
- Belong to the group of bacteria termed the Archaea or archaeobacteria.
- Are strict anaerobes they evolved billions (3.5 billion) years ago when the earth had very low or zero levels of oxygen.
- Utilise simple inorganic substrates such as H_2 , CO_2 or simple organic substrates such as acetate, formate and methanol

The Archaea



Scanning electron micrograph of a cocci-shaped archaea from the genus *Methanosarcina*. Members of the genus *Methanosarcina* can use all 3 routes to methane.



Scanning electron micrograph of archaea from the genus *Methanosaeta*. Members of the genus *Methanosaeta* are acetotrophic, i.e. they produce methane from acetate.

Specific Growth Conditions

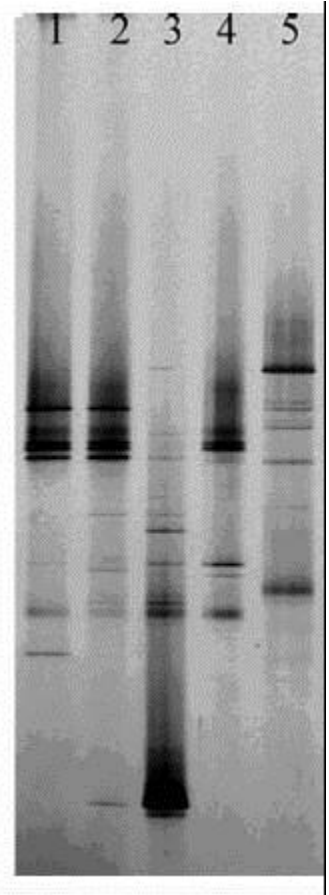
These micro-organisms have been difficult to culture *in vitro* as in nature they are dependant on other bacteria to provide a suitable environment for their growth.

The fermentative bacteria provide a environment with a;

- low redox potential (-330 mV)
- extremely low in O₂
- suitable range of substrates

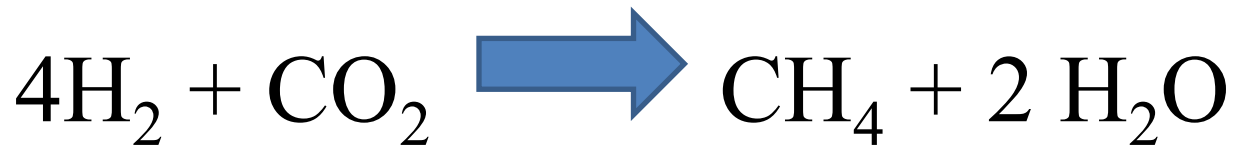
Analysing The Consortia

- Traditional microbiology
 - “petri” dish technology (some problems here)
 - Enzymatic profiles
- Molecular Biology Techniques
 - PCR/RT PCR
 - DGGE
 - FISH
 - Genomics and Proteomics
 - Microarrays



Limited Range of Substrates

Hydrogen (Lithotrophic methanogens)



Very beneficial for the microorganisms as $G_0 = -138.9 \text{ KJ mol}^{-1}$ is gained.

But only 30% of methane produced via this route.

Limited Range of Substrates

Acetate (acetoclastic methanogens)



Not so beneficial for the microorganisms as only $G_0 = -32 \text{ KJ mol}^{-1}$ is gained.

But 70% of methane produced via this route.

Other Anaerobic Processes

- Unfortunately other gases are produced as well as methane and CO_2 .
- These include hydrogen sulphide (H_2S) and ammonia (NH_3)
- Hydrogen Sulphide is produced by a competing group of bacteria to the methanogens called the sulphate reducing bacteria.

What Effects the % and amount of Methane in the Biogas

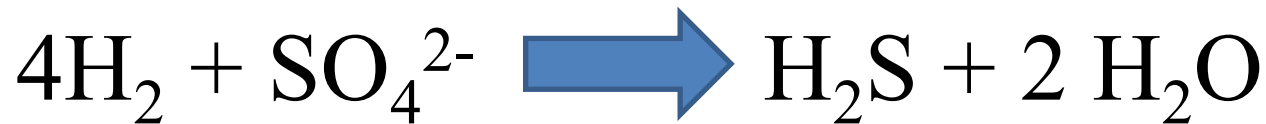
- Relative solubility of the gases
- Is alkalinity being destroyed?
- Biodegradability of substrate
- Chemical composition of biodegradable substrate
 - Buswell equation

Sulphate Reducing Bacteria (SRBs)

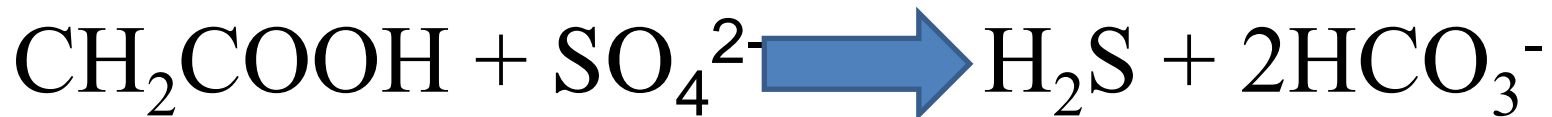
Are a problem for methanogenesis as:

- Compete with methanogens for their source of energy (hydrogen and acetate).
- Can become inhibitory (cellular toxin) at dissolved levels of 50 mg/l.
- More of the dissolved form at lower pH.

Competitive Nature of SRBs



Very beneficial for the micro-organisms as $G_0 = -154 \text{ KJ mol}^{-1}$ is gained.



Not so beneficial for the micro-organisms as only $G_0 = -43 \text{ KJ mol}^{-1}$ is gained BUT more than the methanogens

Keeping “The Workers” Happy

The bacterial consortium requires a number of factors to be controlled to maintain performance. These include:

- Temperature
- pH (related to buffering capacity)
- Essential Nutrients
- Avoid toxic compounds
- Sufficient residence time to reproduce

The Effect of Temperature

- Three temperature optima have been reported for the anaerobic digestion process psychrophilic (around 15°C), mesophilic (around 35°C) and thermophilic (around 55°C) temperatures.
- Methanogenesis has been found to occur up to 75°C but the optimum temperature is thought to be 55-60°C.
- The advantage of the higher temperature ranges is that the process will proceed at a faster rate than the lower temperature ranges as stated by the Arrhenius equation.

The Effect of pH

- Different groups in the anaerobic consortia can cope with different pH levels
 - Methanogens prefer pH 6.5- 7.5
 - Acidogens also prefer pH 6.5-7.5 BUT can cope with pH 5.2

Therefore if pH is lowered methane production will slow and consumption of VFA reduce while VFA production continues. Spiral of Decline.

- Effects methane production but also distribution of volatile fatty acids

Bicarbonate Alkalinity and pH

- pH Scale is the \log_{10} of the H^+ concentration.
- Bicarbonate alkalinity is a measure of the H^+ buffering capacity of the reactor. Measured in $mg/l\ CaCO_3$
 - If you have a alkalinity buffer then difference is between walking a tight rope and a plank when operating the digester.
 - If alkalinity is decreasing may be a sign of trouble before pH drops below critical level.

Nutrient Requirements

- The anaerobic consortia need nutrients to grow their cells and drive their enzymes and metabolic processes.
- Can be divided up into macro and micro nutrients.
- Need to be “available”

Macro nutrients

- Relatively large quantities
 - C/N/P ratios
 - Also need sulphur!

C	N	P	S
500-1000	15-20	5	3

Micronutrients (Trace Elements)

Needed in much lower quantities (g per m³)

Required for enzyme activity

Includes :

Ni, Fe, Co, Se, Mg etc.

Toxic or Inhibitory Compounds

- Divided into two groups:
 - Too much of a good thing
 - Ammonia
 - Sulphate
 - Ca/Na/K
 - Should not be here at all
 - Cleaning compounds
 - Antimicrobials
 - Solvents
 - Heavy metals

Ammonia Toxicity

- Ammonia (NH_3) and ammonium (NH_4^+) is required to provide N for growth.
- Too much of a good thing e.g. abattoir wastes.
- Dependant on pH, Ammonia (NH_3) is toxic and is produced from ammonium (NH_4^+) as pH increases.

ammonia inhibitory at 80 mg/l

ammonium inhibitory at 1500 mg/l

Na/Ca/K

- Cation inhibition
- Useful as used to provide pH and alkalinity control and nutrients
- Ca also contributes to precipitation/scale
- Inhibitory levels
 - Na 5000 mg/l to 30,000 mg/l
 - K 2,500 mg/l to 5000 mg/l
 - Ca 2,500 mg/l to 7,000 mg/l

Avoiding Overloading

If significant “overworking” of the bacteria can lead to stress and eventual failure..

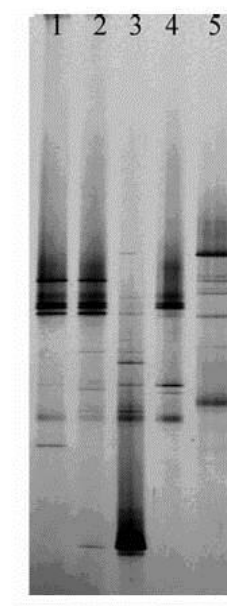
Can be avoided by using rules on hydraulic retention time (HRT) AND organic load (OLR).

Conclusions

- The anaerobic digestion process depends on the effective working of a complex interaction of microorganisms.
- It has been working successfully for 3.5 billion years.
- However there a wide number of ways which we can make it not work very well.
- It can be breaking down and still seem to be working

Thank You

Questions



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