ANAEROBIC PROCESSES

1. General Nature of Anaerobic Operations
   a. H_____ of insoluble organic matter (Reaction 1)
      - carried out by hydrolytic (and other) extracellular enzymes (hydrolases, cellulases, amylases, and proteases)
      - extracellular enzymes are produced by the f_____ bacteria
      - large soluble molecules are cleaved to enable t_____ into cell

   Step 1 - Hydrolysis Reactions
   - breakdown of particulate and high molecular weight organics into soluble organics and long chain volatile fatty acids.

   b. A_____ - acid forming phase (facultative microbes can withstand oxygen)
      i. f_____ reactions (Reaction 2) in which organic compounds serve as both e_____ a_____ and donors
         - principal products are intermediary degradative products such as p_____ and butyric acids, and methane precursors, a_____ acid and molecular hydrogen, H_2.
         - H_2 production originates from the dehydrogenation of p_____ and is not the primary producer of H_2 in anaerobic processes.

      Step 2 - Acid Forming Phase
      - fermentative reactions produce short chain volatile fatty acids (VFA's), acetic acid, and some H_2.
      - the breakdown of fatty acids during anaerobic oxidation results in most of the H_2 produced.

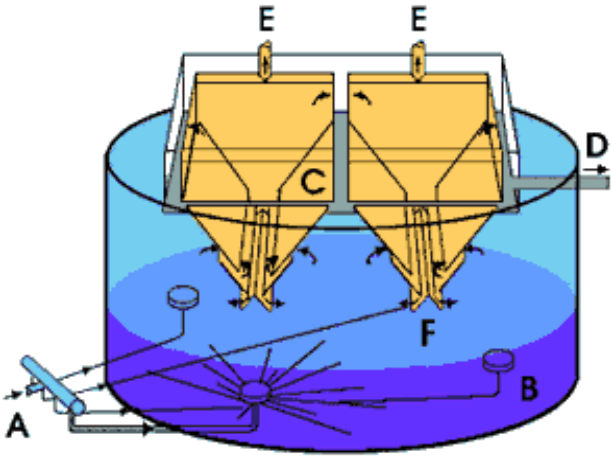
      ii. o_____ of volatile and long chain fatty acids (Reactions 3 & 4) produces most of the H_2 through the transfer of electrons from NADH to hydrogen ions. This has been termed anaerobic o_____ and is inhibited by high partial pressures of ____ (whereas production of H_2 from pyruvate is not).
         - production of H_2 is significant because it represents the major loss of COD from anaerobic treatment systems (i.e., COD leaves the system as a gas)
         - H_2 can be combined with CO_2 by hydrogen o_____ a_____ to form acetic acid (Reaction 5), but this a relatively insignificant reaction.

      Step 3 - Methane Forming Phase
      - aceticlastic methanogens split acetic acid into methane and CO_2.
      - hydrogen oxidizing methanogens combine hydrogen and CO_2 to produce methane.

      iii. m_____ - methane forming phase (strict anaerobes)
         products of a_____ are acetic acid and H_2 which are used by m_____ bacteria to produce methane and CO_2.
         - a_____ methanogens split acetic acid into methane and CO_2 (Rxn 6):
           $H_3CCOOH \rightarrow CH_4 + CO_2$
         - H_2 o_____ methanogens oxidize hydrogen and reduce CO_2 to produce m_____ (Reaction 7):
           $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$

Intermediates

- v_____ f_____ a_____ - measure of acids to alkalinity is a useful process indicator (should be < 0.1 for mesophilic systems and < 0.4 - 0.5 for thermophilic systems)
- a_____ - should be very ;low for a well functioning anaerobic process
  – about 70% of CH_4 is from acetate (aceticlastic methanogens)
  – both acetate and propionate follow A_____ kinetics
- p_____ - may account for about 20% of CH_4 produced
  – broken down to acetate and H_2
  – low propionate levels are indicative of a well functioning process
  – H_2 will inhibit propionate conversion (product inhibition)
  – acetate will inhibit propionate conversion
  – s_____ considerations: for low H_2 concentrations need hydrogen oxidizing methanogens in close proximity to hydrogen producers
  – propionate is inhibitory at high concentrations (5,000 - 8,000 mg/L)
  – u_____ form most inhibitory
- H_____ , H_2
  – very insoluble H= $8 \cdot 10^{-4}$M/atm (1.4 mg/L @ 35 deg C)
  – if need <100 ppm H_2 and average d_____ between bacteria is 100µ there will be less than 1 molecule of H_2 between every two bacteria
  – much of H_2 f_____ can not be measured due to interspecies hydrogen transfer
  – spatial considerations have been overcome by systems with dense biofilms and granules
  – hydrogen may not be in equilibrium w/ bulk liquid or gas phase
  – difficult to measure concentration and flux
  – may be useful as a p_____ indicator



- Anaerobic toxicity assay
  – use serum bottles, place e_____ substrate (e.g., acetate or ethanol) and biomass
  – measure gas production as a function of initial substrate concentration
  – can calculate EC_{50} : concentration that causes 50% inhibition