The Ruminant Microbiome, Biomass Conversion and Carboxylate Production

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Outline

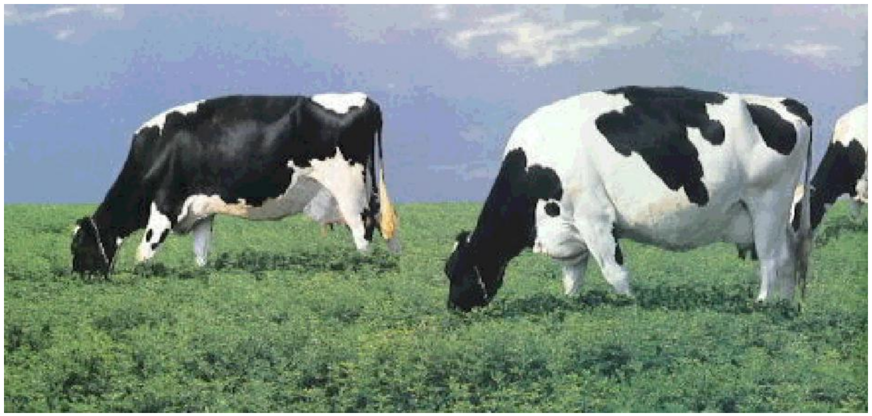
- Role of the rumen in the ruminant animal
- The rumen as a model bioreactor
- Primary differences between the ruminal fermentation and AD
- VFA chain elongation pathways
- Extraruminal fermentations
    - basic characteristics
    - opportunities and limitations

Two Questions

What can the ruminant animal teach us about how to conduct an industrial fermentation of biomass materials?

Can the ruminal fermentation be adapted to an industrial process for converting biomass to fuels or fuel precursors, specifically C_2-C_8 carboxylates?

RUMINANT ANIMALS: Nature's most prolific bioreactors



Worldwide populations (ILRI, 2014):

1.4×10^9 cattle and water buffalo

1.9×10^9 sheep and goats

- **Combined fermentation capacity ~200 billion liters**
- **The world's largest commercial fermentation**
- **On a mass/volume basis, the most intensive cellulosic bioconversion system in nature**

Ruminal biomass fermentation proceeds via a unique type of consolidated bioprocessing

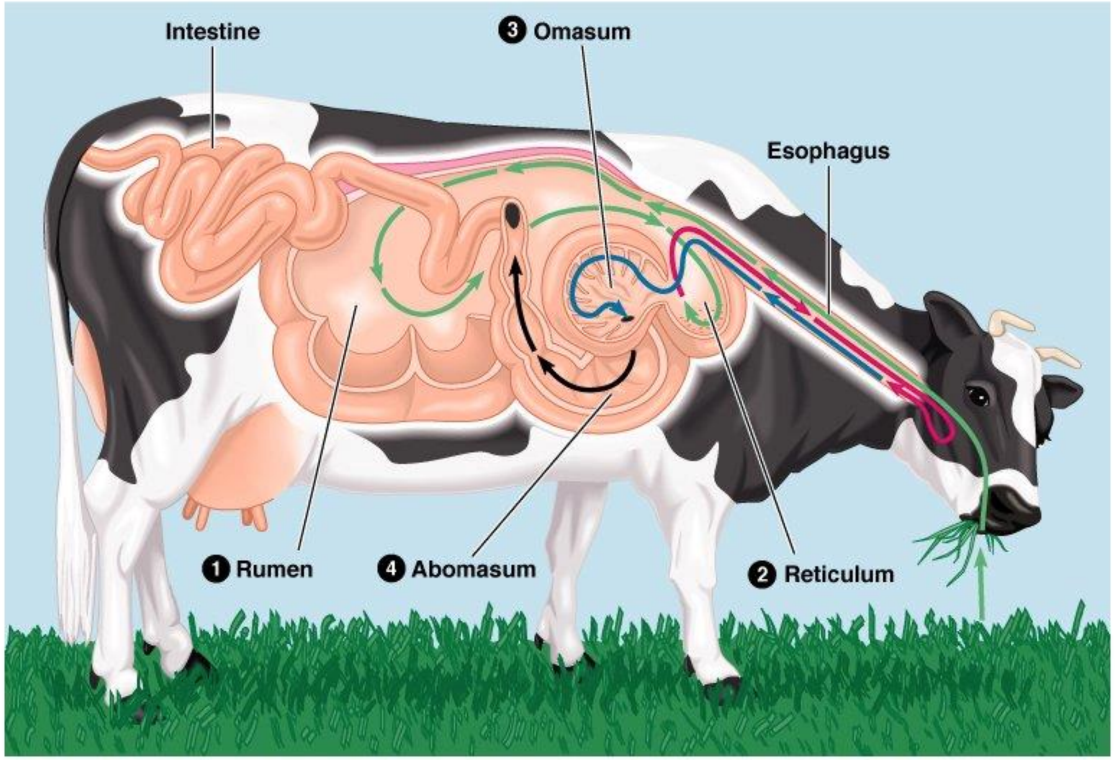
“Four Stomachs”

Rumen - “Fermentation Vat”

Reticulum – Solids separation, retention of large feed particles; additional fermentation

Omasum - Water and salts absorption

Abomasum - Acidic digestion of proteins to amino acids and of carbohydrates



Rumen

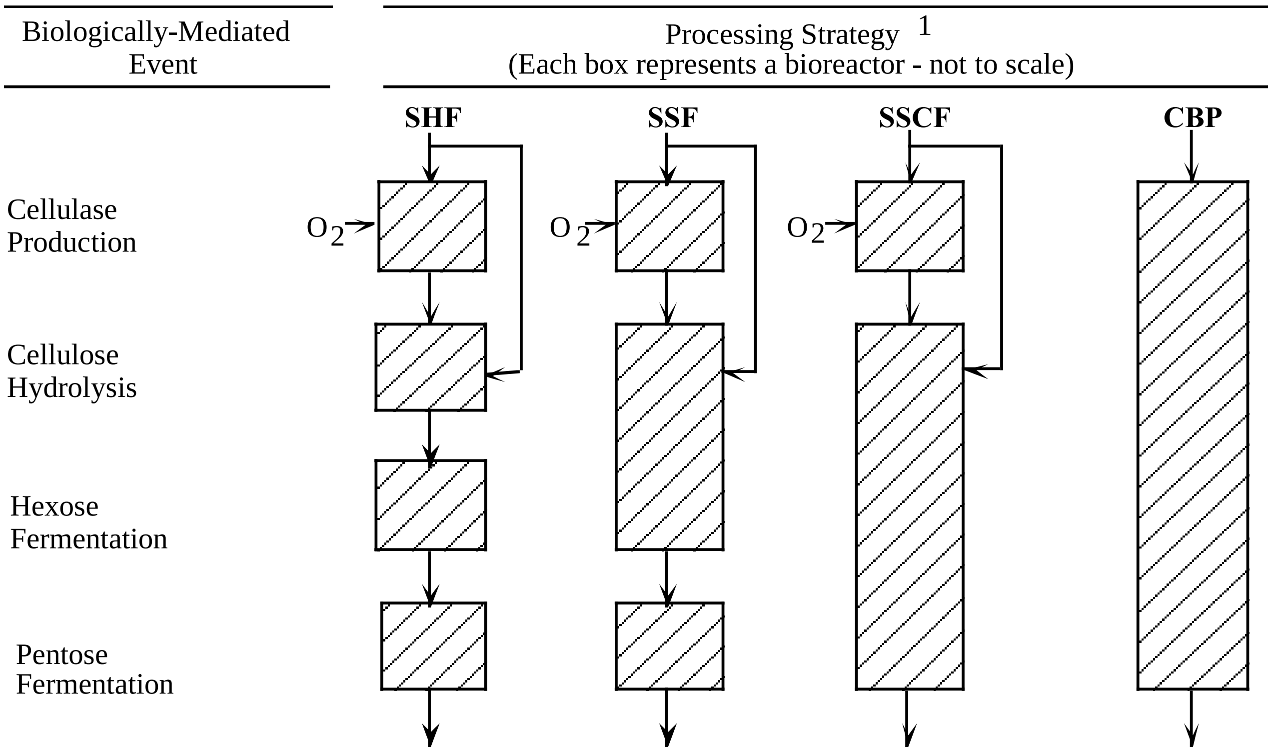
Reticulum

Omasum

Abomasum

courses.washington.edu

Biomass Conversion Schemes

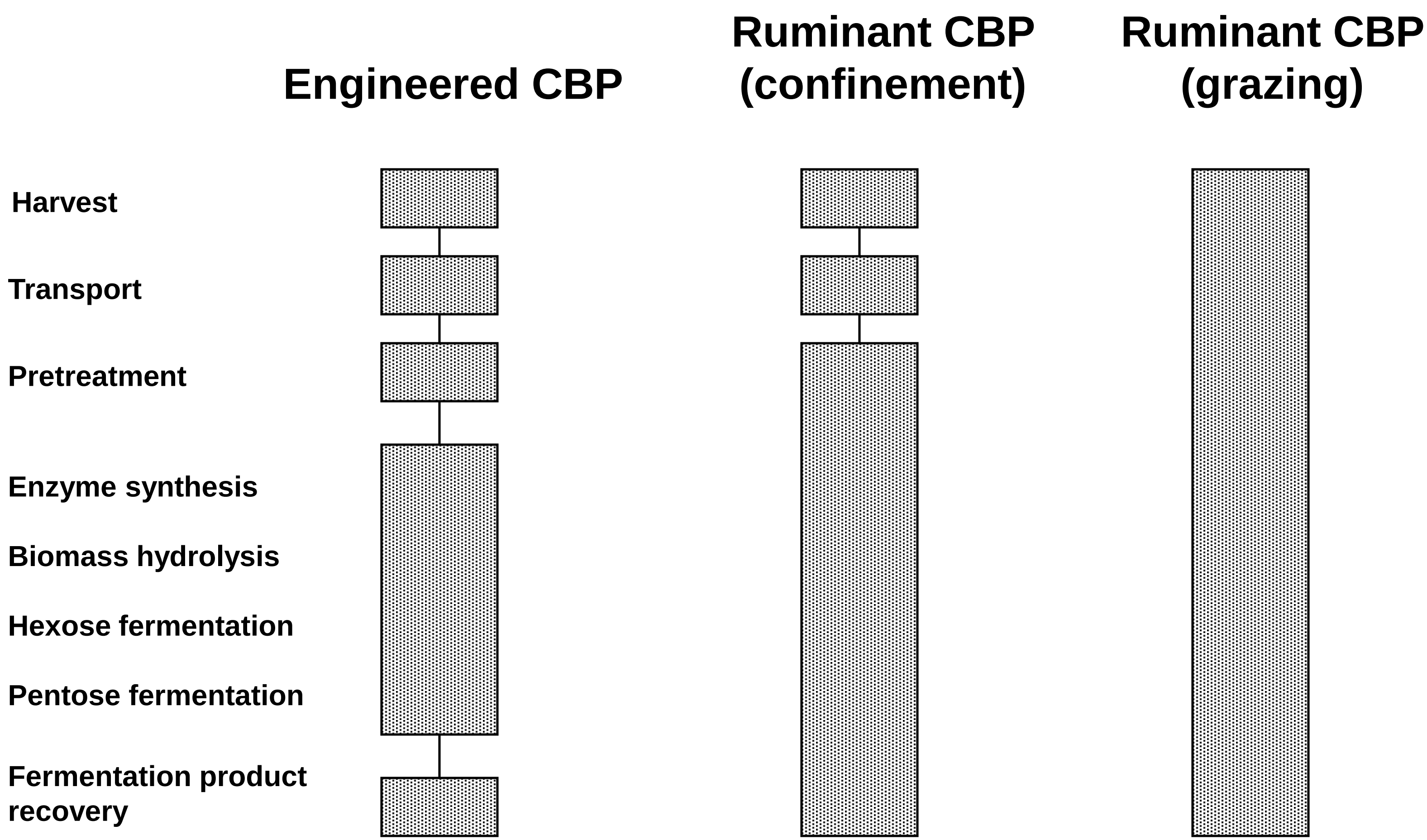


[1] SHF: Separate Hydrolysis & Fermentation; SSF: Simultaneous Saccharification & Fermentation
SSCF: Simultaneous Saccharification & Co-Fermentation; CBP: Consolidated Bioprocessing

Lynd et al., Microbiol. Mol. Biol. Rev. 66:507(2002)

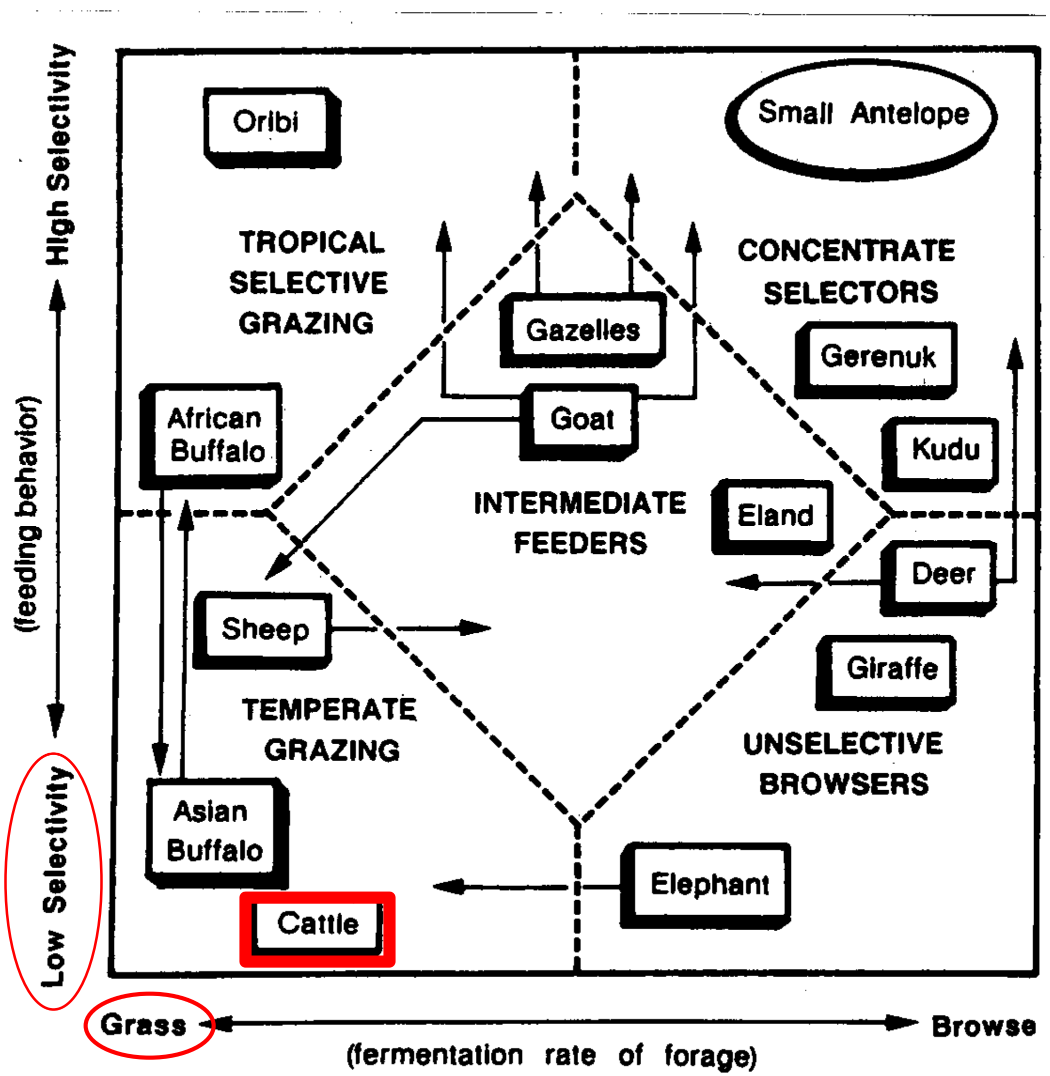
Consolidated Bioprocessing (CBP) is carried out exclusively by anaerobic bacteria that produce their own fibrolytic enzymes and that ferment the products of polysaccharide depolymerization to ethanol and/or other products.

Consolidated Bioprocessing: Extended View



The ruminant animal further consolidates CBP

Diversity in Ruminant Feeding Behavior



P.J. Van Soest, Nutritional Ecology of the Ruminant, 2nd ed., Comstock Publishers, Ithaca, NY (1982)

Cattle are extreme among ruminants with respect to their nonselective feeding on highly fibrous feedstuffs.

Cattle are optimally adapted to utilize the most recalcitrant plant biomass.

Bovine Grazing Behavior

	Low pasture allowance (25 kg DM/cow/d)		High Pasture Allowance (41 kg DM /cow/d)	
Grazing Behavior	Unsuppl.	Suppl.	Unsuppl.	Suppl.
Grazing time (min/d)	609	534	626	522
Bites/min	56	54	56	55
DMI/bite (g)	0.55	0.55	0.60	0.59
Bites/d *	34,100	28,840	35,050	28,700
Intake (kg DM/d)				
Pasture	17.5	15.5	20.5	16.1
Concentrate	--	8.7	--	8.7
Total	17.5	24.2	20.5	24.8

Bargo et al., J.Dairy Sci. 85:1777 (2002)

**** Just eating -- Does not include rumination!***

Biomass Pretreatment

Chemical

(dilute H_2SO_4, AFEX, DEO,etc.)

Can be highly effective

Substantial negatives

- Reagent costs
- Energy costs
- Feedstock loss
- Fermentation inhibitors
- Waste disposal

Physical

(grinding, etc.)

Generally less effective

Primary negatives are capital and energy costs

Physical pretreatment is the only option for the ruminant animal

Rumination: Effective physical pretreatment

Grazing ruminants pioneered the concept of angular, variable speed grinding of biomass.

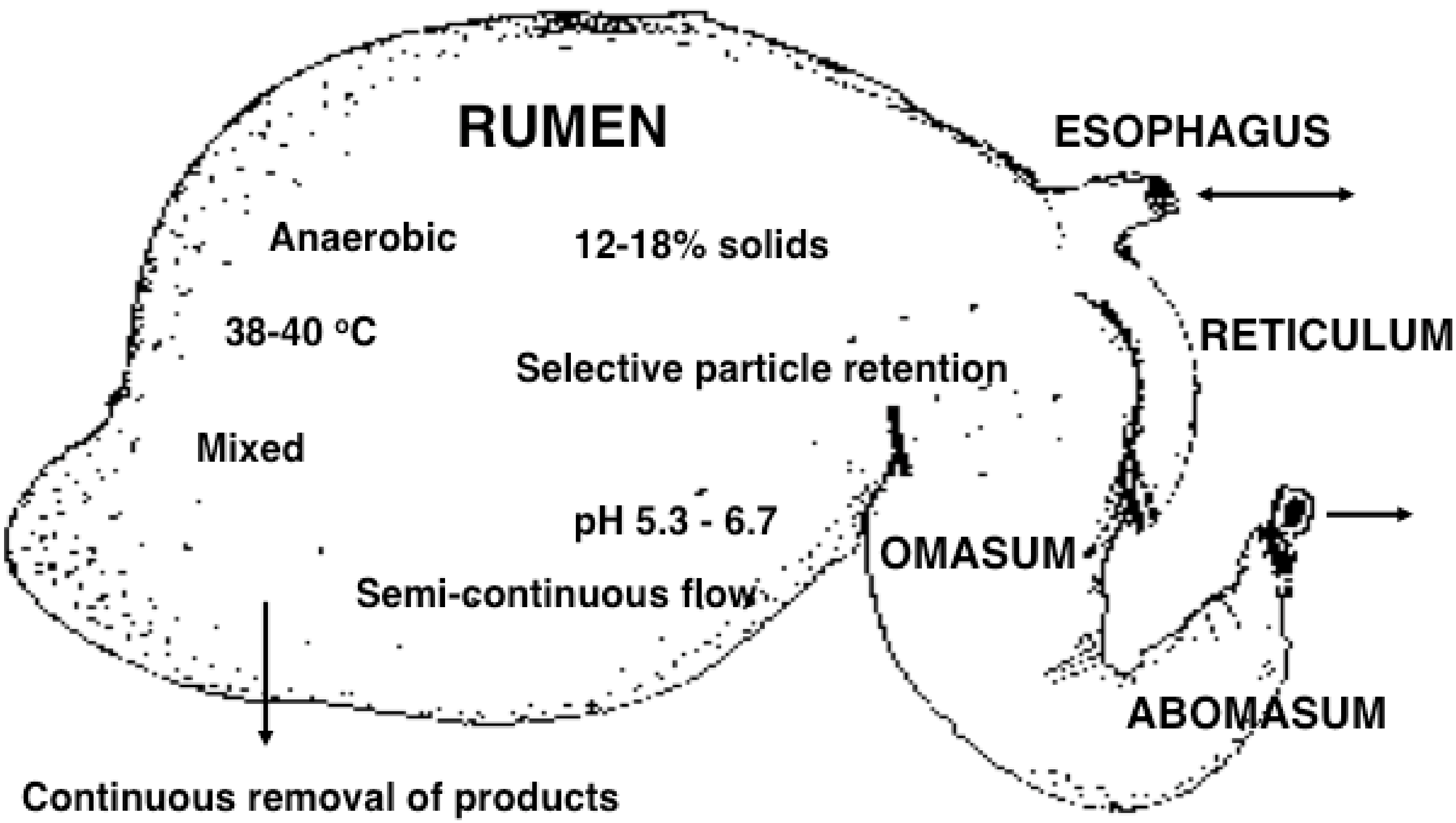
Frequent re-chewing (rumination) of wetted ingested feed delaminates plant fiber, increasing surface area while maintaining fibrous nature of the remaining material.

- Exposes plant tissues to fibrolytic microbes
- Maintains adequate particle size for rumen function

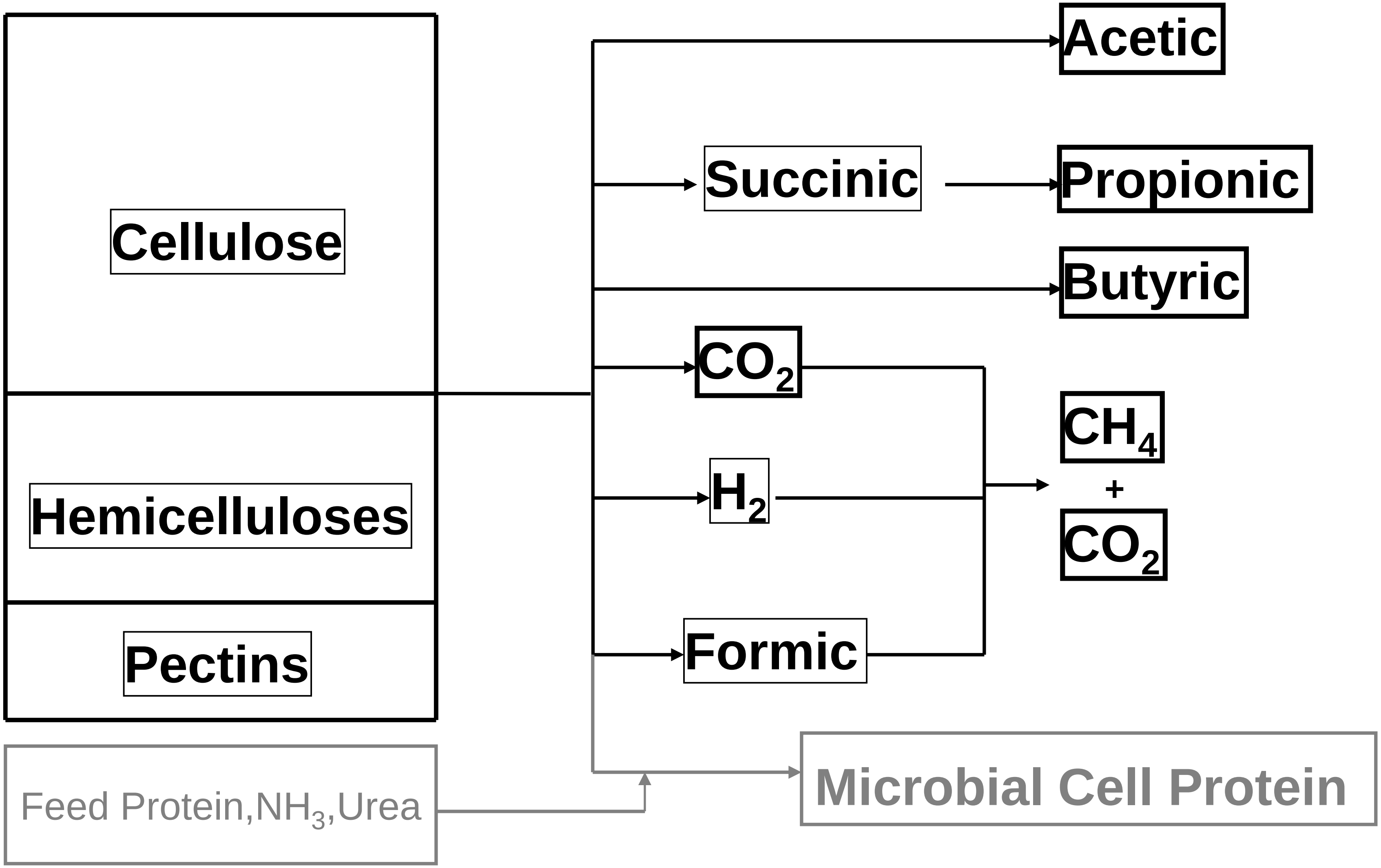
Rumination surprisingly efficient from an energy standpoint (~1% of the gross energy of the feed).

The rumination model might be useful in an industrial biomass fermentation.

The rumen is a highly efficient natural bioreactor for processing plant biomass



Ruminal Biomass Fermentations



The major groups of ruminal microbes and their primary roles

BACTERIA

Ferment fiber, starches and sugars in feeds to VFA, H_2 and CO_2

Produce most of microbial cell protein, but also ferment feed proteins to VFA + NH_3

Alter toxicity of plant metabolites

Bacteroidetes (*Prevotella*)
Firmicutes (*Ruminococcus, Lachnospira, Butyrivibirio*)

ARCHAEA

Thermodynamic displacement, producing methane

Methanobrevibacter

FUNGI **Assist in fiber degradation**

Neocallismastix

PROTISTS

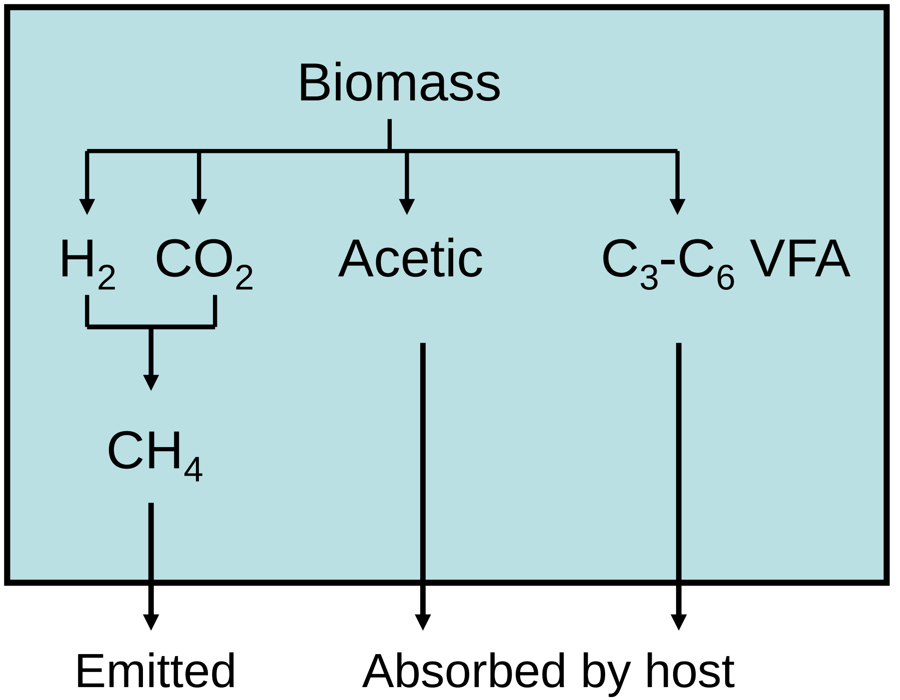
Consume and ferment bacteria to VFA + NH_3

Sequester and ferment starch

Recycle N

Isotrichs
Holotrich ciliates
- many contain endosymbiotic bacteria and archaea

Rumen fermentation

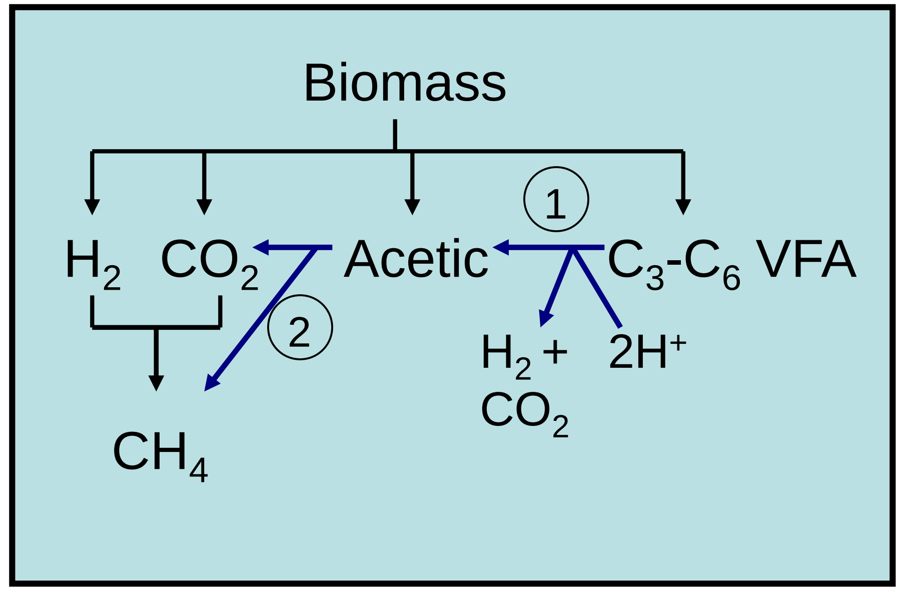


Modest methane production

Most of the feed energy is retained in the form of VFA

Short retention time (0.3 to 2 d)

"Anaerobic Digestion"

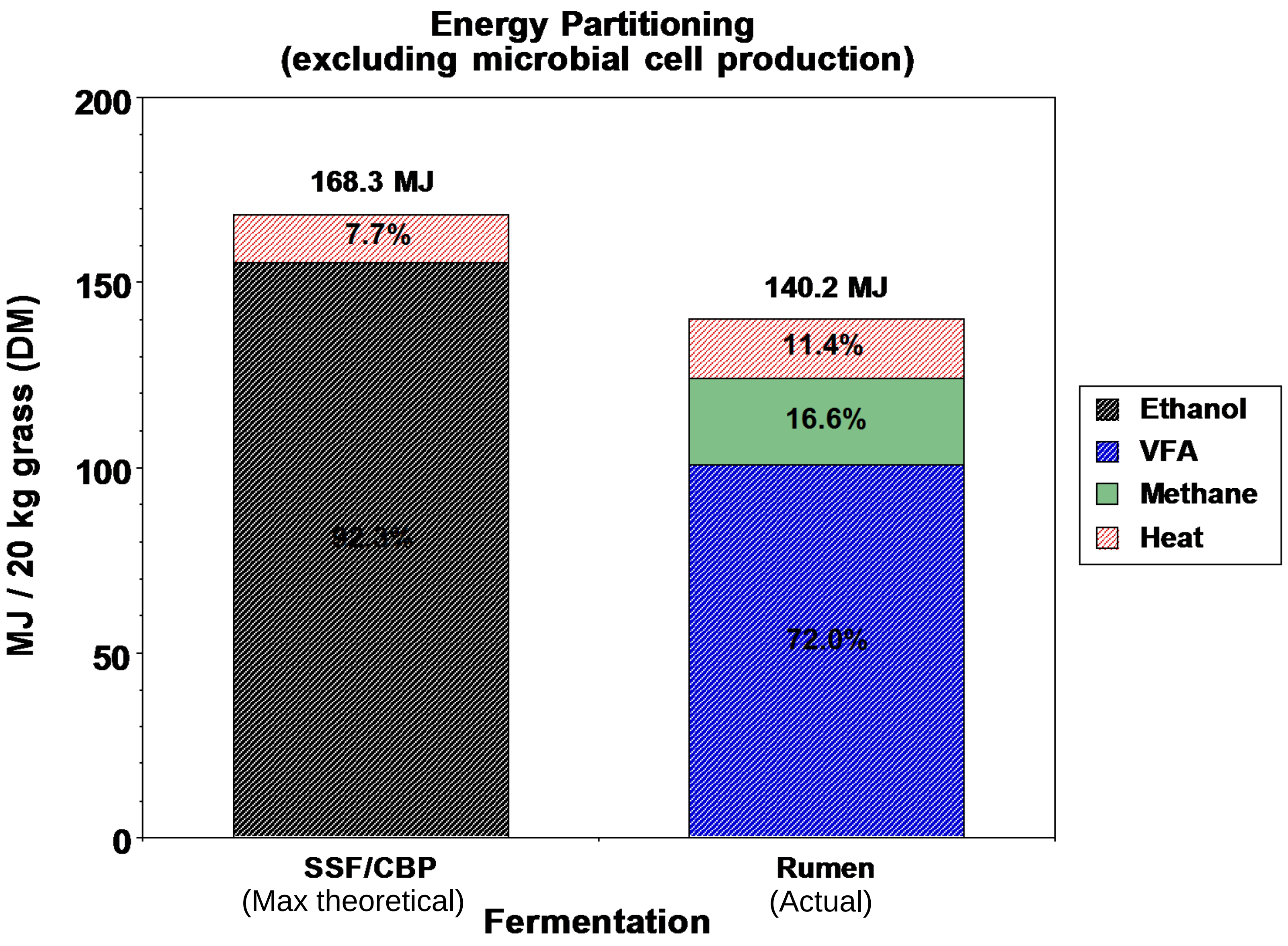


1 Proton-reducing acetogens

2 Aceticlastic methanogens

Long retention time (2 to 3 wks)

Cellulosic ethanol vs. Ruminal VFA production



“Extraruminal” biomass fermentation to VFA

Features in common with AD

- Wide substrate range (not just CHO)
- Non-aseptic operation
- Low operating energy (modest temp, minimal mixing)
- In vitro operability and scalability

Features not typical of AD

- Microbial community proficient in fermentation of fibrous biomass
- Microbial community highly tolerant to VFA
- Short cycle time (2-3 d)

In vitro fermentation of biomass components

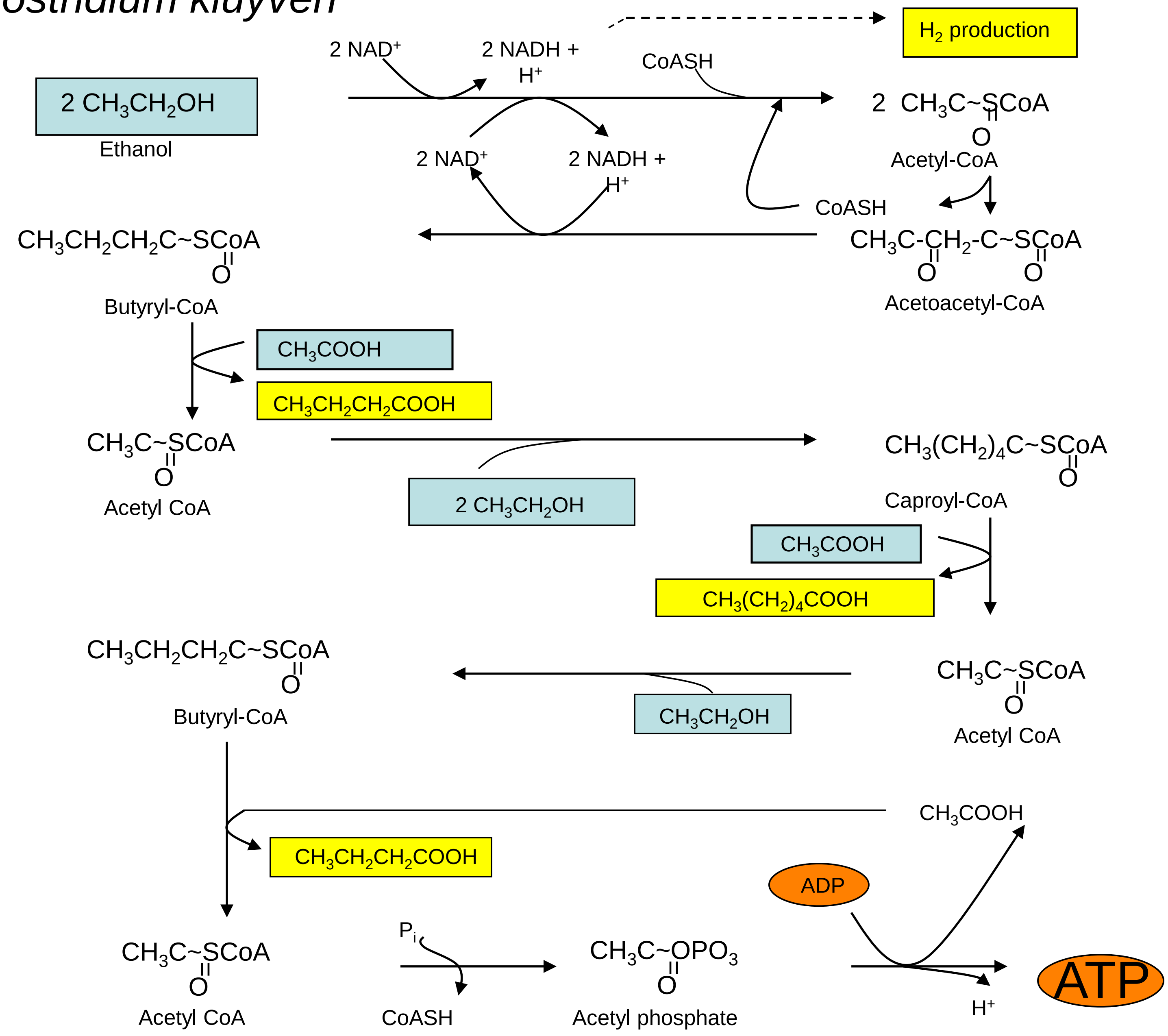
Substrate	*g product / g added substrate*							*mmol alkyl/g*
	Ace	*Pro*	*But*	*Val*	*BCVFA*	*TotVFA*	*Methane*	
Cellulose	**0.258**	**0.337**	**0.040**	**0.015**	**0.011**	**0.661**	**0.027**	**15.75**
TS xylan	**0.297**	**0.285**	**0.031**	**0.003**	**0**	**0.615**	**0.042**	**14.08**
Birch xylan	**0.334**	**0.282**	**0.036**	**0.008**	**0.001**	**0.656**	**0.042**	**14.70**
CS hemicellulose	**0.253**	**0.251**	**0.029**	**0.008**	**0**	**0.534**	**0.049**	**12.75**
Alfalfa pectin	**0.337**	**0.140**	**0.034**	**0.015**	**0.016**	**0.542**	**0.049**	**11.88**
Corn starch	**0.250**	**0.271**	**0.066**	**0.023**	**0**	**0.612**	**0.038**	**14.73**
Soy peptone	**0.154**	**0.065**	**0.084**	**0.082**	**0.136**	**0.520**	**0.026**	**15.53**
Dist dried grains	**0.182**	**0.162**	**0.044**	**0.029**	**0.034**	**0.441**	**0.028**	**10.93**
Bacterial cells	**0.104**	**0.027**	**0.017**	**0.014**	**0.036**	**0.198**	**0.011**	**5.54**
DNA	**0.052**	**0.033**	**0.009**	**0**	**0.008**	**0.102**	**0.006**	**6.49**

Results are from 72 h fermentations and were corrected for product formation in control vials containing ruminal inocula but lacking added substrate. CO_2 was not quantified.

VFA chain elongation

- Mixed ruminal cultures, both in vivo and in vitro, display rapid fermentation of feedstuffs to C_2-C_4 VFA, with only low yields of C_5-C_6
- Prolonged incubation and/or suppression of methanogenesis does not typically result in additional chain elongation
- VFA chain-elongating strains are present in very low densities in ruminal fluid but are readily isolated in pure culture
- Manipulation of substrate and operating conditions can facilitate chain elongation

Clostridium kluyveri



Ruminal VFA chain elongators

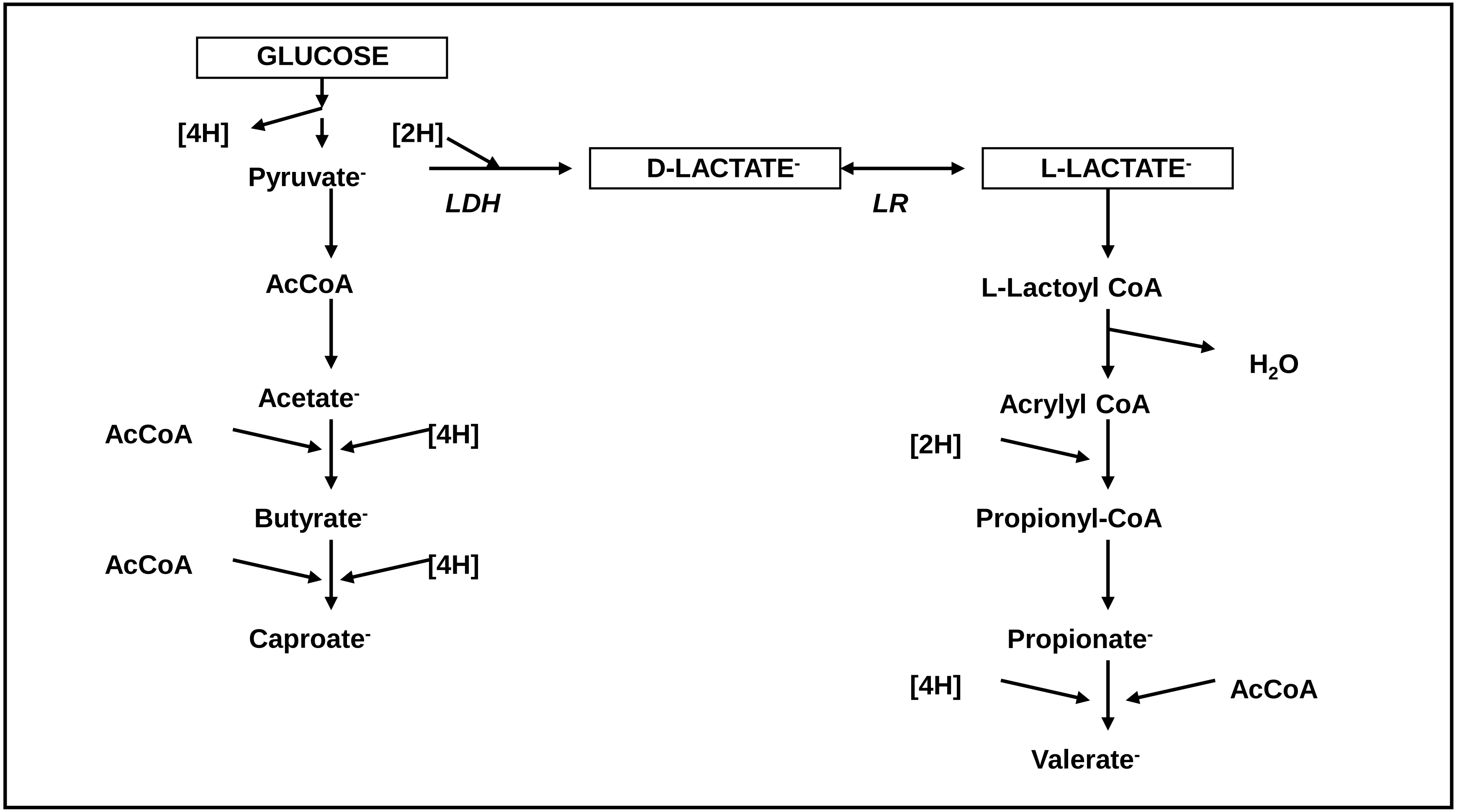
Clostridium kluyveri

- Readily isolated as a transient from the rumen
- Converts EtOH + C_2 → C_4 → C_6 by RBO

Strain ATCC 8527 produced 4.6 g C_6/L in 6 d from cellulose and EtOH in coculture with the ruminal cellulolytic *Rumincoccus flavefaciens* FD-1 (Kenealy et al.1995)

Ruminal isolate 3231B produced 13.2 g/L (110 mM) C_6 in 3 d from EtOH + C_2 (Weimer and Stevenson 2012)

Megasphaera elsdenii



Normal member of ruminal community, <0.01 to 4% of bacterial 16S rRNA gene copy number

M.e. strain ATCC 25940 produced 11.4 g C_6/L in 8.3 d from Glc (Roddick and Britz, 1997)

M.e. strain MH produced 9.7 g C_6/L in 1 d from Fructose + Ace + But (Jeon et al. 2016)

Multiple-cycle in vitro fermentations

Medium (per L): 60 g switchgrass,12 g DDGs, 20 g $CaCO_3$ in Na_2S-reduced, half-strength Goering/Van Soest buffer, CO_2 gas phase, 10% inoculum.

↓ *Non-aseptic, static incubation, 39 C*

Cultures transferred (~10% v/v) on same medium at 3-4 d intervals for 160 d

↓

Same medium + Ethanol (9.4 -15 g/L)

↓ *Non-aseptic, static incubation, 39 C*

Cultures transferred (~10% v/v) at 3-4 d intervals for additional 740 d

Successive twice-weekly transfer of extraruminal cultures

Time range	**mL gas**		**Final pH**		**mM Total VFA**		**mM Alkyl**		**Average chain length**	
SWG	Enrich A	Enrich B	Enrich A	Enrich B	Enrich A	Enrich B	Enrich A	Enrich B	Enrich A	Enrich B
0-202 d	132.3	131.1	5.46	5.47	175.2	176.4	322.7	320.1	2.85	2.82
206-398 d	98.7	99.6	5.88	5.85	150.0	148.0	272.1	268.4	2.82	2.82
402-900 d	88.9	89.3	5.93	5.94	122.2	125.3	205.5	211.1	2.69	2.69
All (3-900 d)	101.1	101.6	5.81	5.81	139.9	140.6	245.7	247.9	2.75	2.74
SWG+EtOH										
3-98 d	109.3	107.2	5.33	5.35	183.6	183.2	326.7	341.0	2.79	2.89
101-200 d	91.5	96.2	5.44	5.31	195.2	216.7	333.5	363.7	2.74	2.71
203-299 d	93.9	94.4	5.49	5.35	235.2	228.1	345.2	331.1	2.49	2.46
302-400 d	92.0	88.9	5.08	5.16	281.8	263.1	383.7	369.2	2.39	2.42
404-498 d	91.5	88.4	5.11	5.19	275.4	252.0	376.3	357.4	2.40	2.44
502-600 d	79.6	79.9	5.20	4.86	215.9	298.7	308.0	395.4	2.48	2.33
603-736 d	80.4	74.9	5.07	5.10	256.2	238.7	340.4	322.9	2.36	2.38
All (3-736 d)	89.5	88.3	5.24	5.17	236.5	238.8	346.7	352.3	2.52	2.52

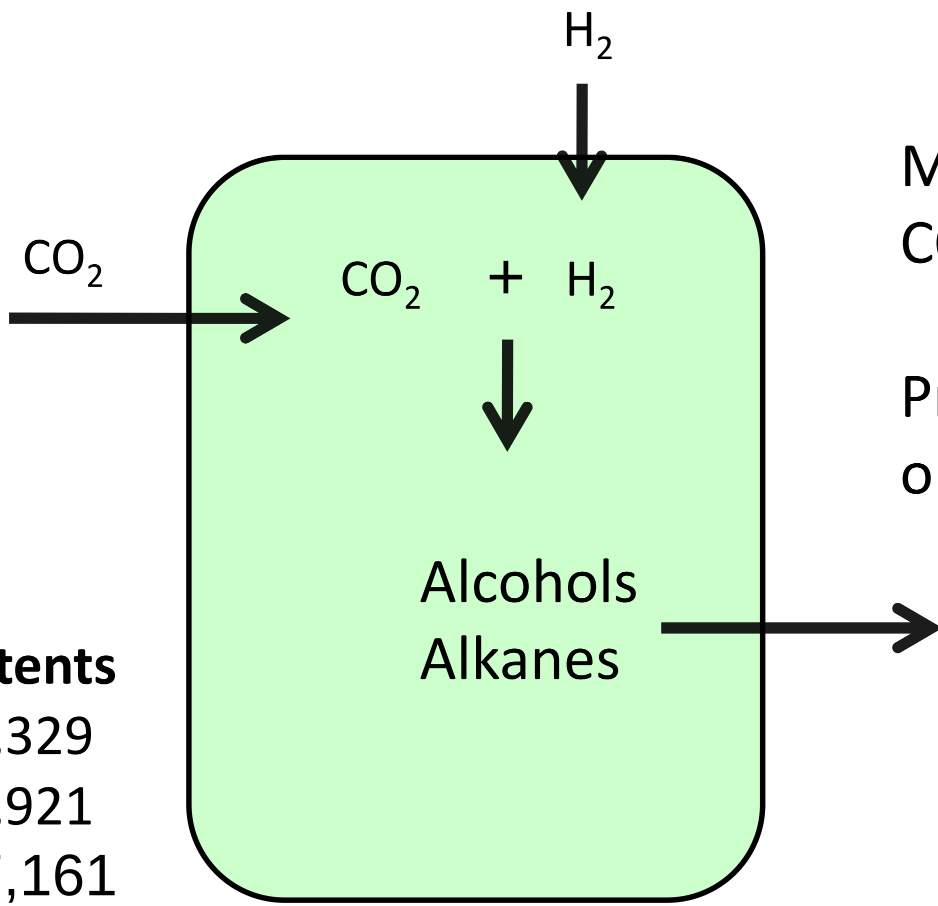
Reduction of VFA by Ruminal Microbes (Kohn, U. of MD)

- Organisms exist in nature that can tolerate high concentrations of alcohols, acids, or even hydrocarbons.

  e.g. organisms are found growing in stored gasoline.

- Whether those organisms degrade or synthesize desired products depends on thermodynamics.
  - Conditions can be created to make it thermodynamically feasible to produce high concentrations of the desired products, and under those conditions, the products are made and organisms can be isolated.

Electrofuels
(Kohn, U. of MD)



Microorganisms use
CO_2 and H_2

Produce lower alkyl alcohols
or alkanes

Electrofuels

US Patents
8,178,329
8,535,921
9,217,161

Summary

- Ruminants combine a novel physical pretreatment with a diverse microbial community to enable fermentation of relatively recalcitrant biomass to VFA
- Unlike conventional AD, ruminal fermentations rapidly produce VFA in high yield with only modest methane production
- Extraruminal fermentations are relatively robust but currently require exogenous electron donors to produce C_5-C_6 VFA in high yield
- Additional metabolic capabilities, such as VFA reduction to alcohols and hydrocarbons, merit further investigation