AnSc 5311
Ruminant Nutrition

Microbial Fermentation of Carbohydrates
(Source – Notes for AnSc 607 – Microbiology of the Rumen – J. B. Russell, Cornell University)

I. Degradation of carbohydrate polymers

A. Carbohydrate polymers must be degraded to molecules that can be into microbial cells before fermentation

1. Starch – three enzymes are components of the process: 1) α-amylase hydrolyzes α 1, 4 bonds; 2) debranching enzyme hydrolyzes α 1, 6 bonds at branch points; 3) amyloglucosidase hydrolyzes both α 1, 4 and α 1, 6 bonds

a) α-amylase and amyloglucosidase are common in microbes

b) Debranching enzyme is not well defined with respect to mechanism in microbes

2. Cellulose – cellulase hydrolyzes cellulose to and some glucose

a) Ruminal cellulolytic organisms – cellulase activity is cell-associated, not found in cell-free ruminal fluid

3. Hemicellulose (xylans) – have β-type bonds like cellulose

a) Composed of arabinans, galactans, mannans, and xylans

b) A wide variety of hemicellulose-degrading enzymes have been described in microbes

(1) Enzymes are specific for the sugar component in the polymer

4. Pectin – a polymer of

a) Can be degraded by a variety of ruminal microbes

II. Transport of carbohydrates into microbial cells

A. Extracellular concentrations of soluble sugars and oligosaccharides are low, so concentration gradient does not favor entry into microbial cell

1. These compounds are not lipid soluble, which further decreases the likelihood of entry into microbial cells in the absence of active or facilitated transport mechanisms

B. Types of transport mechanisms

1. Active transport

a) Involves a transport protein to bind the substrate, coupled to hydrolysis of high-energy phosphate (e.g., ATP)

(1) Many Gram– bacteria have binding proteins (with a high affinity for the substrate) between their outer membrane and cell wall that first bind the substrate and pass it on to the transport protein

(a) organisms do not have an outer membrane and probably lack such binding proteins

(2) Hydrolysis of high-energy phosphate causes conformational change in the transport protein that facilitates binding

2. Secondary active transport

a) Typically involves pumping of protons (or Na^+) from the cell to establish an electrochemical gradient that is used to drive uptake of the substrate

(1) Proton pumping is energy-dependent – usually coupled to or electron transport systems

(2) Result is negative charge (and high pH) inside the cell and positive charge (and low pH) outside the cell

(a) Positively charged substrates flow into the cell because the electrochemical gradient favors positive charge inflow

(b) Uptake of neutral and negatively charged substrates is coupled with proton influx

3. Group translocation (the phosphotransferase system - PTS)

a) The high-energy phosphate bond of is hydrolyzed and transferred to a series of enzymes and ultimately to the incoming sugar

(1) One of the enzymes in the series serves as the transport protein for the sugar

(2) *S. bovis*, *S. ruminantium*, and *M. elsdenii* have been shown to have this type of transport system for glucose

C. Trapping of substrates inside cells

1. Polar compounds are less lipophylic and less likely to leak out of the cell, so a common mechanism of trapping substrates is to make them more polar by phosphorylation

a) This is a built in component of the phosphotransferase system of transport – this would tend to conserve use of high-energy phosphate compared with active transport

b) is a common mechanism of phosphorylation for sugars

(1) Sugar + ATP $\rightarrow$ Sugar~P + ADP

2. Disaccharides that are taken up by cells are cleaved to simple sugars before metabolism – two mechanisms used

a) Hydrolytic cleavage – cellobiose is cleaved by β-glucosidase to two molecules of glucose

(1) Glucose would then need to be phosphorylated

(2) Similar mechanisms for other disaccharides

b) Phosphorylytic cleavage – cleavage of cellobiose plus inorganic P is catalyzed by the cellobiose phosphorylase to yield glucose-1-P and glucose

(1) Similar phosphorylases for other disaccharides

(2) Some ruminal bacteria (e.g. *R. flavefaciens*) have been shown to possess such phosphorylases

(3) Phosphorylytic cleavage would tend to conserve high-energy phosphate compared with hydrolytic cleavage

III. General pathways of carbohydrate fermentation in the rumen

A. Primary pathway is fermentation of hexose to pyruvate via pathway

1. Overall: Glucose-6-P →

B. A second alternative pathway would be hexose monophosphate shunt

1. Glucose-6-P metabolized via ribulose-5-P to glyceraldehyde-3-P and seduheptulose-7-P to fructose-6-P and glyceraldehyde-3-P (see any standard biochemistry text for full pathway)

C. A third alternative pathway would be the heterofermentive pathway

1. Glucose → Glucose-6-P → 6-P-gluconate → ribulose –5-P → xylulose-5-P → glyceraldehyde-3-P + acetyl~P

a) is the enzyme that converts xylulose-5-P to G3P and acetyl~P

D. Energetic comparisons of the three pathways of hexose metabolism (all using PTS transport)

1. Embden-Meyerhof – yield is 2 ATP + 2 $NADH_2$

2. Hexose monophosphate shunt – yield is $1^{2/3}$ ATP + $1^{2/3}$ $NADH_2$ + 2 $NADPH_2$

3. Heterofermentive pathway – yield is 1 ATP + 3 $NADH_2$

4. Primary pathway is the Embden-Meyerhof because overall yield of ATP is greater and less reducing equivalents are produced

a) Disposal of reducing equivalents is a problem in anaerobic systems

E. Fermentation of pentoses would occur via the hexose monophosphate shunt (HMS) or the heterofermentive pathway

1. Yield of ATP is greater with HMS

2. Estimates in ruminal microbes indicate 75% of pentose is fermented by HMS and 25% by the heterofermentive pathway

F. Metabolic fates of pyruvate

1. Production of lactate

a) L-, D-, or D,L-lactate can be produced from pyruvate via NAD-linked lactate dehydrogenase

b) Pyruvate + $NADH_2 \rightarrow$ Lactate + NAD

c) This enzyme has been found in *Selenomonas*, *Megasphaera*, lactobacilli, and streptococci spp.

(1) Most organisms produce L-lactate, but lactobacilli produce D-lactate and D,L-lactate

d) The reverse reaction (lactate to pyruvate) via a FMN-linked lactate dehydrogenase has been reported in *Megasphaera*

2. Production of acetyl CoA via pyruvate–ferredoxin oxidoreductase

a) Has been reported in clostridial spp. and in *Megasphaera*, *Selenomonas ruminantium*, *Butyrivibrio fibrisolvens*, and ruminococci

b) Pyruvate + CoASH $\rightarrow$ 2-α-lactyl-TPP-CoA Enzyme $\rightarrow$ 2-Hydroxyethyl-TPP-CoA Enzyme + FD $\rightarrow$ Acetyl CoA + FDH_2 + CO_2

(1) Thiamine pyrophosphate (TPP) is a cofactor and ferredoxin (FD) is used to accept H^+ in the final step

(2) Mg^{++} is a cofactor with TPP in the first step of the reaction

(3) CO_2 is evolved in the second step of the sequence

3. Production of acetyl CoA and formate via

a) Has been reported in *E. Coli* and *Viellonella*

(1) Pyruvate + CoASH $\rightarrow$Acetyl CoA + Formate

(a) TPP and Mg^{++} are cofactors

4. Production of acetyl CoA and formate via CO_2 reduction

a) Has been reported in ruminococci, *Selenomonas ruminantium*, *Butyrivibrio fibrisolvens*, and *Butyrivibrio succinogenes*

b) Pyruvate + CoASH $\rightarrow$ Acetyl CoA + CO_2

c) $CO_2 + XH_2 \rightarrow$ Formate + X

(1) X and XH_2 are the oxidized and reduced forms of various electron carriers (e.g., ferredoxin and flavin nucleotide)

IV. Pathways for formation of acetate, propionate, butyrate and other VFA

A. Acetate – two major pathways have been identified

1. Phosphotransacetylase and acetate kinase

a) Phosphotransacetylase: Acetyl CoA + Pi $\rightarrow$ Acetyl~P + CoASH

b) Acetate kinase: Acetyl~P + ADP $\rightarrow$ Acetate + ATP

2. Acetyl CoA lyase – only identified in anaerobic protozoa

a) Acetyl CoA + ADP + Pi $\rightarrow$ Acetate + ATP + CoASH

3. Overall pathway of acetate formation in *Ruminococcus albus* – **see Figure 1 (attached)**

B. Butyrate

1. Formation of butyrate from Acetyl CoA – six steps – **see Figure 2 (attached)**

a) Acetylacetyl CoA thiolase

(1) 2 Acetyl CoA $\rightarrow$ Acetylacetyl CoA + CoASH

b) β-hydroxybutyrate dehydrogenase

(1) Acetylacetyl CoA + $NADH_2$ $\rightarrow$ β-hydroxybutyrl-CoA + NAD

c) Enoyl-CoA dehydratase

(1) β-hydroxybutyrl-CoA $\rightarrow$ Crotonyl CoA + H_20

d) Butyrl CoA dehydrogenase

(1) Crotonyl CoA + $NADH_2$ $\rightarrow$ Butyrl CoA + NAD

(a) Reduced flavoprotein replaces $NADH_2$ in *Butyrivibrio fibrisolvens*

e) Phosphate butyrl transferase

(1) Butyrl CoA + Pi $\rightarrow$ Butyrl-P

f) Butyrate kinase

(1) Butyrl~P + ADP $\rightarrow$ Butyrate + ATP

C. Propionate

1. is often a precursor – **see Figure 3 (attached)** for overview of pathway for succinate production in *Bacteroides succinogenes*

2. Formation of propionate from pyruvate, lactate, and succinate – the pathway

a) Pyruvate or lactate can serve as a precursor, or succinate can be converted to propionate via methylmalonyl CoA mutase/racemase

(1) Methylmalonyl CoA mutase is a B_{12} enzyme

b) Referred to as the randomizing pathway because when ^{14}C glucose is labeled at the 2 position, the label will show up in either the 2 or 3 position of propionate

c) ATP yield is per mole of lactate

d) See **Figure 4 (attached)** for overview of the randomizing pathway and **Figure 5 (attached)** for an overview or propionate production from glucose for *Selenomonas ruminantium*

3. The direct reductive pathway (pathway)

a) Identified in *Megasphaera elsdenii* and *Bacteroides ruminicola*

b) ATP yield seems to be 1 mole per 3 moles of lactate because the acyldehydrogenase does not seem to be linked to phosphorylation

c) See **Figure 6 (attached)** for an overview of the acrylate pathway

D. Other VFA

1. See **Figure 7 (attached)** for an overview of production of valerate and caproate

V. Formation of methane

A. Methanogenic bacteria can grow on CO_2, H_2, formate, methanol, or VFA

B. CO_2 and H_2 are the principle substrates for CH_4 production

1. Formate is a substrate, but it is degraded to CO_2 and H_2 by formate hydrogen lyase

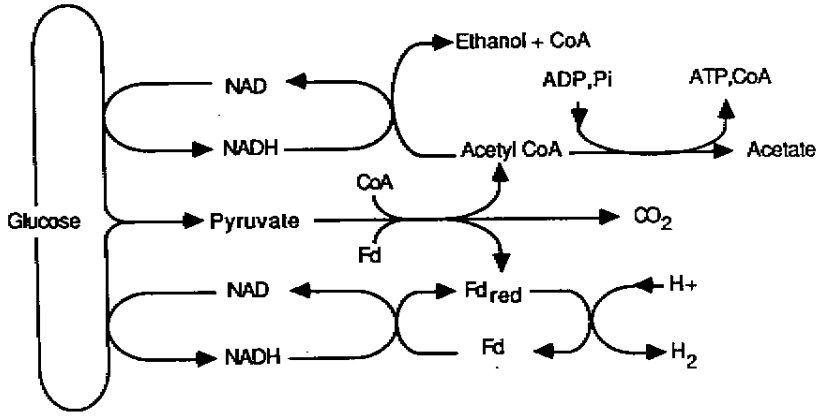
2. ATP synthesis occurs as a result of electron transport coupled with phosphorylation

C. See **Figure 8 (attached)** for an overview of methane production from CO_2 and H_2

1. In this figure, X is a carrier of unknown structure, B_{12} is hydridocobalamin, and CoM is coenzyme M

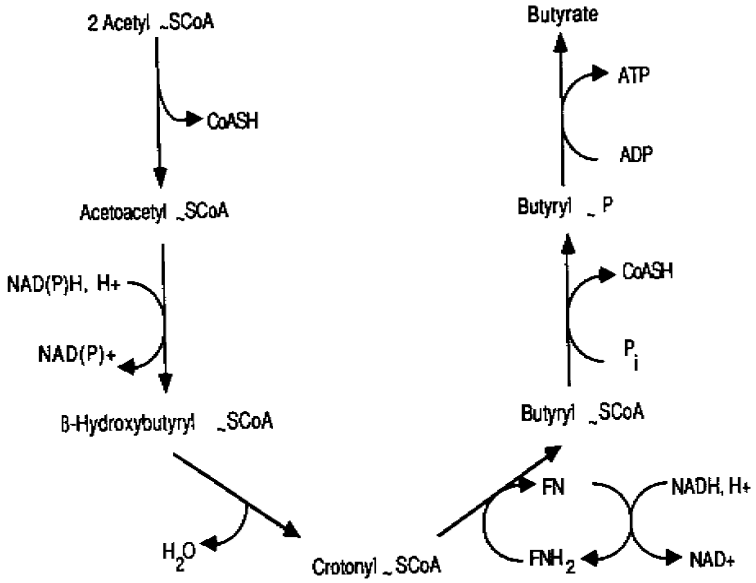
a) CoM is 2-mecaptoethane sulfonic acid

The following figures were reproduced from Notes for AnSc 607 – Microbiology of the Rumen – J. B. Russell, Cornell University



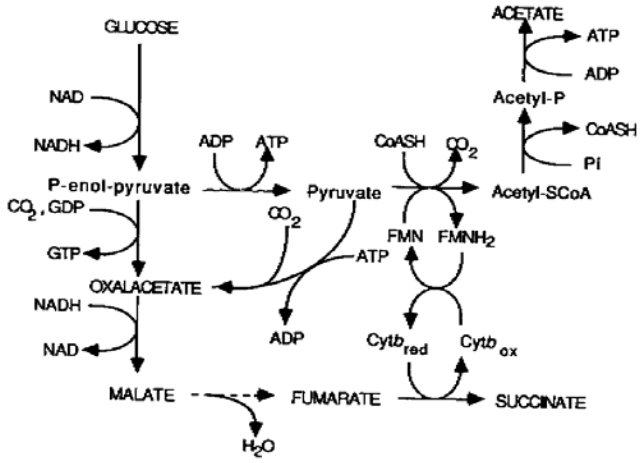
Pathway of acetate, H_2, and ethanol formation by *R. albus*

Figure 1. Acetate production



Pathway of butyrate formation by *B. fibrisolvens.*

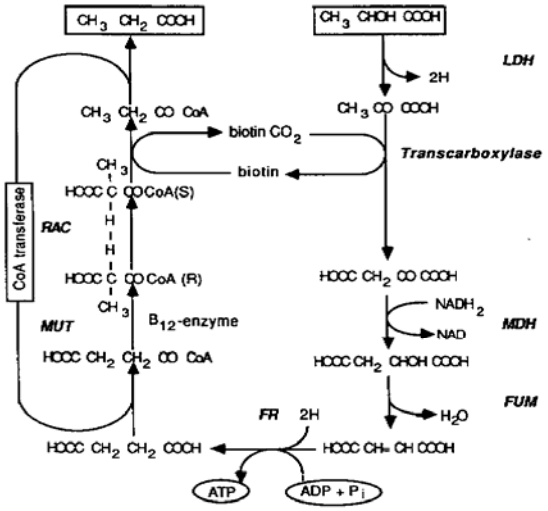
Figure 2. Butyrate production



Pathway of succinate and acetate formation by *B. succinogenes*.

Figure 3. Succinate production

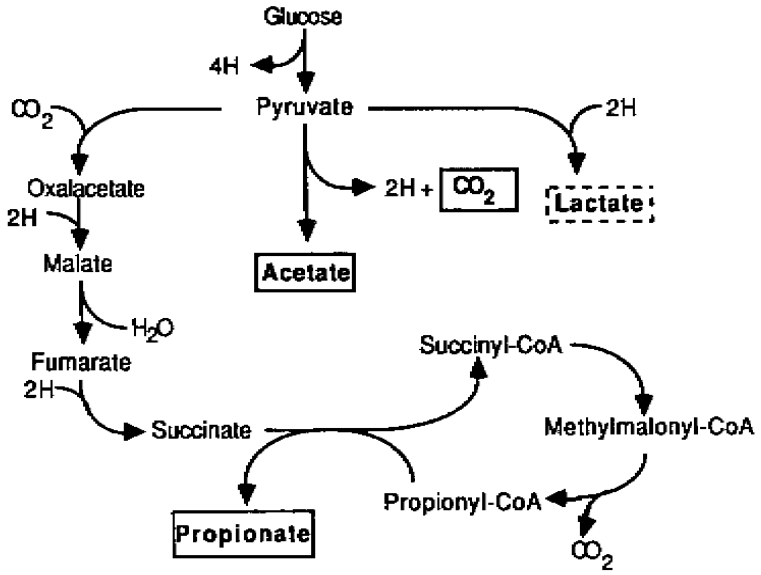
Formation of propionate via succinate (randomizing pathway)



sum: lactate + $NADH_2$ + ADP + P_i ⟶ propionate + NAD + ATP

This is the primary pathway of propionate formation in the rumen and is used by *Selenomonas ruminantium*. Note that pyruvate or lactate can serve as a precursor.

Figure 4. Propionate production



Pathway of propionate formation by *S. ruminantium.*

Figure 5. Production of propionate – overview from glucose to propionate

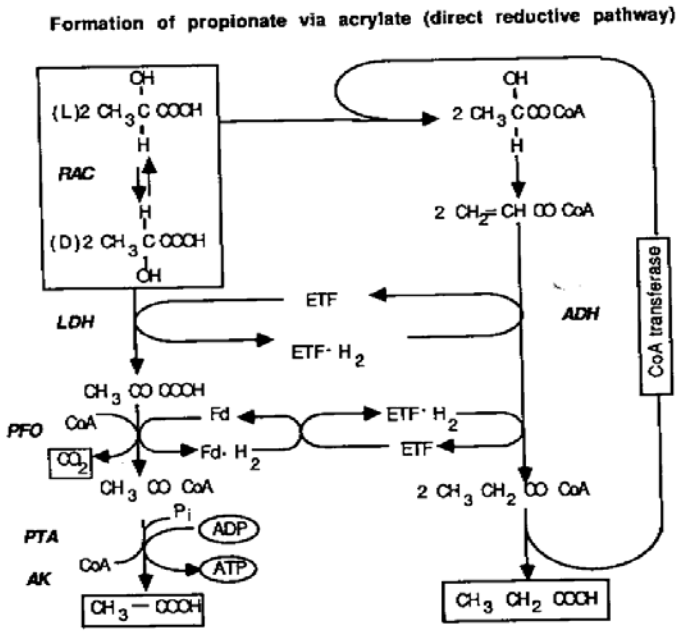


Figure 6. Propionate production by the acrylate pathway

Summary of Fermentation Products

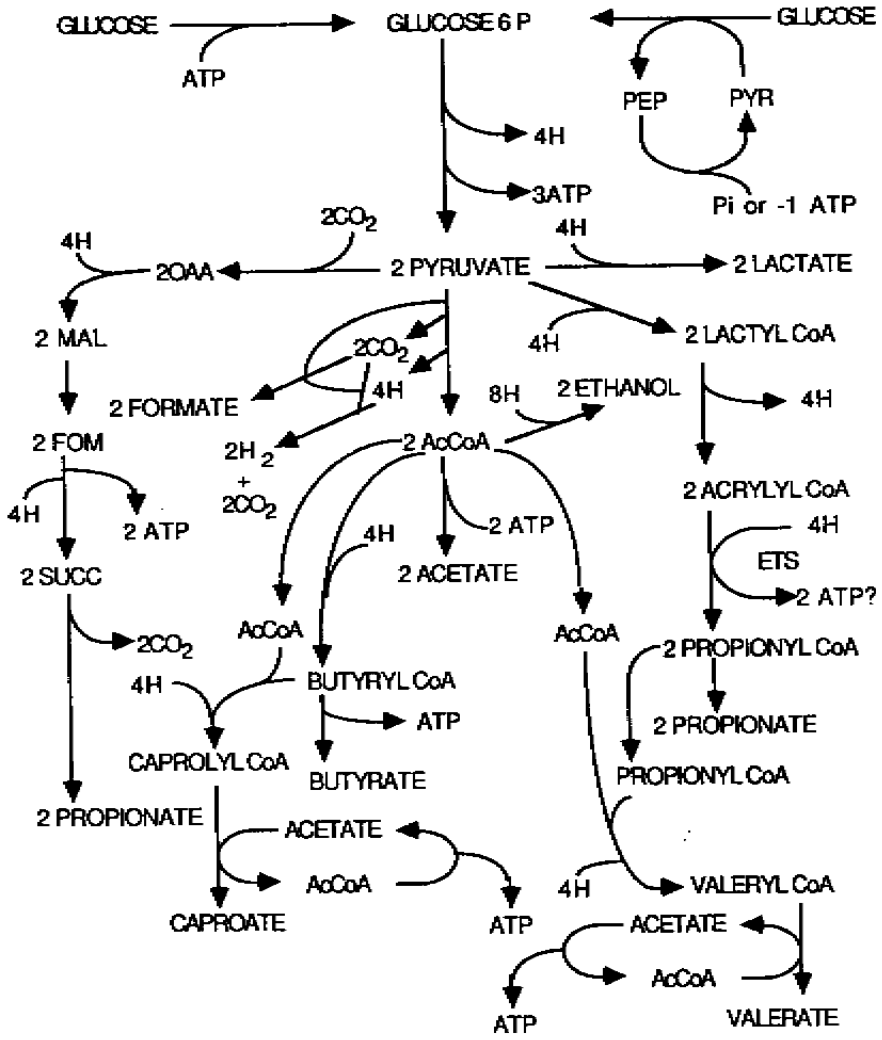
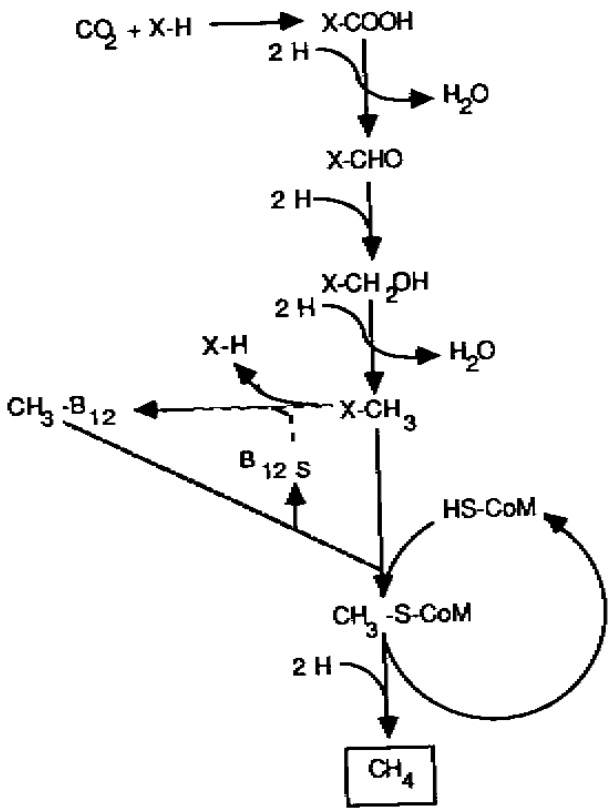


Figure 7. Overall summary of fermentation products



Scheme for the reduction of CO_2 to methane. X, carrier of unknown structure; B_{12}, hydridocobalamin; HS-CoM, coenzyme M.

Figure 8. Production of methane