Chapter 13.
Anaerobic Treatment by Methanogenesis

All the figures and tables in this material are from the reference below unless specified otherwise.
Reference: Bruce E. Rittmann and Perry L. McCarty, "Environmental Biotechnology: Principles and Applications", McGraw-Hill, 2001.

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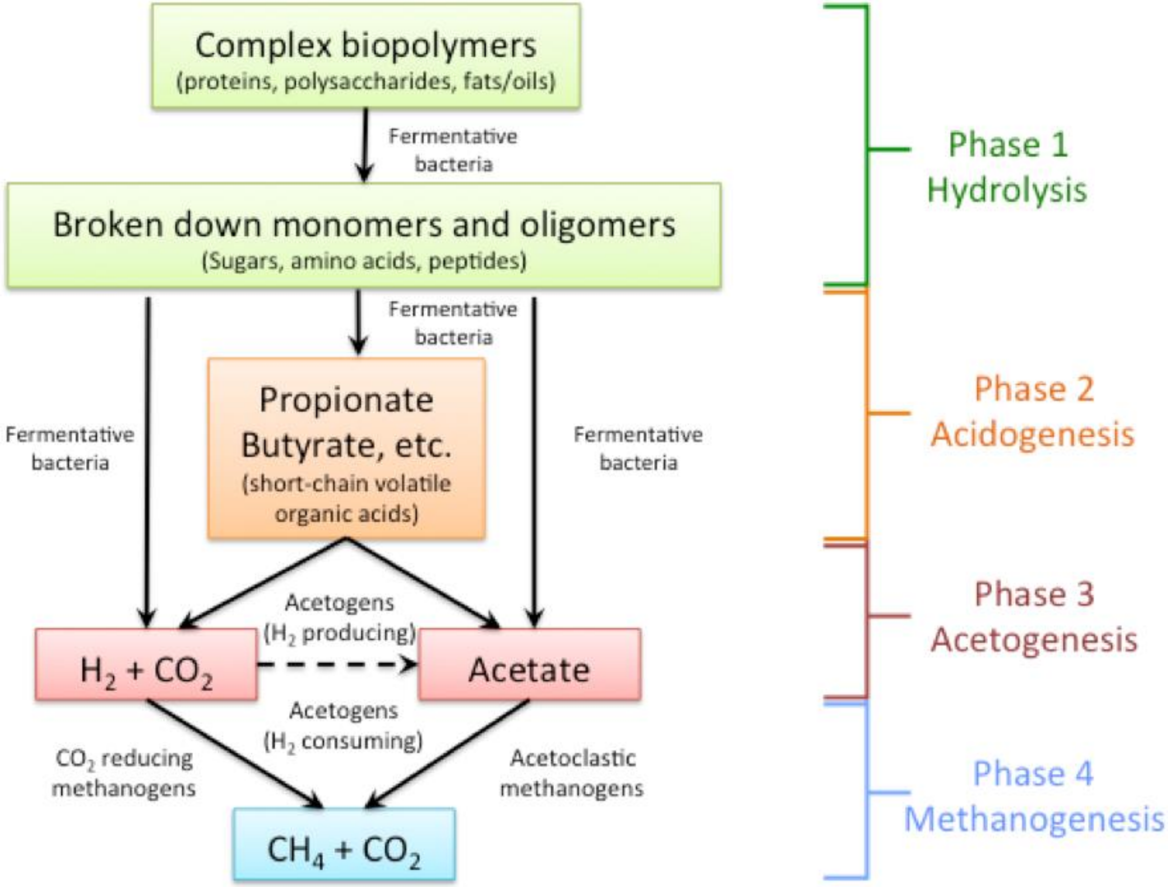
Intro: Anaerobic Digestion

√ Anaerobic digestion

- Anaerobic digestion refers to the process in which microorganisms break down biodegradable substrates under anaerobic conditions, producing biogas



Complex biopolymers
(proteins, polysaccharides, fats/oils)

Fermentative bacteria

Broken down monomers and oligomers
(Sugars, amino acids, peptides)

Fermentative bacteria

Fermentative bacteria

Fermentative bacteria

Propionate
Butyrate, etc.
(short-chain volatile organic acids)

Acetogens
(H_2 producing)

$H_2 + CO_2$

Acetate

Acetogens
(H_2 consuming)

CO_2 reducing methanogens

Acetoclastic methanogens

$CH_4 + CO_2$

Phase 1
Hydrolysis

Phase 2
Acidogenesis

Phase 3
Acetogenesis

Phase 4
Methanogenesis

Source: BEEMS Module B7 - Anaerobic Digestion

Intro: Methanogenesis

√ Methanogenesis

- **Methanogenesis refers to an anaerobic process in which the electron equivalents in organic matter (BOD_L) are used to reduce carbon to CH_4.**

- CH_4 has 8 electron equivalents (64 g BOD_L or COD)

  $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$

- Methanogenesis takes place by methanogens, the unique group of Archaea that produces methane

- Electron donor : BOD_L or H_2

  Electron acceptor : BOD_L (fermentation) or CO_2

- BOD_L can be used as both an e^--donor and an e^--acceptor (partially oxidized and reduced).

Intro: Methanogenesis

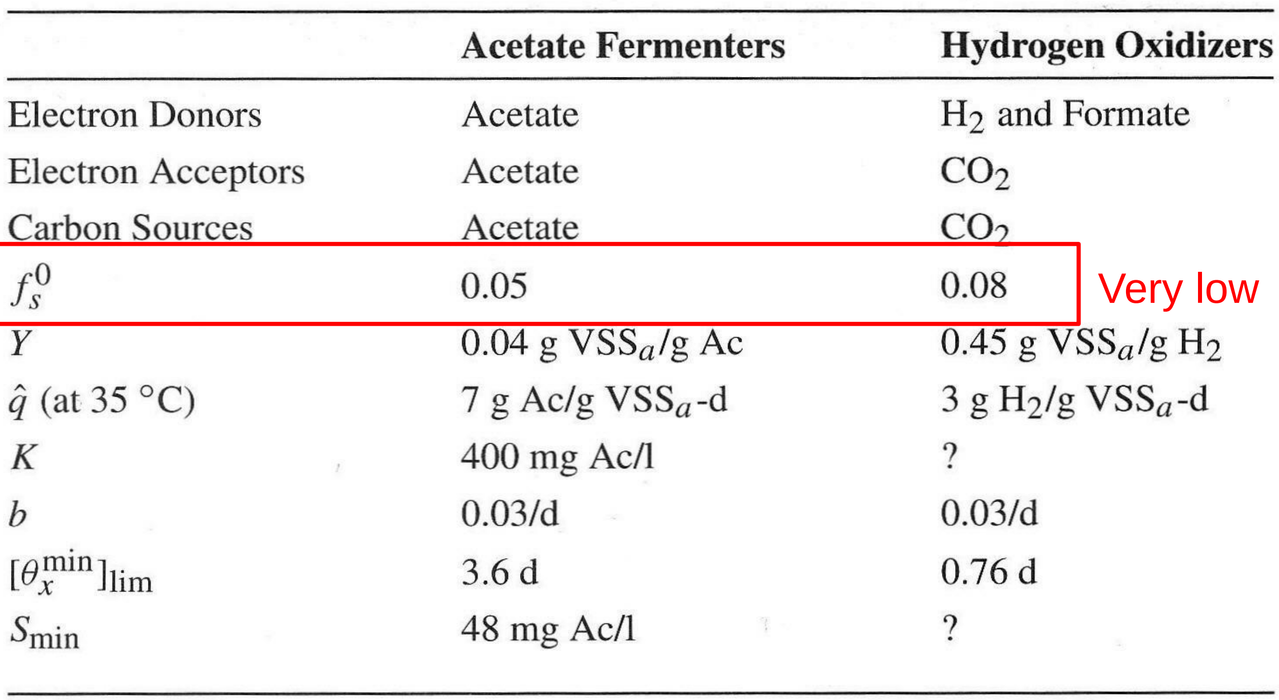
√ Methanogens

- Acetate Fermenters ; $CH_3COOH \rightarrow CH_4 + CO_2$

  Hydrogen Oxidizers: $4\ H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ (*e^- acceptor*; *CO_2*)

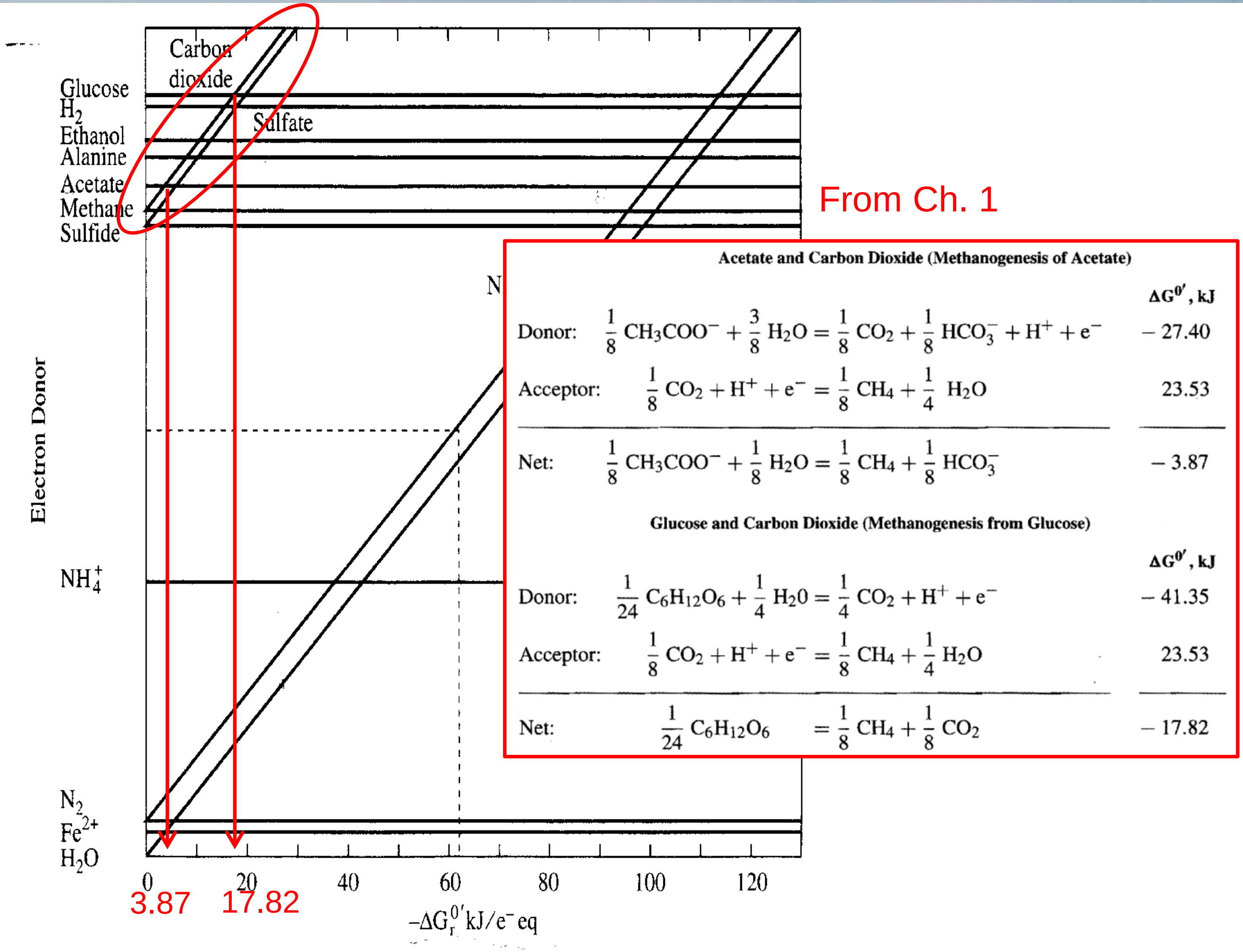
- Methanogens are slow-growing microorganisms.

- All the e- originally present in the input BOD must be ultimately be funneled into acetate, H_2, or formate.



	Acetate Fermenters	**Hydrogen Oxidizers**
Electron Donors	Acetate	H_2 and Formate
Electron Acceptors	Acetate	CO_2
Carbon Sources	Acetate	CO_2
f_s^0	0.05	0.08
Y	0.04 g VSS_a/g Ac	0.45 g VSS_a/g H_2
$\hat{q}$ (at 35 °C)	7 g Ac/g VSS_a-d	3 g H_2/g VSS_a-d
K	400 mg Ac/l	?
b	0.03/d	0.03/d
$[\theta_x^{min}]_{lim}$	3.6 d	0.76 d
S_{min}	48 mg Ac/l	?

Very low



From Ch. 1

Acetate and Carbon Dioxide (Methanogenesis of Acetate)

		$\Delta G^{0'}$, kJ
Donor:	$\frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O = \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^-$	– 27.40
Acceptor:	$\frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	23.53
Net:	$\frac{1}{8}CH_3COO^- + \frac{1}{8}H_2O = \frac{1}{8}CH_4 + \frac{1}{8}HCO_3^-$	– 3.87

Glucose and Carbon Dioxide (Methanogenesis from Glucose)

		$\Delta G^{0'}$, kJ
Donor:	$\frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O = \frac{1}{4}CO_2 + H^+ + e^-$	– 41.35
Acceptor:	$\frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	23.53
Net:	$\frac{1}{24}C_6H_{12}O_6 = \frac{1}{8}CH_4 + \frac{1}{8}CO_2$	– 17.82

Figure 2.2 Relationship between various electron donors and acceptors and resulting reaction free energy.

13.1 Uses for Methanogenic Treatment

√ Applications

- **Digestion and stabilization of sludge and other residues**
- **Treatment of high strength industrial wastewaters**
  - Food processing, pulp & paper, leachate, etc.
  - Especially useful with more concentrated wastewaters with COD > 5,000 mg/L.
- **Treatment of more dilute wastewaters (domestic sewage).**
  - It is gaining popularity particularly where the climate is warm for most of years.

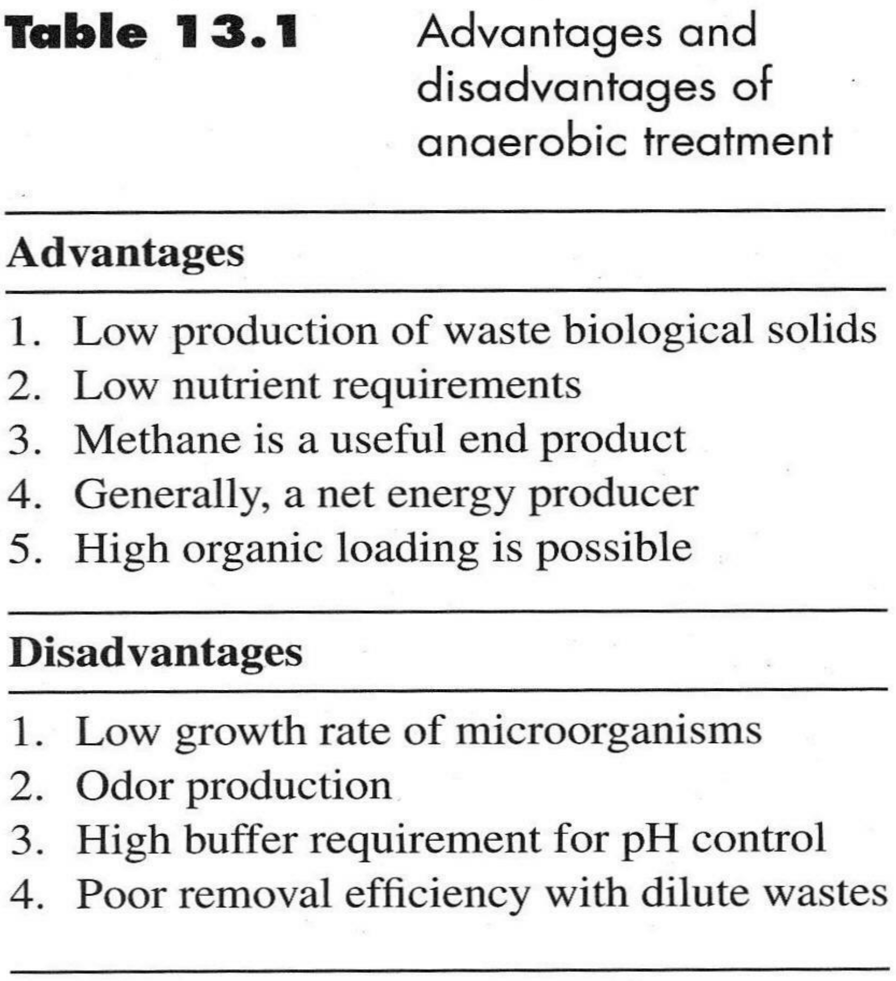


Table 13.1 Advantages and disadvantages of anaerobic treatment

Advantages
1. Low production of waste biological solids
2. Low nutrient requirements
3. Methane is a useful end product
4. Generally, a net energy producer
5. High organic loading is possible
Disadvantages
1. Low growth rate of microorganisms
2. Odor production
3. High buffer requirement for pH control
4. Poor removal efficiency with dilute wastes

13.2 Reactor Configurations

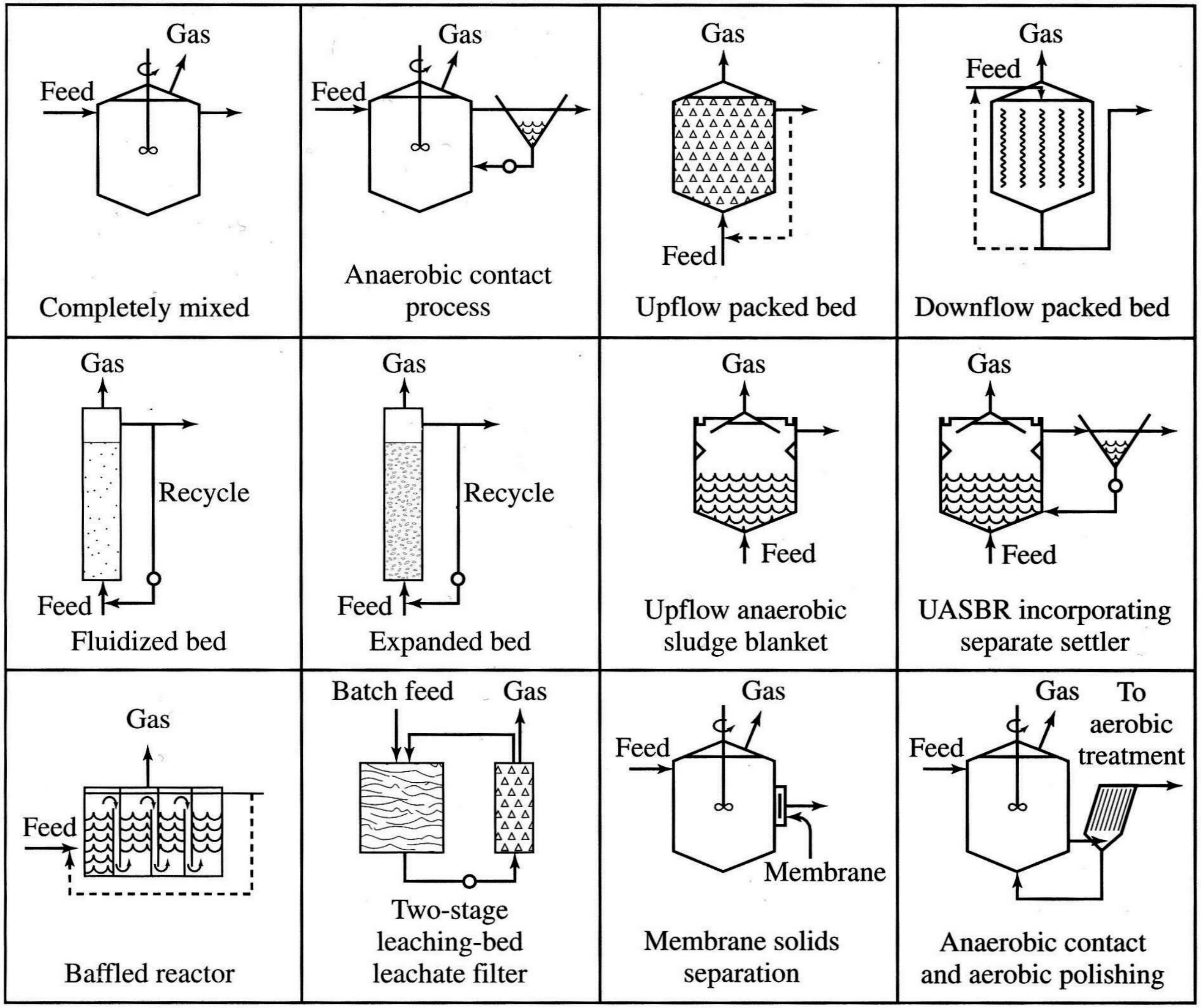


Figure 13.1 Typical anaerobic reactor configurations. SOURCE: Speece, 1983.

13.2 Reactor Configurations

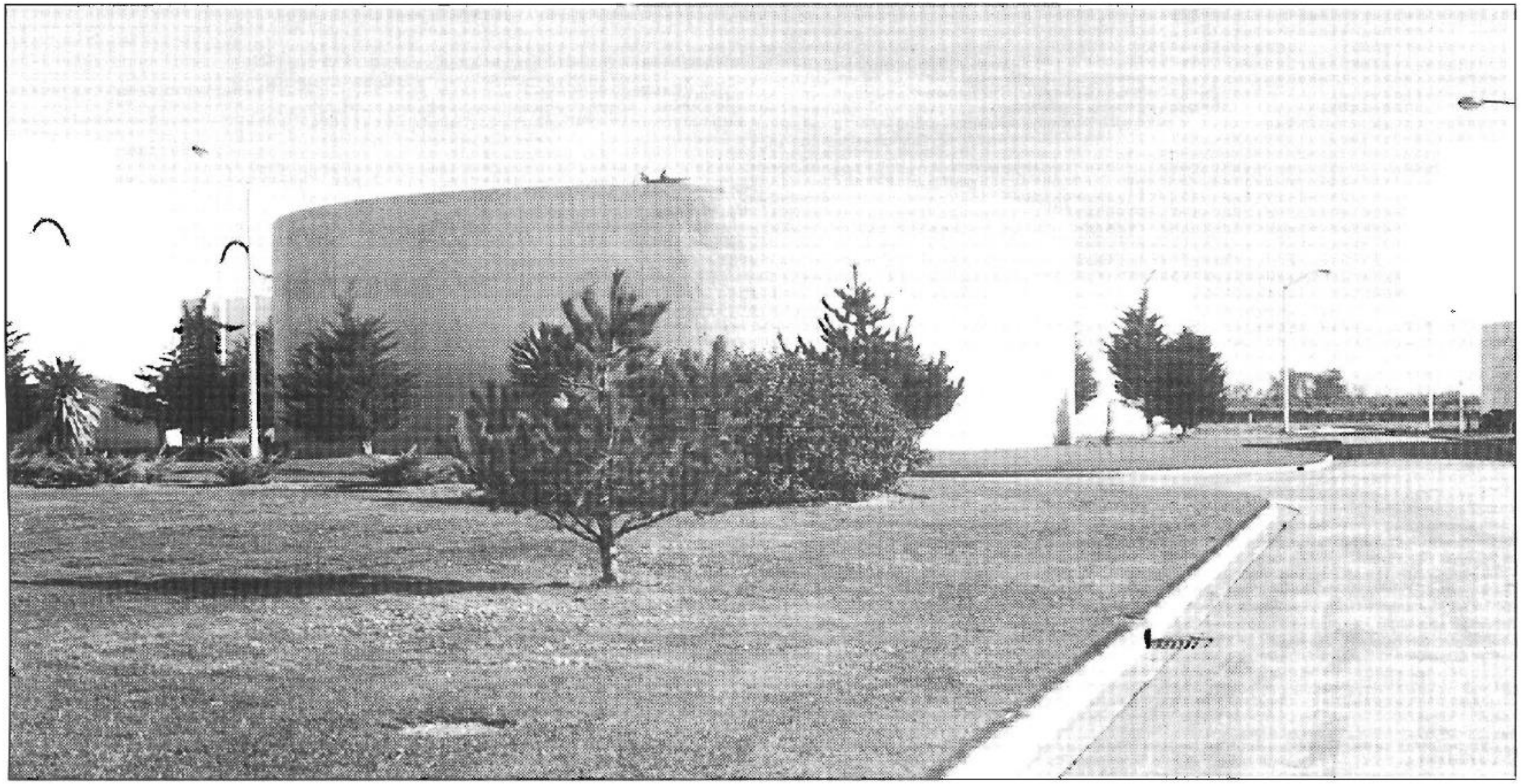


Photo 13.1 Mesophilic anaerobic sludge digester with a floating cover.

13.2 Reactor Configurations

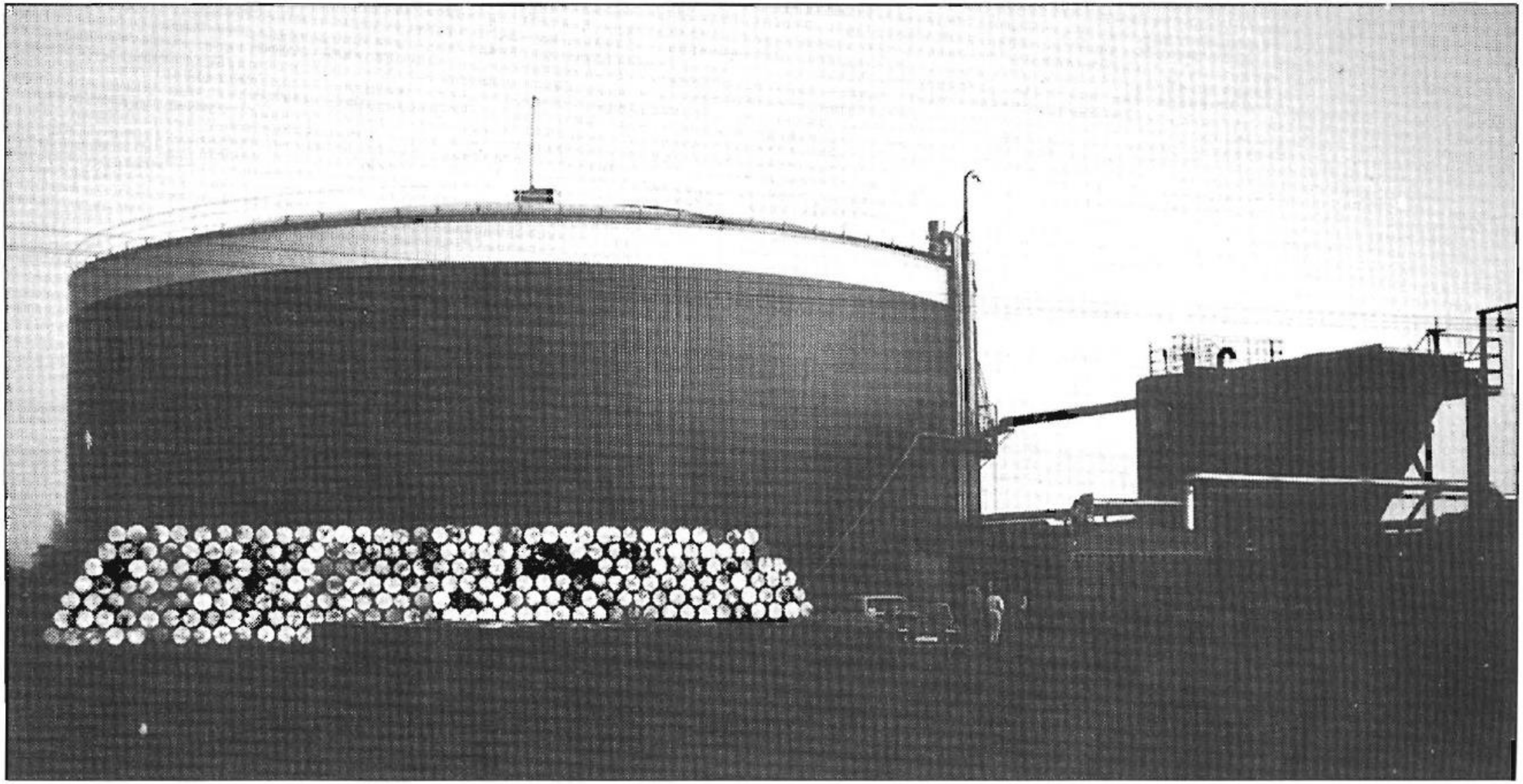


Photo 13.2 Anaerobic contact process. Anaerobic reactor is to the left and the settler to the right.

13.2 Reactor Configurations

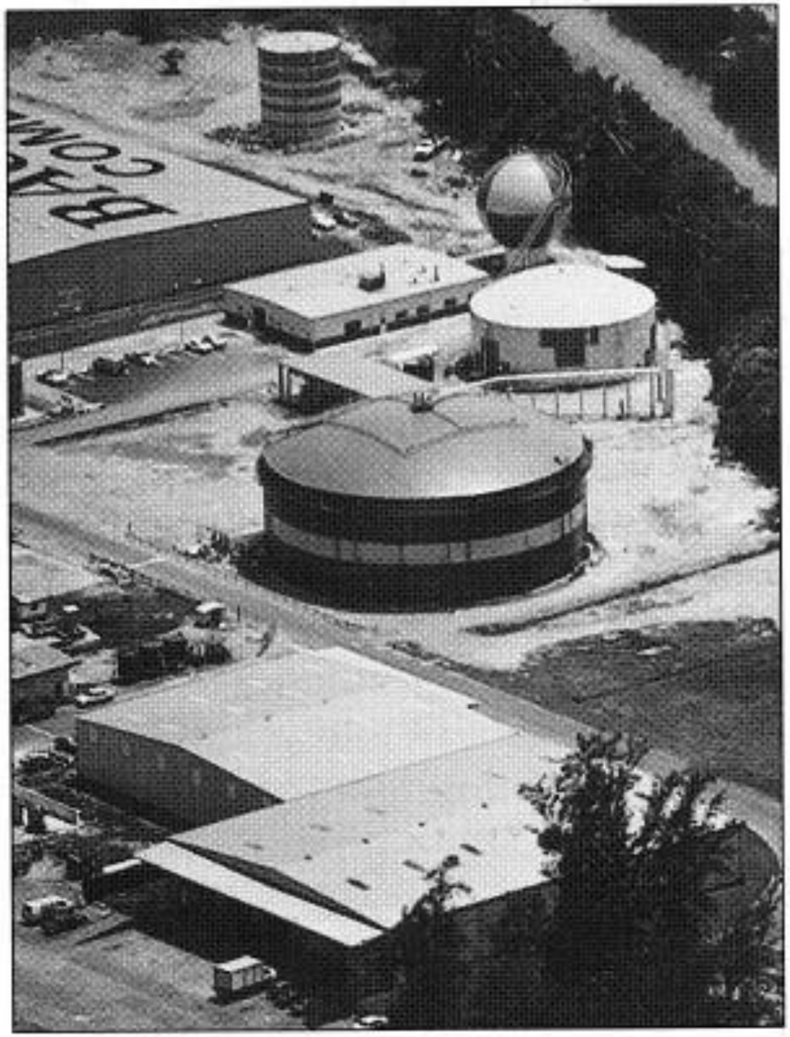


Photo 13.3 Packed-bed anaerobic filter with plastic media used to treat rum wastewater.

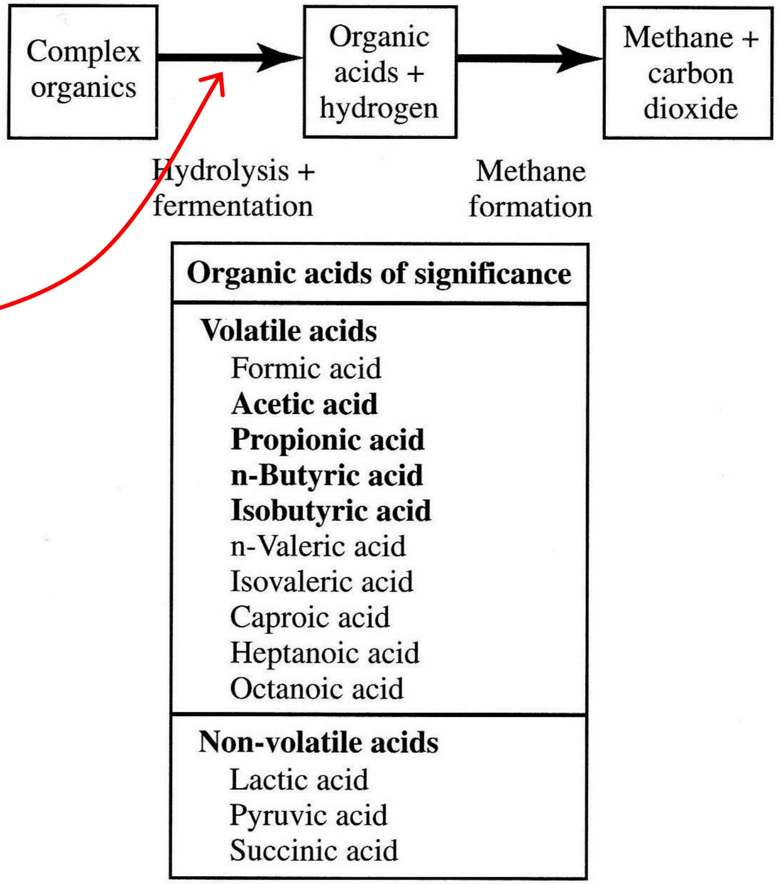
13.3 Process Chemistry and Microbiology

√ Process microbiology

Hydroysis

Acidogenesis

Acetogenesis



Complex organics → Organic acids + hydrogen → Methane + carbon dioxide

Hydrolysis + fermentation

Methane formation

Organic acids of significance

Volatile acids
- Formic acid
- **Acetic acid**
- **Propionic acid**
- **n-Butyric acid**
- **Isobutyric acid**
- n-Valeric acid
- Isovaleric acid
- Caproic acid
- Heptanoic acid
- Octanoic acid

Non-volatile acids
- Lactic acid
- Pyruvic acid
- Succinic acid

Figure 13.2 Simplified two-step view of the overall conversion of complex organics into methane in anaerobic wastewater treatment. Volatile acids shown in bold are the most prevalent intermediates found in the process.

13.3 Process Chemistry and Microbiology

√ Process chemistry

- It is important to know that methanogens at the last stage can only use a limited number of substrates for the formation of methane.

- Currently, it is known that methanogens use the following substrates:
  acetate, $CO_2 + H_2$, formate, methanol, methylamines, CO

- Typical energy-yielding conversion reactions involving these compounds are as follows:

  $CH_3COOH \rightarrow CH_4 + CO_2$

  $4H_2 + CO_2 \rightarrow CH_4 + 2 H_2O$ (e^- acceptor, CO_2)

  $4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O$

  $4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$

  $4(CH_3)_3N + H_2O \rightarrow 9 CH_4 + 3 CO_2 + 6 H_2O + 4 NH_3$

- The two principal pathways involved in the formation of methane are from acetate and $H_2 + CO_2$.

13.3 Process Chemistry and Microbiology

√ Stoichiometry

- **While CO_2 is not the true e^- acceptor for methanogens (it's a fermentation in reality), the exact pathway is not important for maintaining a mass balance.**

- **Example: acetate to methane**

Energy reaction ($R_e = R_a - R_d$)

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$$

$$-R_d:\ \frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O \rightarrow \frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^-$$

$$R_a:\ \frac{1}{8}CO_2 + H^+ + e^- \rightarrow \frac{1}{8}CH_4 + \frac{1}{4}H_2O$$

$$R:\ \frac{1}{8}CH_3COO^- + \frac{1}{8}H_2O \rightarrow \frac{1}{8}CH_4 + \frac{1}{8}HCO_3^-$$

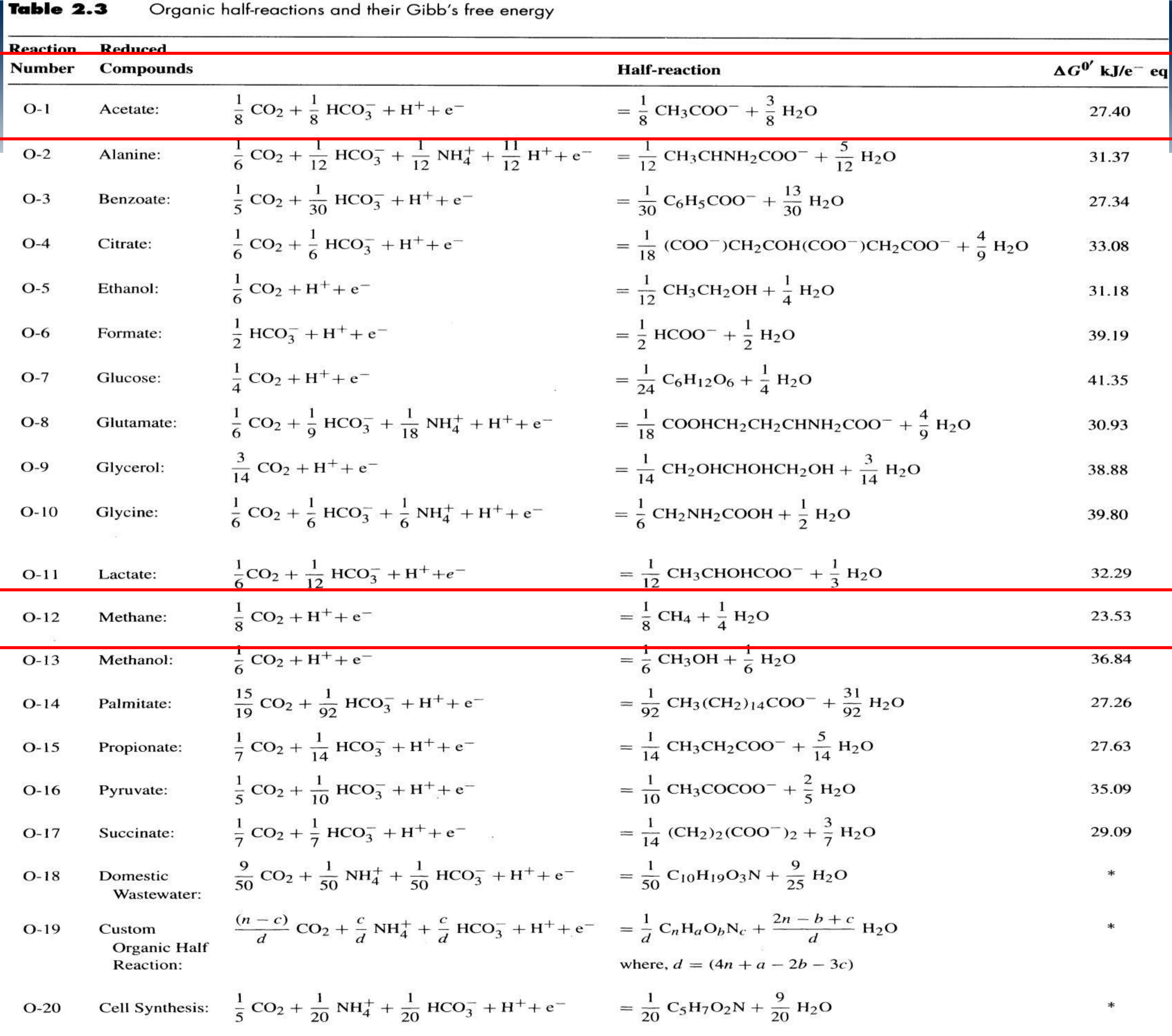


Table 2.3 Organic half-reactions and their Gibb's free energy

Reaction Number	Reduced Compounds	Half-reaction	$\Delta G^{0'}$ kJ/e⁻ eq
O-1	Acetate:	$\frac{1}{8}CO_2 + \frac{1}{8}HCO_3^- + H^+ + e^- = \frac{1}{8}CH_3COO^- + \frac{3}{8}H_2O$	27.40
O-2	Alanine:	$\frac{1}{6}CO_2 + \frac{1}{12}HCO_3^- + \frac{1}{12}NH_4^+ + \frac{11}{12}H^+ + e^- = \frac{1}{12}CH_3CHNH_2COO^- + \frac{5}{12}H_2O$	31.37
O-3	Benzoate:	$\frac{1}{5}CO_2 + \frac{1}{30}HCO_3^- + H^+ + e^- = \frac{1}{30}C_6H_5COO^- + \frac{13}{30}H_2O$	27.34
O-4	Citrate:	$\frac{1}{6}CO_2 + \frac{1}{6}HCO_3^- + H^+ + e^- = \frac{1}{18}(COO^-)CH_2COH(COO^-)CH_2COO^- + \frac{4}{9}H_2O$	33.08
O-5	Ethanol:	$\frac{1}{6}CO_2 + H^+ + e^- = \frac{1}{12}CH_3CH_2OH + \frac{1}{4}H_2O$	31.18
O-6	Formate:	$\frac{1}{2}HCO_3^- + H^+ + e^- = \frac{1}{2}HCOO^- + \frac{1}{2}H_2O$	39.19
O-7	Glucose:	$\frac{1}{4}CO_2 + H^+ + e^- = \frac{1}{24}C_6H_{12}O_6 + \frac{1}{4}H_2O$	41.35
O-8	Glutamate:	$\frac{1}{6}CO_2 + \frac{1}{9}HCO_3^- + \frac{1}{18}NH_4^+ + H^+ + e^- = \frac{1}{18}COOHCH_2CH_2CHNH_2COO^- + \frac{4}{9}H_2O$	30.93
O-9	Glycerol:	$\frac{3}{14}CO_2 + H^+ + e^- = \frac{1}{14}CH_2OHCHOHCH_2OH + \frac{3}{14}H_2O$	38.88
O-10	Glycine:	$\frac{1}{6}CO_2 + \frac{1}{6}HCO_3^- + \frac{1}{6}NH_4^+ + H^+ + e^- = \frac{1}{6}CH_2NH_2COOH + \frac{1}{2}H_2O$	39.80
O-11	Lactate:	$\frac{1}{6}CO_2 + \frac{1}{12}HCO_3^- + H^+ + e^- = \frac{1}{12}CH_3CHOHCOO^- + \frac{1}{3}H_2O$	32.29
O-12	Methane:	$\frac{1}{8}CO_2 + H^+ + e^- = \frac{1}{8}CH_4 + \frac{1}{4}H_2O$	23.53
O-13	Methanol:	$\frac{1}{6}CO_2 + H^+ + e^- = \frac{1}{6}CH_3OH + \frac{1}{6}H_2O$	36.84
O-14	Palmitate:	$\frac{15}{19}CO_2 + \frac{1}{92}HCO_3^- + H^+ + e^- = \frac{1}{92}CH_3(CH_2)_{14}COO^- + \frac{31}{92}H_2O$	27.26
O-15	Propionate:	$\frac{1}{7}CO_2 + \frac{1}{14}HCO_3^- + H^+ + e^- = \frac{1}{14}CH_3CH_2COO^- + \frac{5}{14}H_2O$	27.63
O-16	Pyruvate:	$\frac{1}{5}CO_2 + \frac{1}{10}HCO_3^- + H^+ + e^- = \frac{1}{10}CH_3COCOO^- + \frac{2}{5}H_2O$	35.09
O-17	Succinate:	$\frac{1}{7}CO_2 + \frac{1}{7}HCO_3^- + H^+ + e^- = \frac{1}{14}(CH_2)_2(COO^-)_2 + \frac{3}{7}H_2O$	29.09
O-18	Domestic Wastewater:	$\frac{9}{50}CO_2 + \frac{1}{50}NH_4^+ + \frac{1}{50}HCO_3^- + H^+ + e^- = \frac{1}{50}C_{10}H_{19}O_3N + \frac{9}{25}H_2O$	*
O-19	Custom Organic Half Reaction:	$\frac{(n-c)}{d}CO_2 + \frac{c}{d}NH_4^+ + \frac{c}{d}HCO_3^- + H^+ + e^- = \frac{1}{d}C_nH_aO_bN_c + \frac{2n-b+c}{d}H_2O$ where, $d = (4n + a - 2b - 3c)$	*
O-20	Cell Synthesis:	$\frac{1}{5}CO_2 + \frac{1}{20}NH_4^+ + \frac{1}{20}HCO_3^- + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$	*

13.3 Process Chemistry and Microbiology

- **Example: acetate to methane**

Synthesis reaction ($R_s = R_c - R_d$) & Overall reaction ? (Homework)

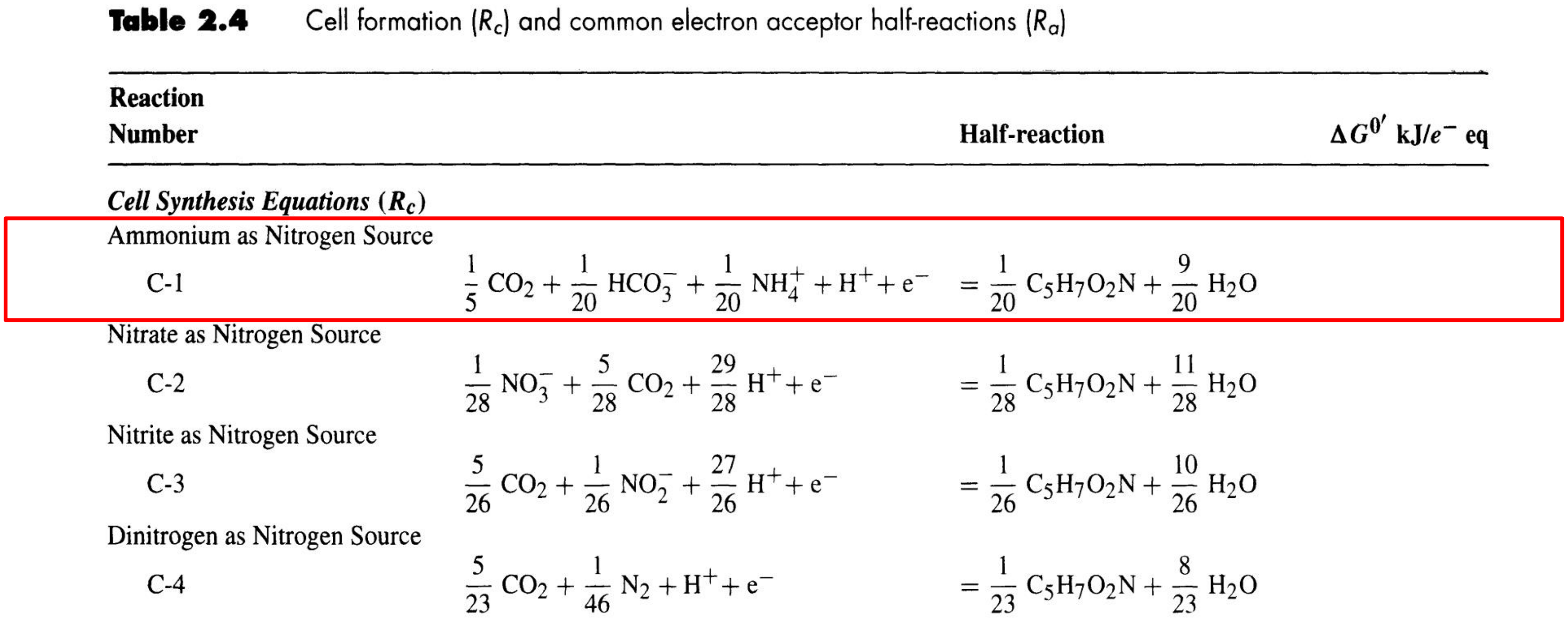


Table 2.4 Cell formation (R_c) and common electron acceptor half-reactions (R_a)

Reaction Number	Half-reaction	$\Delta G^{0'}$ kJ/e^- eq
Cell Synthesis Equations (R_c)		
Ammonium as Nitrogen Source		
C-1	$\frac{1}{5}CO_2 + \frac{1}{20}HCO_3^- + \frac{1}{20}NH_4^+ + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$	
Nitrate as Nitrogen Source		
C-2	$\frac{1}{28}NO_3^- + \frac{5}{28}CO_2 + \frac{29}{28}H^+ + e^- = \frac{1}{28}C_5H_7O_2N + \frac{11}{28}H_2O$	
Nitrite as Nitrogen Source		
C-3	$\frac{5}{26}CO_2 + \frac{1}{26}NO_2^- + \frac{27}{26}H^+ + e^- = \frac{1}{26}C_5H_7O_2N + \frac{10}{26}H_2O$	
Dinitrogen as Nitrogen Source		
C-4	$\frac{5}{23}CO_2 + \frac{1}{46}N_2 + H^+ + e^- = \frac{1}{23}C_5H_7O_2N + \frac{8}{23}H_2O$	

13.3 Process Chemistry and Microbiology

- **General organic substrates**

1. Most of electron equivalents in the organic matter are conserved in CH_4 (e^- sink)
2. Organic matter is converted in many intermediate steps before CH_4 is formed.
3. A certain portion of its electron equivalents, f_s , is (net)synthesized into biomass.
4. N of the organic matter is converted to $NH_{4,}$ which is then the source of cell nitrogen.
5. Assume CO_2 is the e^- acceptor.

$$C_nH_aO_bN_c + \left(2n + c - b - \frac{9df_s}{20} - \frac{df_e}{4}\right) H_2O \rightarrow$$

$$\frac{df_e}{8} CH_4 + \left(n - c - \frac{df_s}{5} - \frac{df_e}{8}\right) CO_2 + \frac{df_s}{20} C_5H_7O_2N$$

$$+ \left(c - \frac{df_s}{20}\right) NH_4^+ + \left(c - \frac{df_s}{20}\right) HCO_3^-$$

$$f_s = f_s^0 \left[\frac{1 + (1 - f_d)b\theta_x}{1 + b\theta_x}\right]$$

where

$$d = 4n + a - 2b - 3c$$

13.3 Process Chemistry and Microbiology

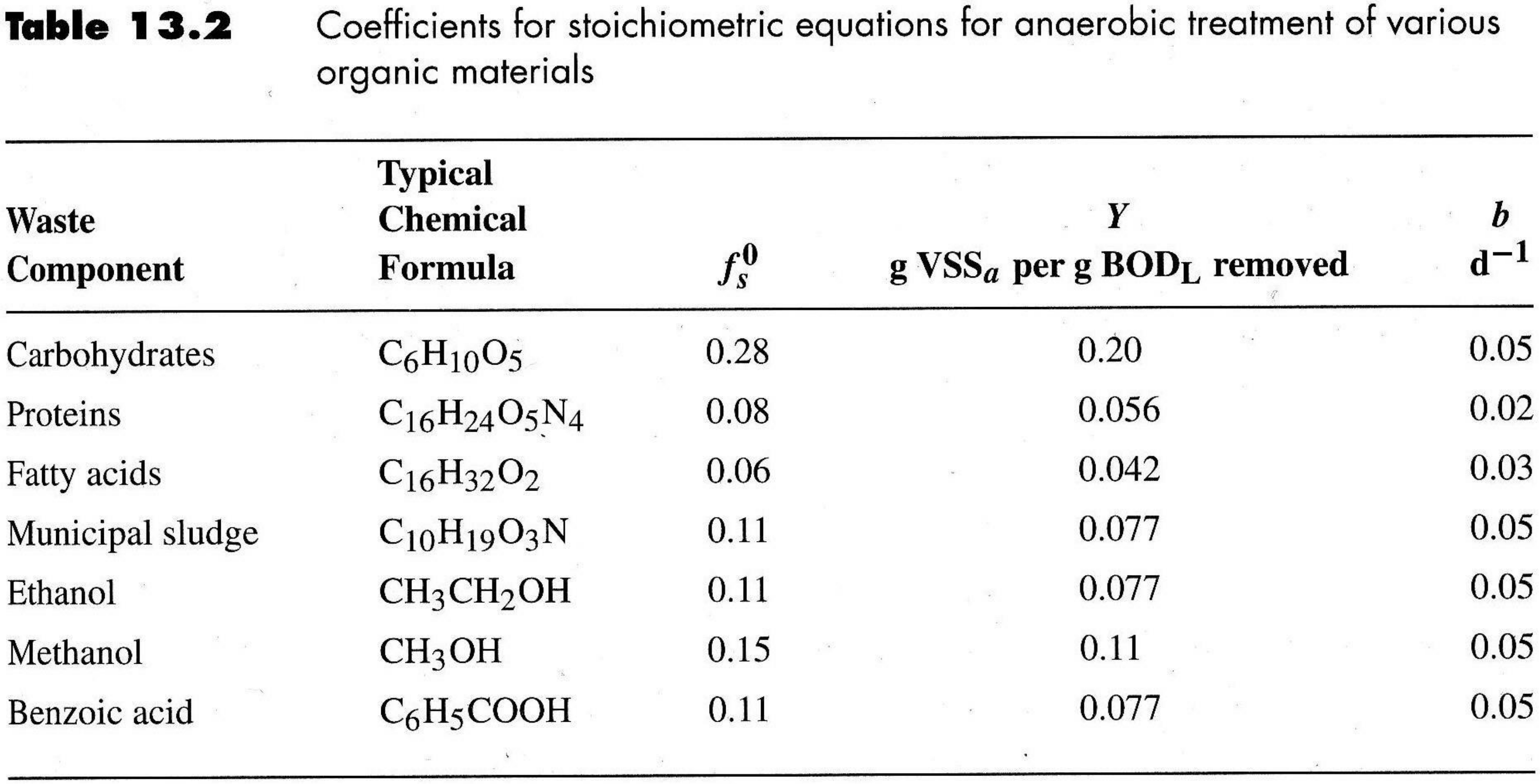


Table 13.2 Coefficients for stoichiometric equations for anaerobic treatment of various organic materials

Waste Component	Typical Chemical Formula	f_s^0	Y g VSS$_a$ per g BOD$_L$ removed	b d^{-1}
Carbohydrates	$C_6H_{10}O_5$	0.28	0.20	0.05
Proteins	$C_{16}H_{24}O_5N_4$	0.08	0.056	0.02
Fatty acids	$C_{16}H_{32}O_2$	0.06	0.042	0.03
Municipal sludge	$C_{10}H_{19}O_3N$	0.11	0.077	0.05
Ethanol	CH_3CH_2OH	0.11	0.077	0.05
Methanol	CH_3OH	0.15	0.11	0.05
Benzoic acid	C_6H_5COOH	0.11	0.077	0.05

* f_s^0 values include the methanogens and all bacteria needed to convert the original organic matter to intermediates (e.g., acetate and H_2).

$$f_s = f_s^0 \left[\frac{1 + (1 - f_d) b \theta_x}{1 + b \theta_x} \right]$$

13.3 Process Chemistry and Microbiology

√ Gas production

- **The end products of anaerobic digestion are methane gas (CH_4) and CO_2.**

- **The quantity of methane gas,**

  It can be calculated from the stoichiometry of the overall reaction (previous slides) & another way below

$$V_{CH_4} = V^{\circ}_{CH_4} \cdot [\Delta S - 1.42(r_{tbp})]$$

V_{CH_4} : total methane production rate (m^3/d)

$V^{\circ}_{CH_4}$: volume of methane produced per kg of COD or BOD_L oxidized (m^3/kg)

ΔS : COD or BOD_L removal rate (kg/d)

r_{tbp} : biomass production rate (kg/d)

13.3 Process Chemistry and Microbiology

- Conversion of biomass weight to OD (BOD or COD)

$$C_5H_7O_2N \quad + \quad 5O_2 \quad \rightarrow \quad 5CO_2 \quad + \quad 2H_2O \quad + \quad NH_3$$

Biomass

113 5*32

$$\frac{5*32}{113} = 1.42$$ units O_2 / unit biomass oxidized

- **Conversion of BOD_L (glucose) to methane gas**

$$C_6H_{12}O_6 \quad + \quad 6O_2 \quad \rightarrow \quad 6CO_2 \quad + \quad 6H_2O$$

180 **6 x 32 = 192**

$$C_6H_{12}O_6 \quad \rightarrow \quad 3CO_2 \quad + \quad 3CH_4$$

180 **16 x 3 = 48**

13.3 Process Chemistry and Microbiology

- 180g of glucose (e.g., 192g as BOD_L) produces 48g of CH_4.

$$\therefore \frac{kg\ CH_4}{kg\ BOD_L} = \frac{48}{192} = \frac{0.25kg\ CH_4\ \text{produced}}{1kg\ BOD_L\ \text{stabilized}}$$

$$\therefore V^{\circ}_{CH_4} = (0.25)\left(\frac{10^3 g}{kg}\right)\left(\frac{1mol}{16g}\right)\left(\frac{22.4l}{mol}\right)\left(\frac{10^3 l}{m^3}\right)^{-1}$$

$$= \frac{0.35m^3\ CH_4}{kg\ BOD_L\ \text{stabilized}}\ (\text{at standard condition, } 0°\text{C (273 K)}, 1\text{atm})$$

$$V_2 = \frac{T_1}{T_2} V_1, \quad T_1 = 273K, \quad T_2 : \text{fermentation temp.}$$

13.3 Process Chemistry and Microbiology

$$\Delta S = (S_o - S) \times Q \times (\frac{kg}{10^3 g})$$

$$= \frac{S_o - S}{S_o} \times S_o \times Q \times (\frac{kg}{10^3 g})$$

$$= (S_o - S) \times Q \times (\frac{kg}{10^3 g})$$

$Q : m^3 / d,$ $S_o : \ g / m_3 = mg / L,$

$\Delta S : kg/d,$

$$V_{CH_4} = V^{\circ}_{CH_4} \cdot [\Delta S - 1.42(r_{tbp})]$$

$$\therefore V^{\circ}_{CH_4} = \frac{0.35 m^3 CH_4}{kg \ BOD_L \text{ stabilized}} \quad \text{(at standard condition (STP), 0°C,1atm)}$$

$$\therefore V_{CH_4} = (0.35 m^3 / kg) \cdot [\{Q \cdot (S_o - S) - 1.42(r_{tbp})\}(\frac{10^3 g}{kg})^{-1}]$$

Or $$V_{CH_4} = (0.35 m^3 / kg) \cdot [Q \cdot (S_0 - S) \cdot f_e]$$

13.3 Process Chemistry and Microbiology

$$r_{tbp} = Y_n \cdot Q(S_o - S)$$

$$= 0.706\, f_s \cdot Q(S_o - S)$$

From Ch. 2

$$Y = f_s^0 \frac{113}{20 \times 8} = 0.706\, f_s^0$$

⇨ $$1.42\, r_{tbp} = 1.42 * 0.706\, f_s \cdot Q(S_o - S) = f_s \cdot Q(S_o - S)$$

⇨ $$Q(S_o - S) - 1.42\, r_{tbp} = (1 - f_s) \cdot Q(S_o - S) = f_e \cdot Q(S_o - S)$$

13.3 Process Chemistry and Microbiology

√ pH and alkalinity requirements

- The pH values outside the desired pH (6.6~7.6) can be detrimental to the process, particularly to methanogenesis.
- The biggest problem generally is to maintain the pH above 6.6.
- The main chemical species controlling pH in the anaerobic treatment are those related to carbonic acid system.

$$CO_2(aq) = CO_2(g)$$

$$CO_2(aq) + H_2O = H_2CO_3$$

$$H_2CO_3 = H^+ + HCO_3^-$$

$$HCO_3^- = H^+ + CO_3^{2-}$$

$$H_2O = H^+ + OH^-$$

- At the normal pH of anaerobic treatment, carbonate (CO_3^-) is not important.

13.3 Process Chemistry and Microbiology

$$\frac{[\mathrm{H}^+][\mathrm{HCO}_3^-]}{[\mathrm{H_2CO}_3^*]} = K_{a,1} = 5 \cdot 10^{-7} (35\ °\mathrm{C})$$

$$\mathrm{pH} = \mathrm{p}K_{a,1} + \log \frac{[\mathrm{HCO}_3^-]}{[\mathrm{H_2CO}_3^*]}$$

$$\frac{\text{Alkalinity(bicarb)}}{50{,}000} = [\mathrm{HCO}_3^-]$$

$$\frac{[CO_2(g)]}{[H_2CO_3^*]} = K_H = 38\ atm/mol\ (35°C)$$

$$\mathrm{pH} = \mathrm{p}K_{a,1} + \log \frac{\dfrac{\text{Alkalinity(bicarb)}}{50{,}000}}{\dfrac{[\mathrm{CO_2(g)}]}{K_H}}$$

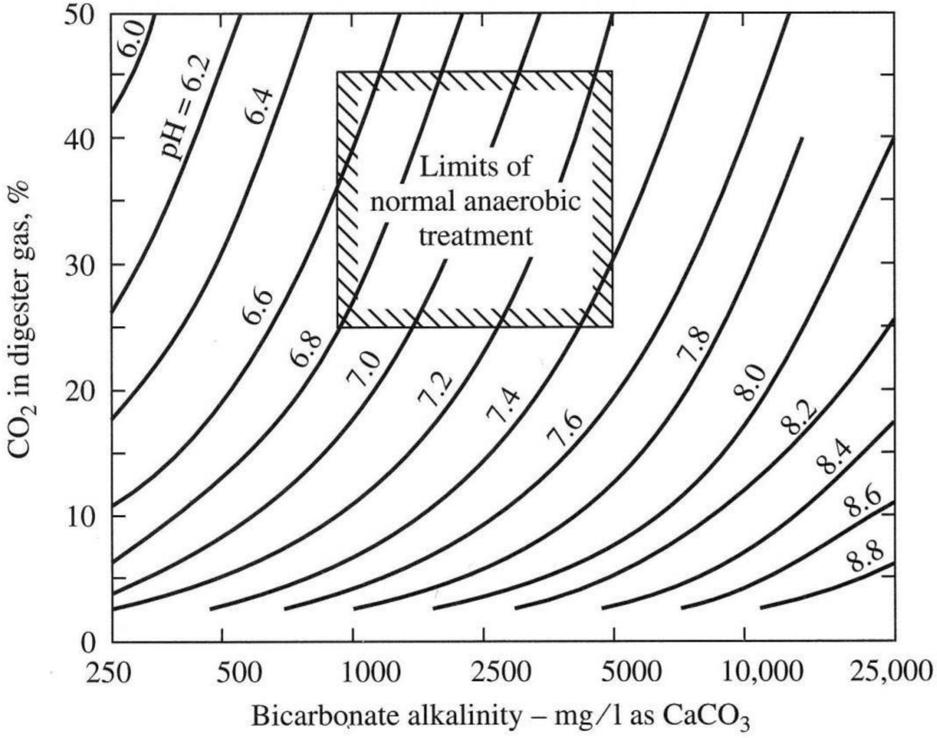


Figure 13.3 Relationship among bicarbonate alkalinity, the percentage of carbon dioxide in the gas phase (at 1 atm total pressure), and reactor pH in anaerobic treatment.