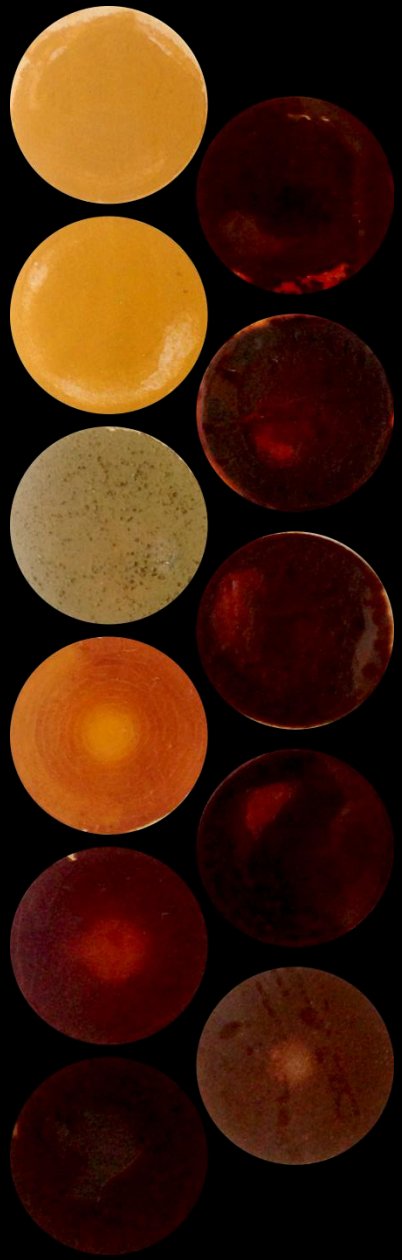
Microbiology of mixed culture beers

Caleb Levar




ET'S
DO
THIS.

Mixed cultures and their influence on beer production

Prior to Pasteur, ALL beer production influenced by microbes in addition to *Saccharomyces spp.*

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Prior to Pasteur, ALL beer production influenced by microbes in addition to *Saccharomyces spp.*

1) Suppress influence by hops, cold temps, rapid consumption, etc.

OR

2) Embrace the influence of “wild” yeast and bacteria

Why mixed cultures?

"…if the application of the pure culture method has improved the average quality of the beer, if it has decreased the chances of infection, **it has given us beer with less character than before**."

Marc H. Van Laer, 1920's

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Dry (0-2 °P, vs 2-4+ °P for pure cultures)

Tart → Sour (pH 3.2-3.9, lactic and acetic acid)

Funky (Phenols, acids, esters)

A note on nomenclature…

“Sour” beer…

vs.

“Wild” beer…

vs.

Something else entirely

Similar microbes across techniques

Where to get the microbes?

- Commercial organisms
- Barrel microbes, bottle dregs
- “Spontaneous” inoculation

Beijerinck and Baas-Becking : “Everything is everywhere, but the environment selects”

What's happening here?

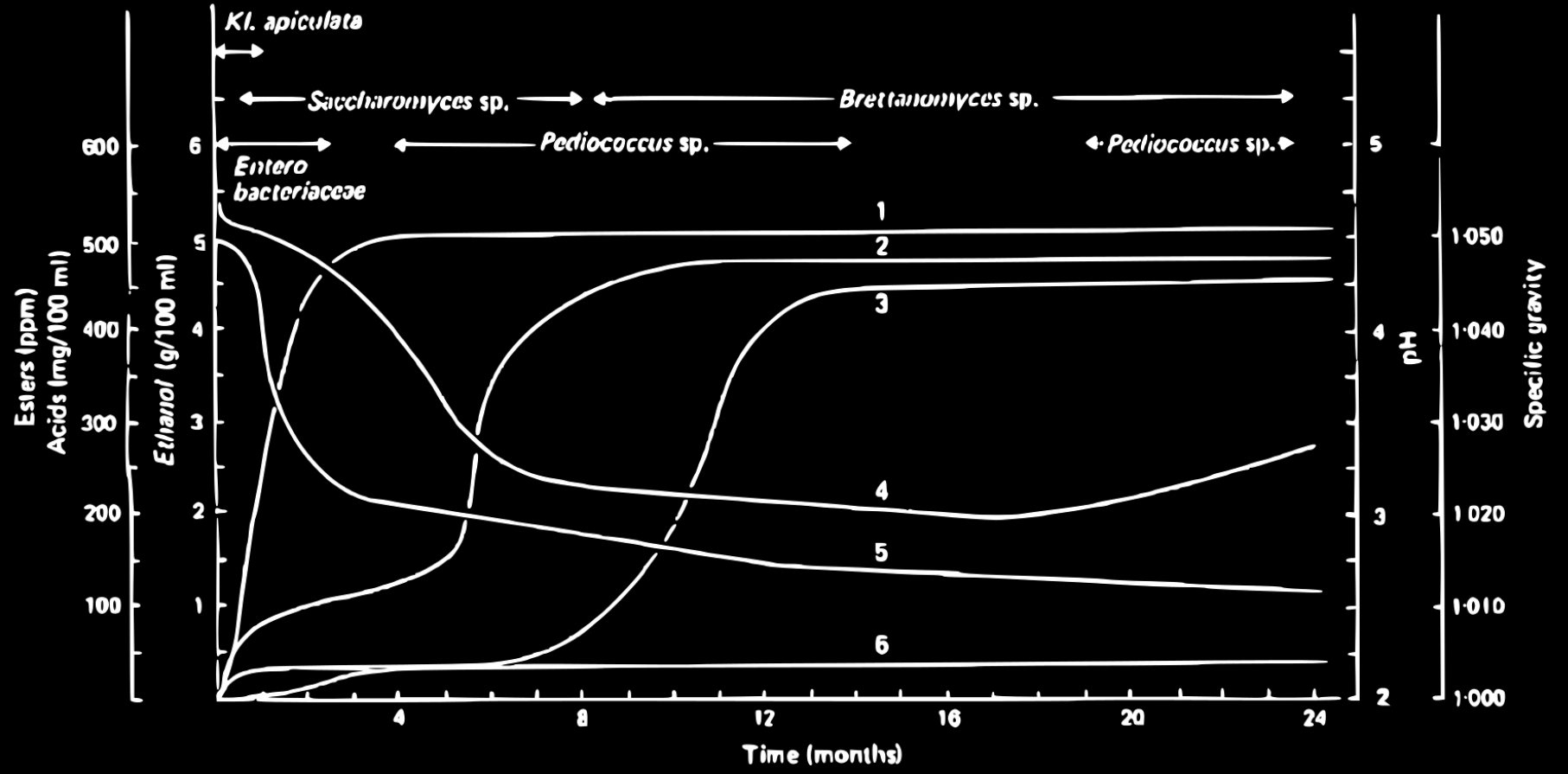
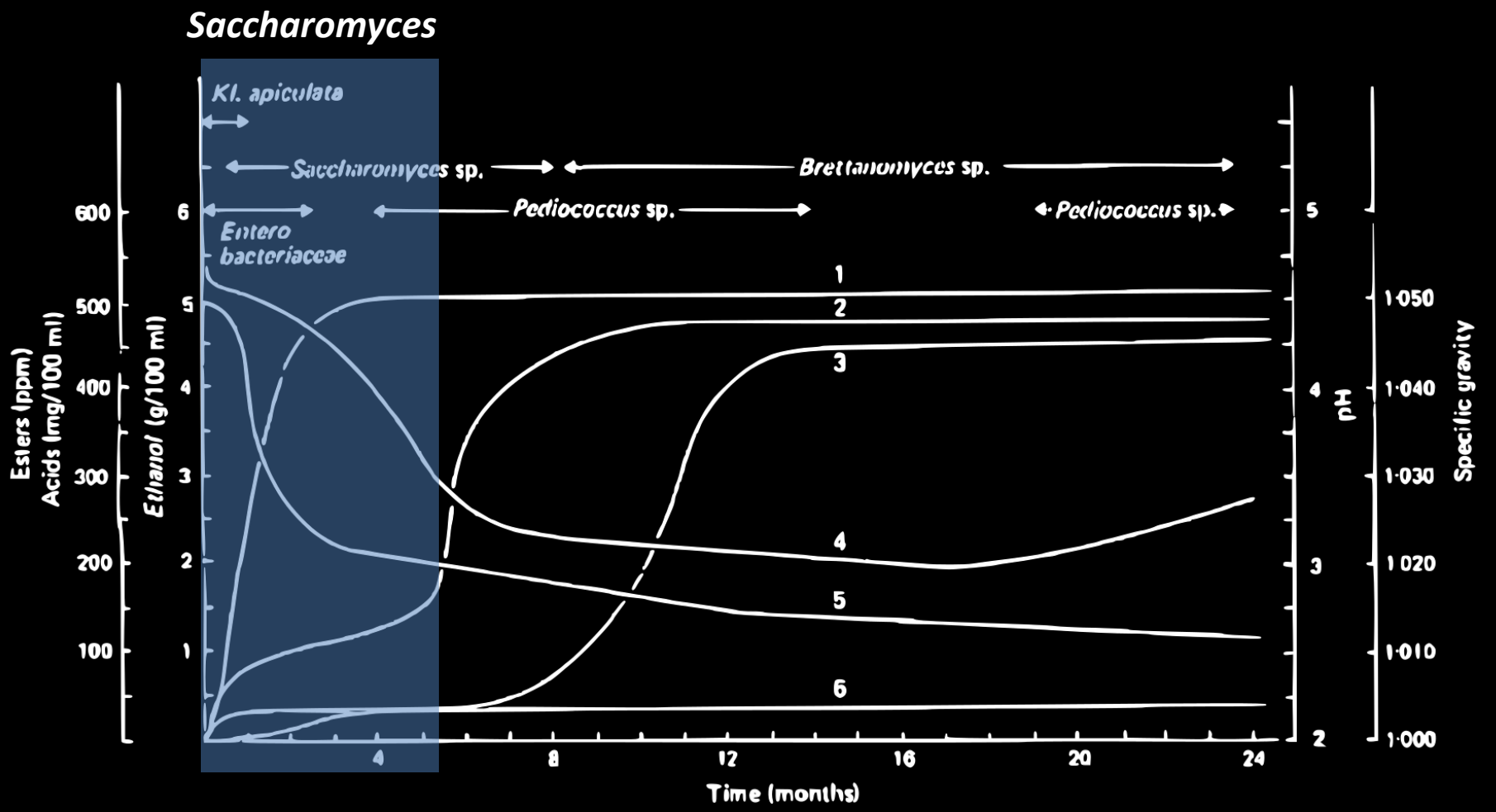


Fig. 3. Evolution of some important parameters of spontaneous Lambic fermentation: 1 = ethanol; 2 = lactic acid; 3 = ethyl-lactate; 4 = pH; 5 = real extract content; 6 = acetic acid.

van Oevelen et al, 1977

Microbial succession in wort/beer



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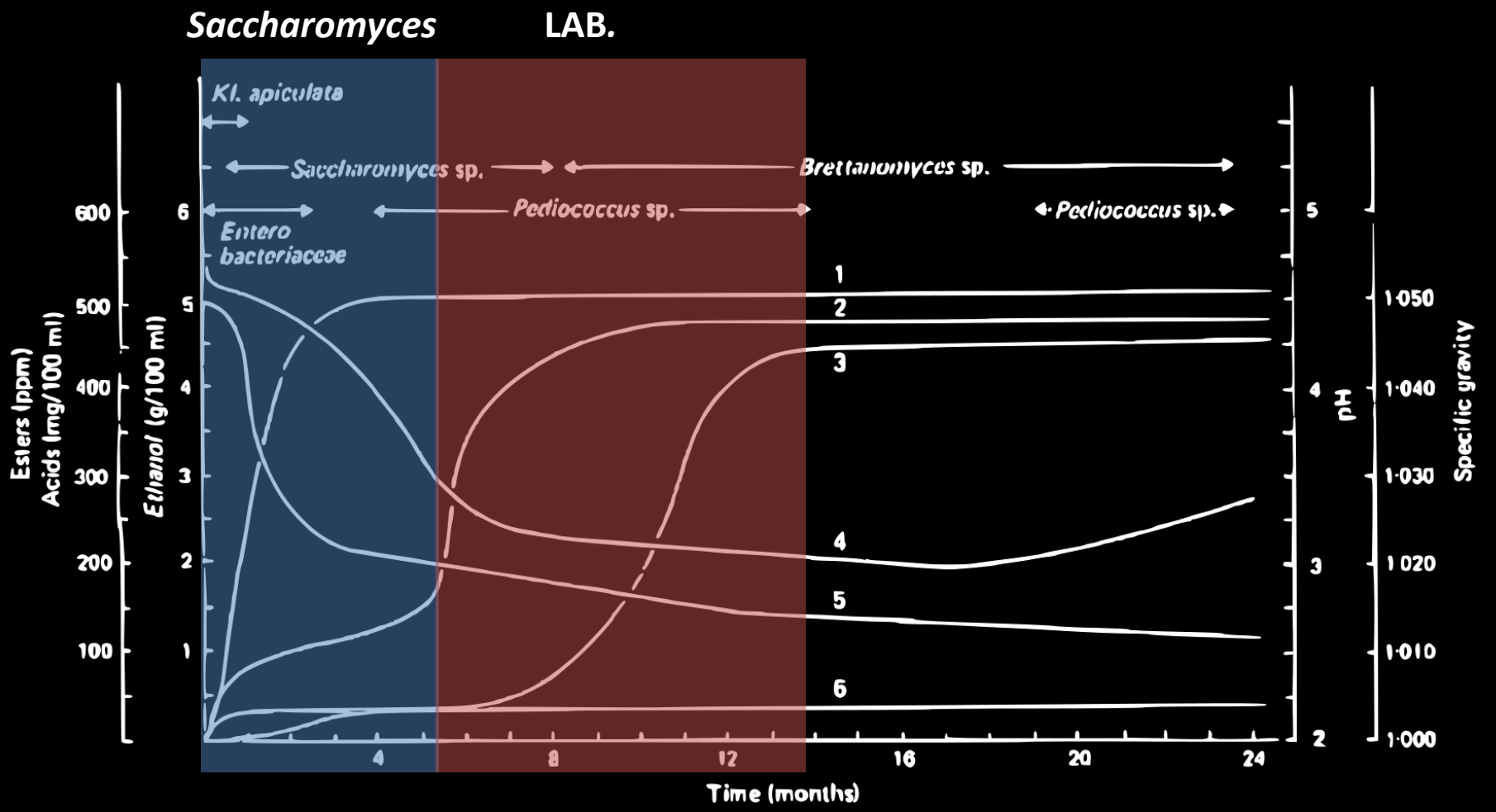

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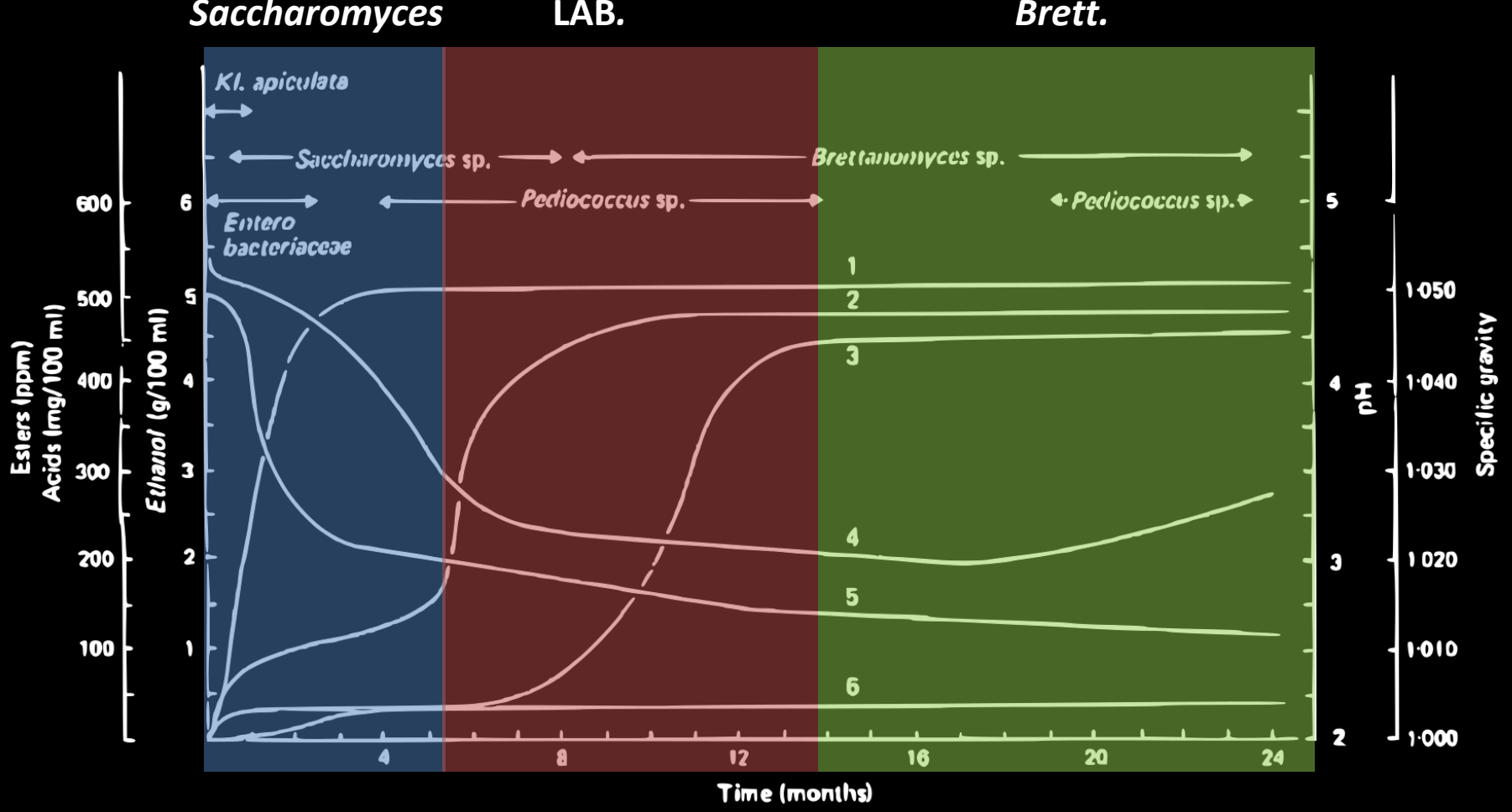

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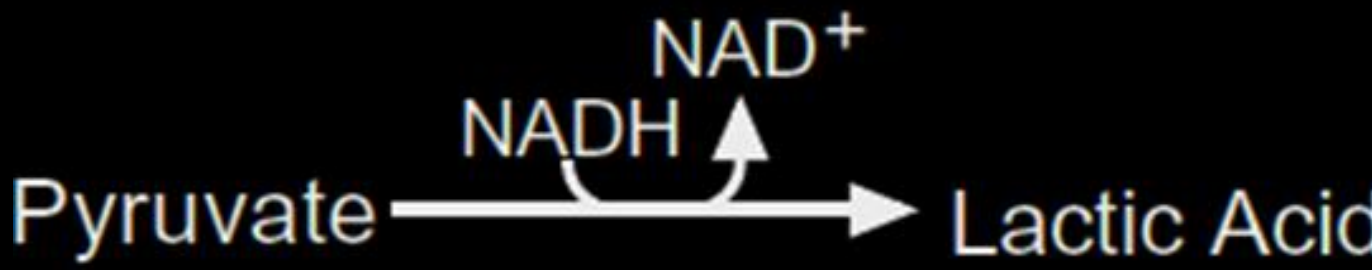
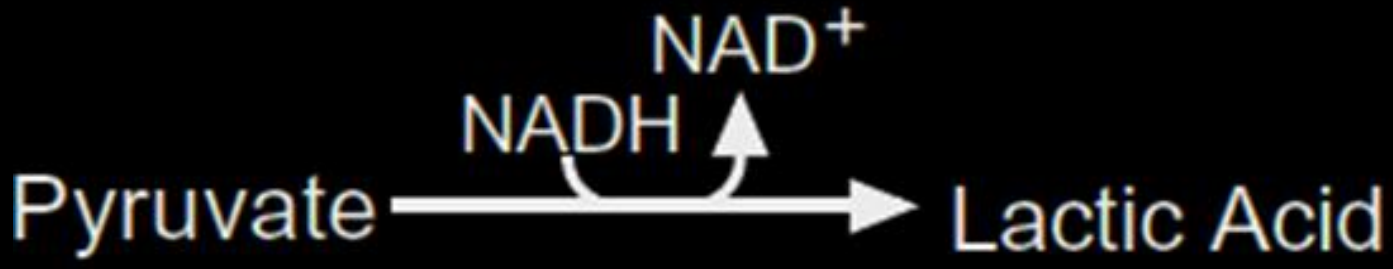
Main microbial players

- *Saccharomyces spp.*
  - Standard brewers yeast: sugar → alcohol, CO_2
- *Lactobacillus spp.*
  - Lactic acid bacteria: sugar → lactic acid
- *Pediococcus spp.*
  - Lactic acid bacteria: sugar/starch → lactic acid
- *Brettanomyces spp.*
  - "Wild" yeast: sugar → alcohol, CO_2, "funk"

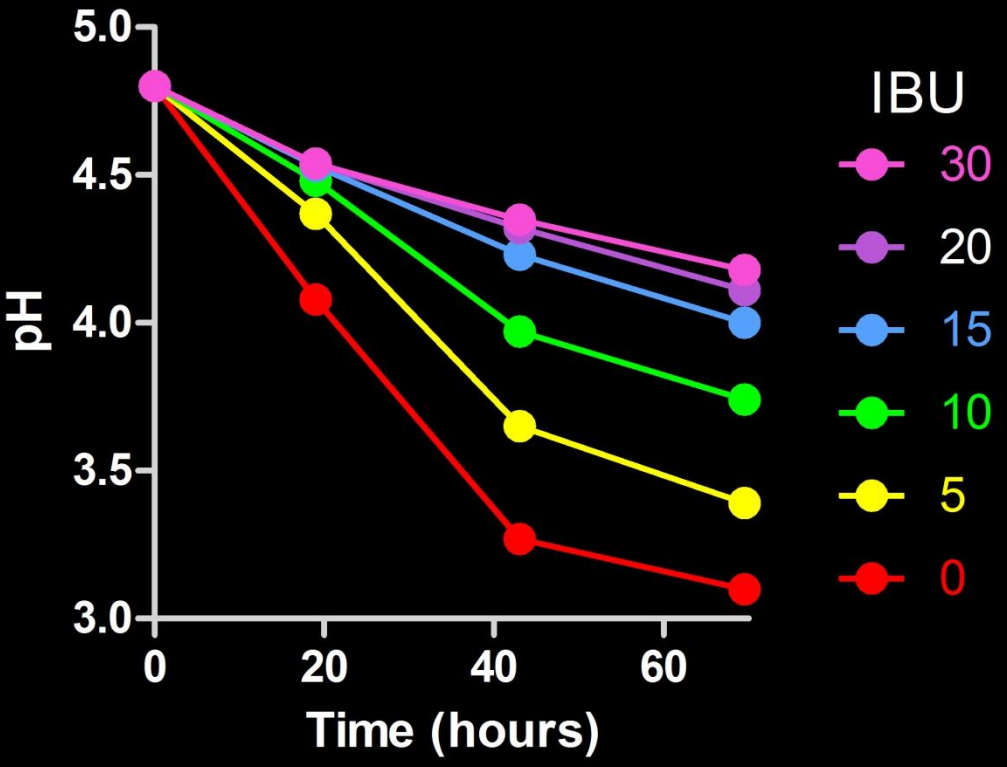
Lactobacillus for hot side sours



Lactobacillus for hot side sours

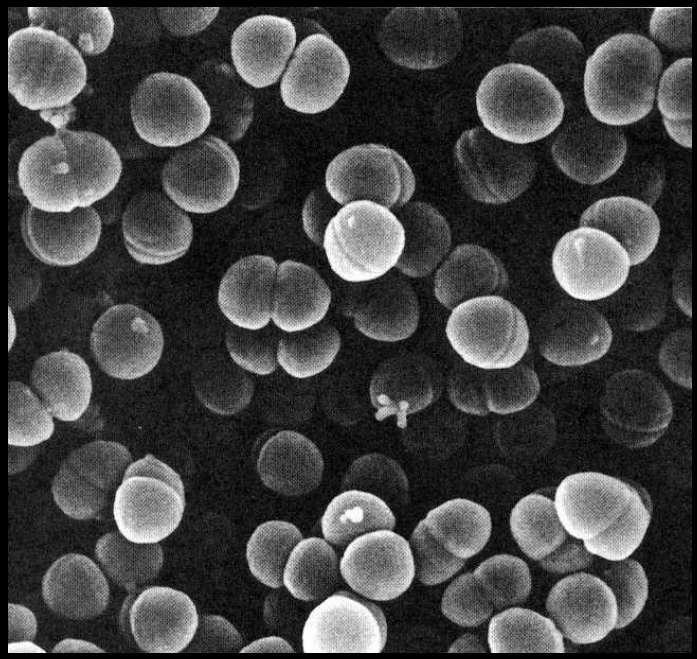


- LAB added to wort
- pH drop monitored
- Wort boiled to kill LAB
- *Sacch.* pitched into cooled wort



Pediococcus for acid production

- Beer conditions favor *Pedio. spp.* over other LAB
  - More tolerant of low pH
  - More tolerant of hops
  - Able to hydrolyze starch



Undesirable byproduct formation by *Pedio. spp.*

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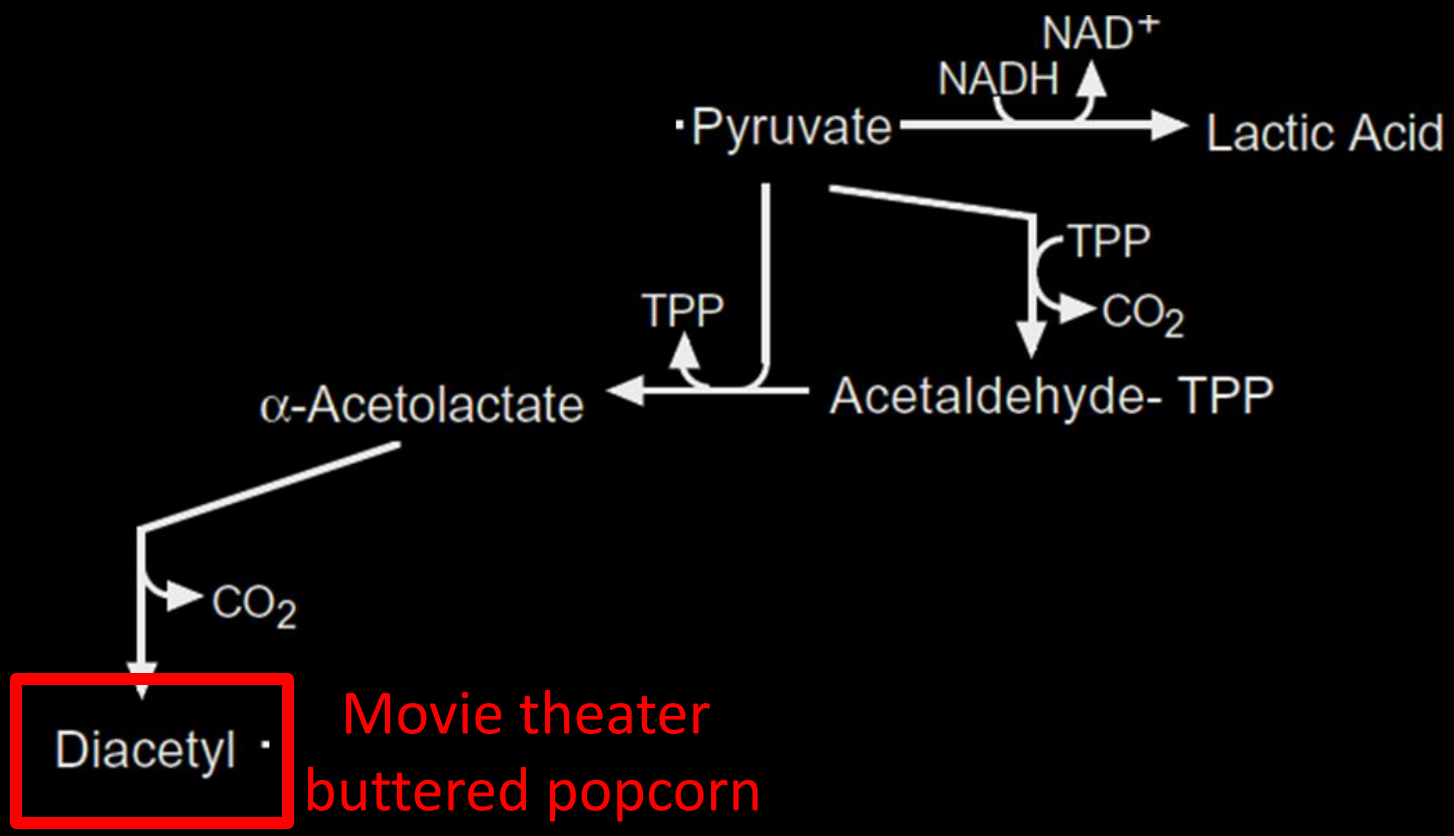


Movie theater buttered popcorn

Undesirable byproduct formation by *Pedio. spp.*

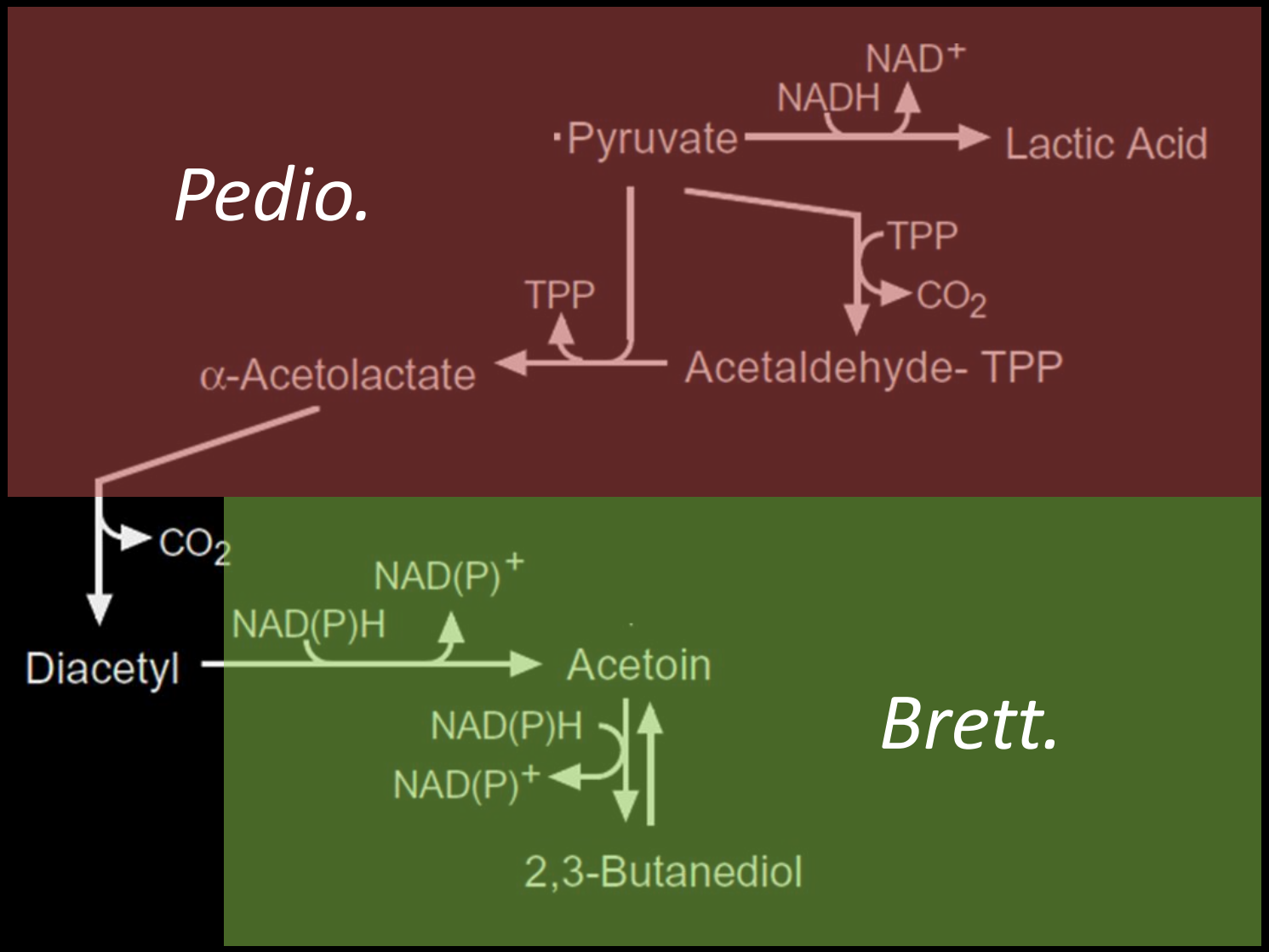
TABLE III
Effect of Carbon Source on Slime Production

Substrates	Concentration (g/L)	Relative Amount of Slime[a]
Glucose	5	++
	10	+++
	20	+++
	40	+++
Maltose	10	+++
Saccharose	10	+
Galactose	10	±
Lactose	10	±
Fructose	10	+++
Glucose and fructose	5 + 5	+++

[a] ± = Only cell deposit is viscous.
+ = Threads < 1 cm.
+++ = Threads > 5 cm.

D. Van Oevelen and H. Verachtert, 1979

Brettanomyces for cleanup



Brettanomyces for cleanup

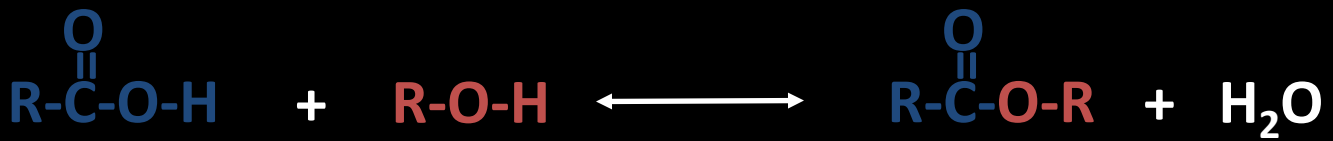
Yeast Added to 3-Week Culture	Viscosity (% S)	
	At Inoculation	After 2 Weeks
None	87	51
S. cerevisiae	87	55
B. bruxellensis	87	27
B. lambicus	87	32

D. Van Oevelen and H. Verachtert, 1979

Brettanomyces for "funk"

Brettanomyces **yeasts show esterase activity towards a large number of esters but especially towards esters normally found in Gueuze: ethyl acetate, ethyl lactate, *iso*-amyl acetate and phenethyl acetate. The esterase activity is expressed by whole cells of all *Brettanomyces* strains in**

- Esterification!



- Need an acid and an alcohol
- It's all about the precursors
  - Can come from *Sacch.*, *Brett.,* other microbes, ingredients, etc.

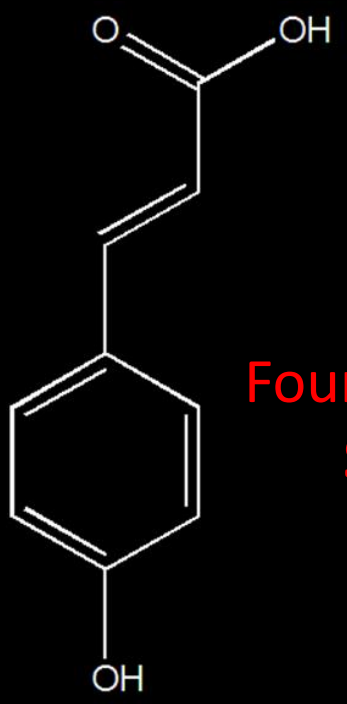
M. Spaepen and H. Verachtert, 1982

Brettanomyces for "funk"

Acid	Character	Ester	Character
Acetic	Sour, Pungent, Vinegary	Ethyl Acetate	Sharp, Musty, Fruity, Pineapple, Solvent, Nail Polish
Lactic	Tart, Tangy, Sour	Ethyl Lactate	Soft, Tart, Fruity, Buttery
Caproic	Soapy	Ethyl Caprate	Waxy, Oil, Fruity, Brandy
Caprylic	Goaty, Fatty, Zoo-like	Ethyl Caprylate	Waxy, Wine, Floral, Tropical fruit, Brandy
Butyric	Rubber, Rancid, Cheesy, Fatty	Ethyl Butyrate	Fruity, Juicy Fruit, Pineapple, Cognac
Isobutyric	Rancid, Sweaty, Cheesy, Fatty	Ethyl Isobutyrate	Citrus, Fruity
Isovaleric	Rancid, Cheesy, Horsy	Ethyl Isovalerate	Fruity, Sweet, Apple, Pineapple, Tutti Frutti

From "Wild Brews", Jeff Sparrow

Brettanomyces for “funk”

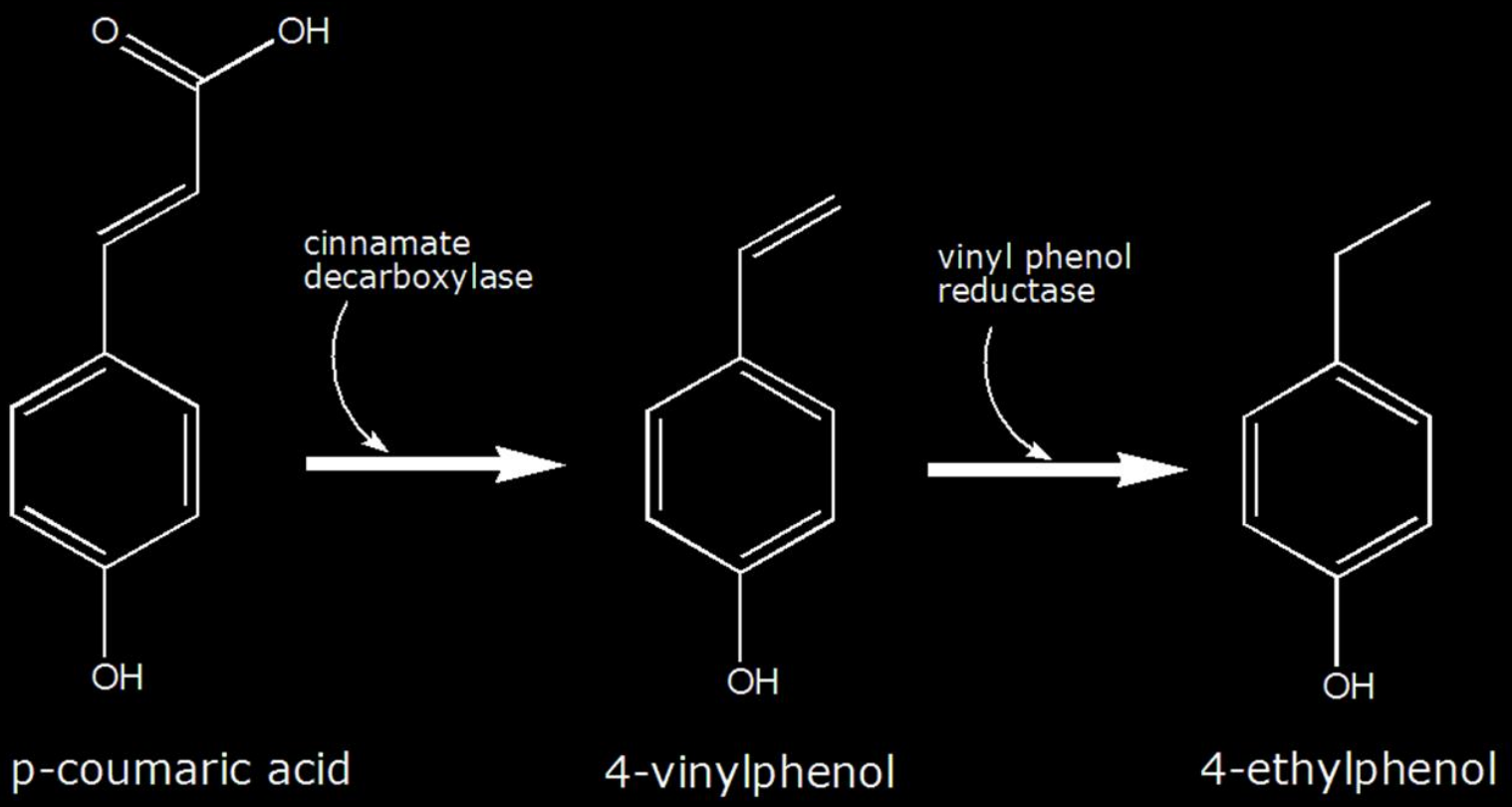


p-coumaric acid

Found in wood, barley husks, etc

Somewhat antimicrobial

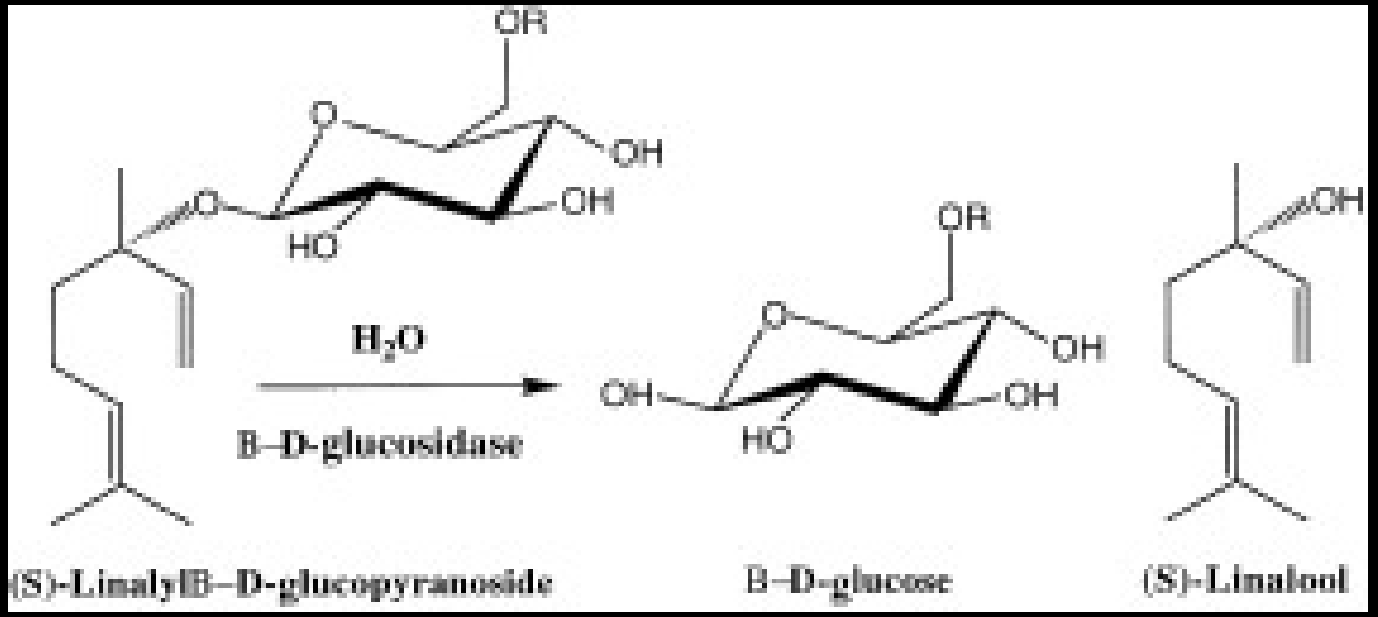
Brettanomyces for “funk”



“Barnyard, mousy, Band-Aid”

Biotransformation by *Brett.*

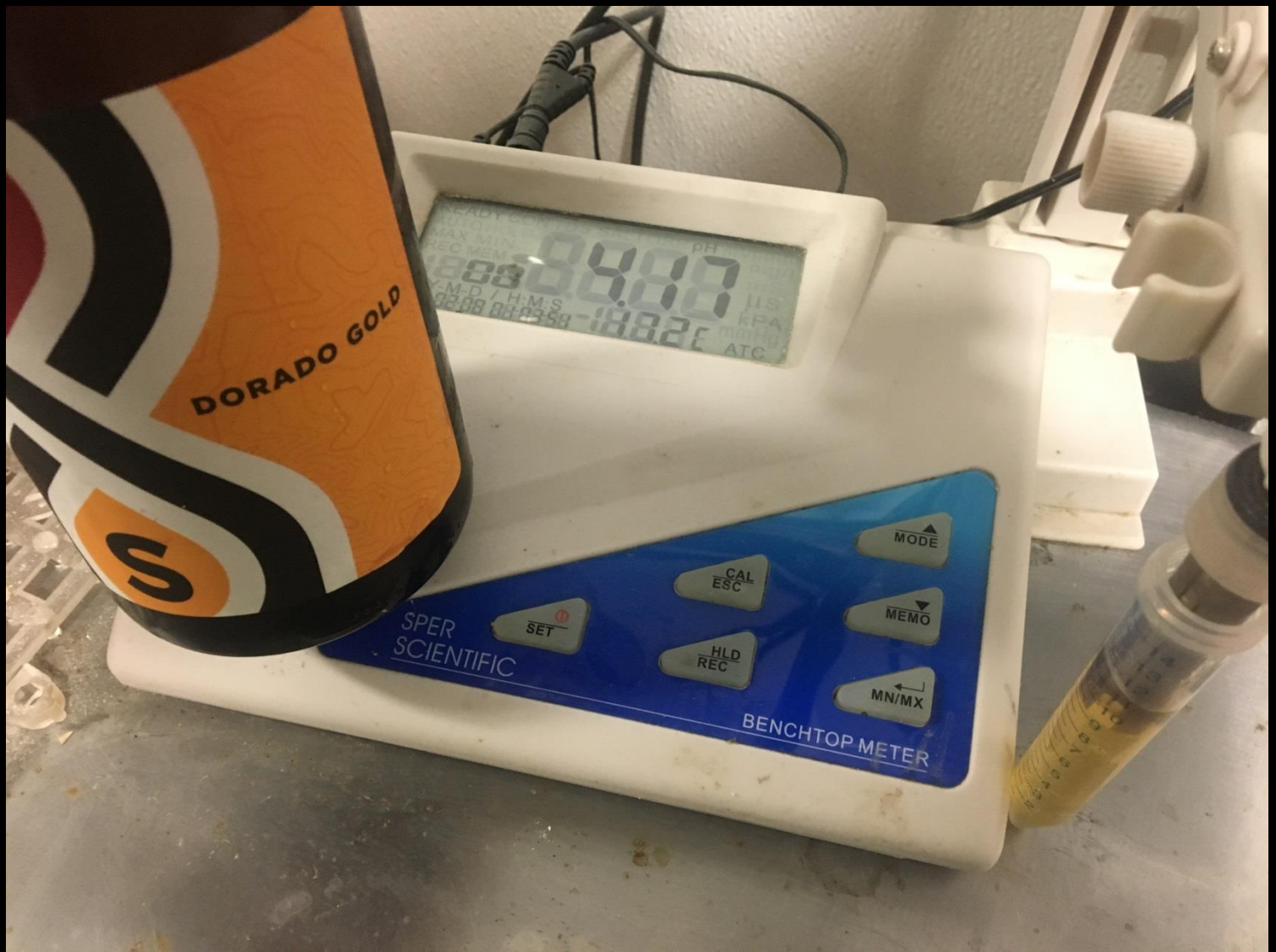
- Some yeasts able to liberate aroma compounds via enzymatic activity
  - β-glucosidase produced by some *Brett. spp.*



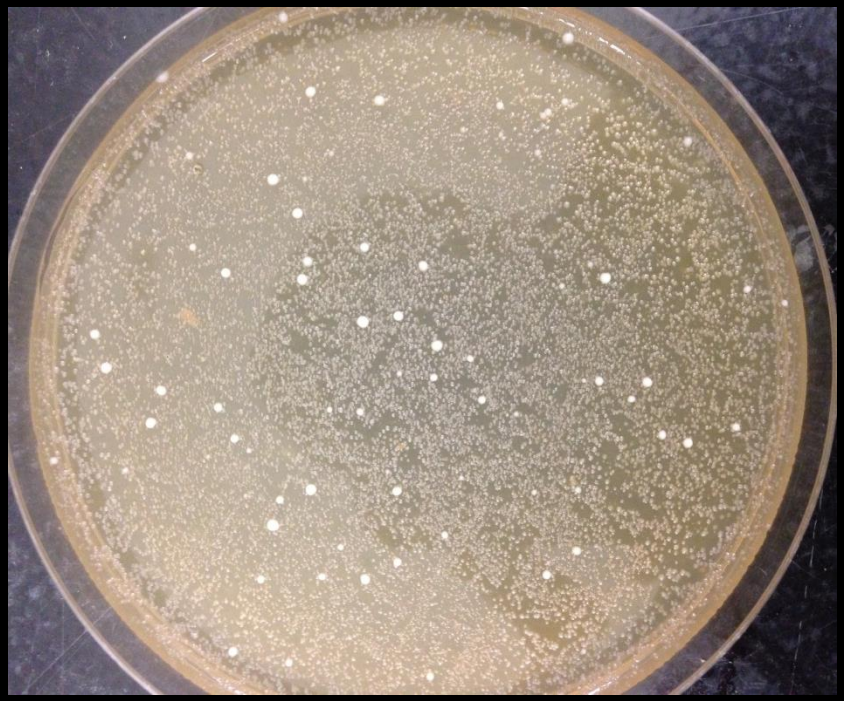
Raguso et al 1999

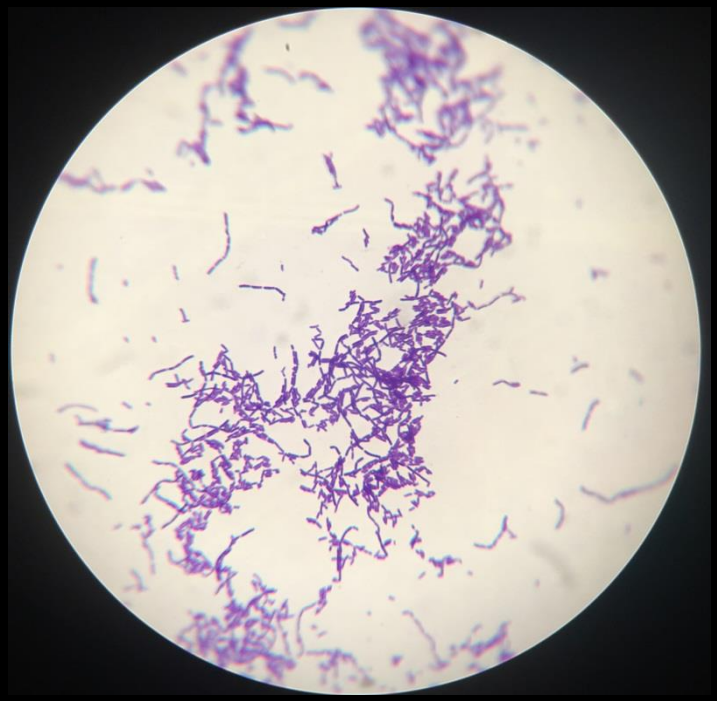
Brett. can do a lot….

Brett. beers are not sour



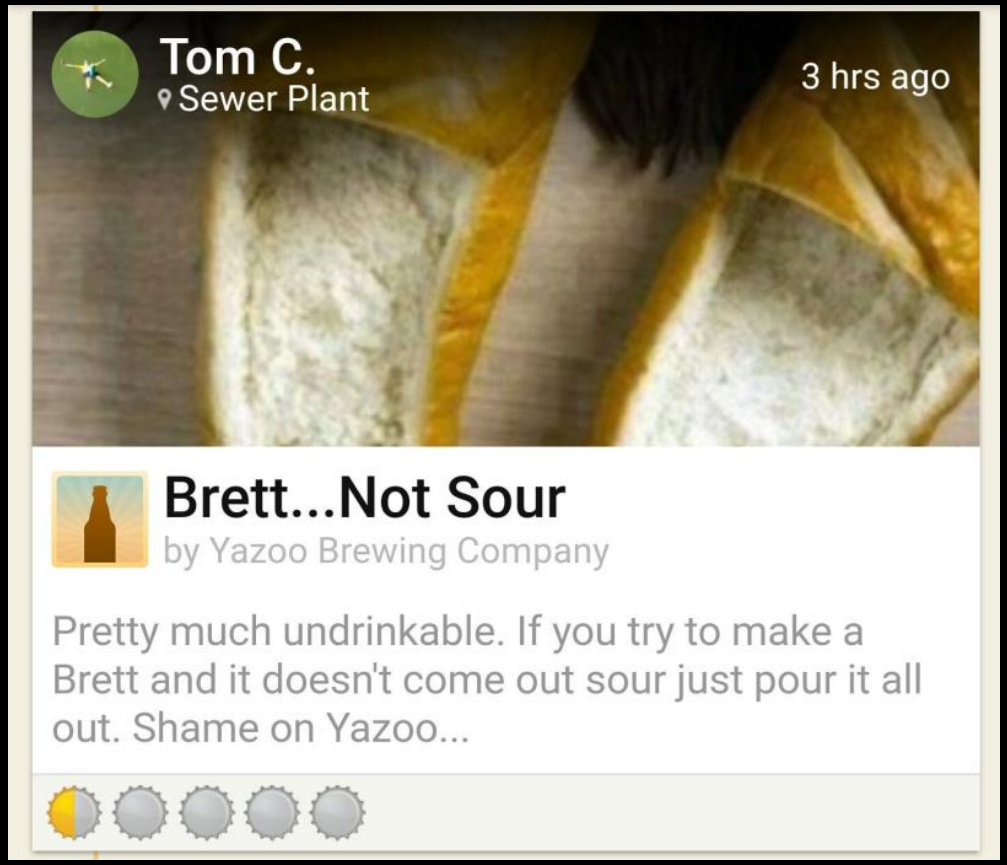
Sour beers utilize more than *Brett.*





Why does this matter?

- Knowing what each microbe can do informs recipes and chosen microbes



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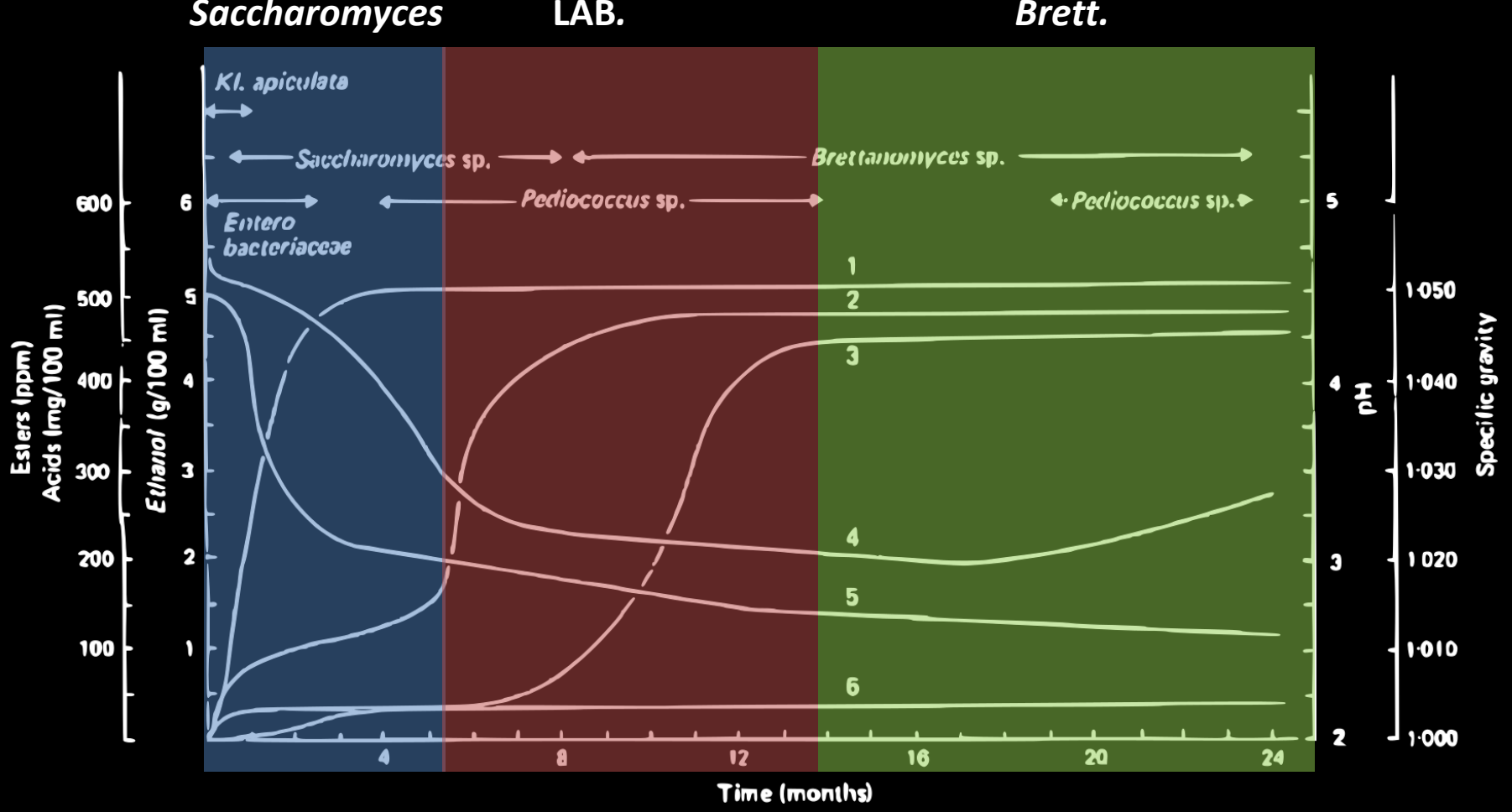

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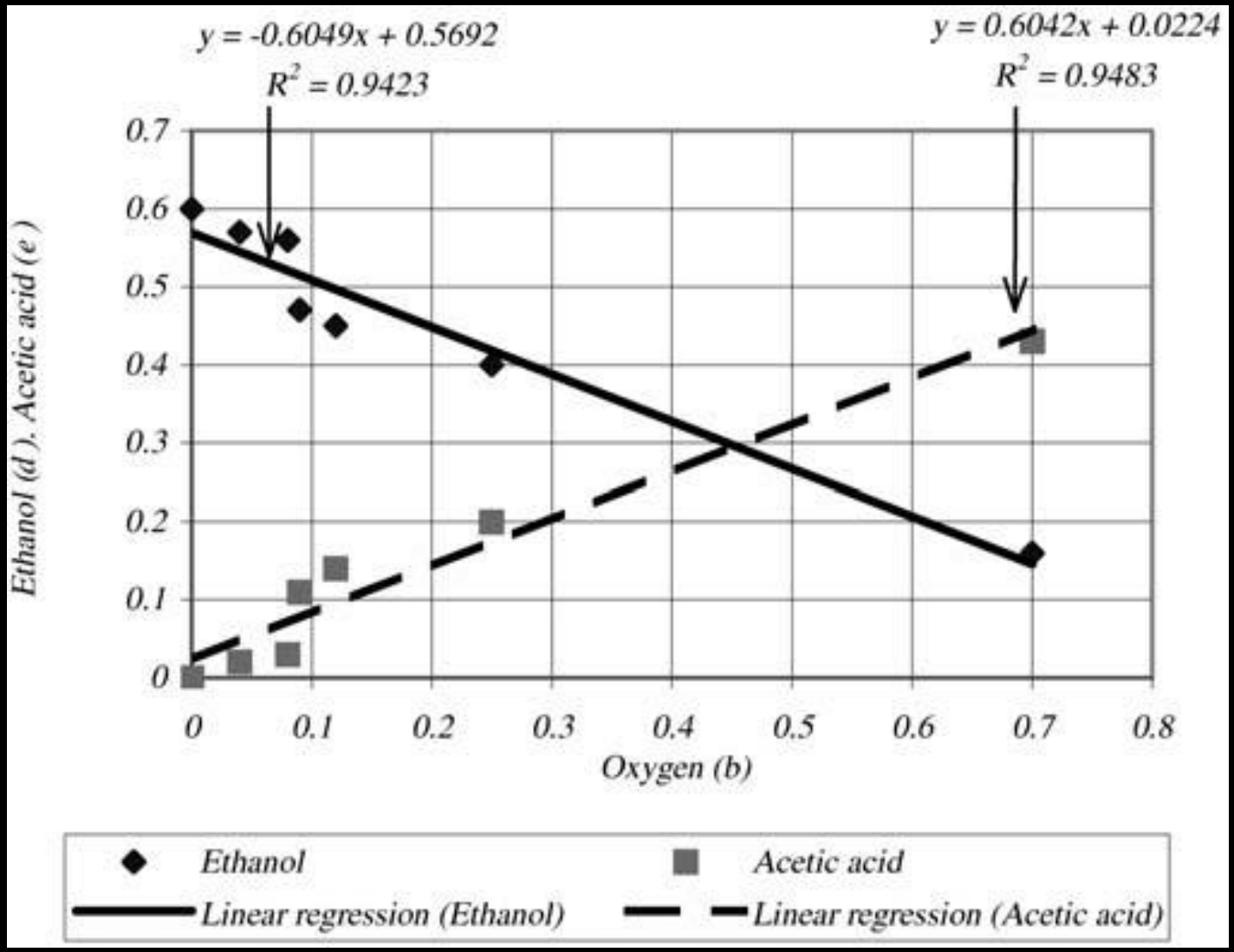
van Oevelen et al, 1977

Beijerinck and Baas-Becking:
"Everything is everywhere, but the environment selects"

Beijerinck and Baas-Becking:
*"Everything is everywhere, **but brewers must apply appropriate selection**"*


“Messieurs, c'est les microbes qui auront le dernier mot."

Brett. and acid production



Brett. and acid production

Anaerobic

Culture[a] (NRRL)	Growth (A_{600})	Acetate (g/l)	Ethanol (g/l)	Residual glucose (g/l)
B. bruxellensis				
Y-1412	25.84	1.1	45.6	0.0
Y-1411	24.78	0.0	48.5	0.0
B. lambicus				
Y-1330	16.05	0.0	51.9	0.0
B. claussenii				
Y-2290	33.89	3.0	49.0	0.0
Y-1414	30.44	0.0	49.4	0.0
B. anomalus				
Y-1415	30.40	0.4	47.1	2.8
Y-12670	31.17	0.0	50.4	0.0
Brettanomyces sp.				
YB-5260	13.67	3.4	48.9	0.3
D. intermedia				
YB-4553	41.37	0.0	48.9	0.0
Y-1092	38.11	1.0	48.5	0.0
YB-4241	32.01	1.3	49.4	0.0
D. bruxellensis				
Y-17534	37.65	1.0	45.0	0.0
Y-12961	21.74	1.0	50.6	0.0
D. anomala				
Y-17521	29.79	0.0	48.9	0.0
Y-17520	29.60	0.0	48.7	0.0
Y-17522	29.30	0.0	50.1	0.0
Z. bailii				
Y-2227	8.09	1.0	49.6	3.4
Z. bisporus				
Y-7558	21.31	0.0	46.1	3.0
P. fermentans				
Y-1619	14.19	2.0	45.6	0.0

Aerobic

Culture[a] (NRRL)	Growth (A_{600})	Acetate (g/l)	Ethanol (g/l)	Residual glucose (g/l)
B. bruxellensis				
Y-1412	35.37	22.9	21.8	14.4
B. claussenii				
Y-2290	34.61	24.6	15.4	41.2
B. anomalus				
Y-1415	39.51	22.4	23.8	17.1
B. custersianus				
Y-6653	82.63	0.0	0.0	53.4
B. intermedius				
Y-2394	19.45	29.6	10.5	40.2
Y-2395	32.25	29.1	13.5	31.2
YB-3363	32.33	23.7	11.0	43.6
D. intermedia				
YB-5164	33.99	31.7	6.1	48.1
YB-4553	33.72	30.0	24.3	57.4
Y-1092	32.09	21.2	16.7	33.4
YB-4241	33.15	21.6	21.6	20
D. bruxellensis				
Y-17523	27.13	24.0	6.2	67.9
Y-17535	28.56	18.7	10.5	49.1
Y-17525	34.96	31.6	15.7	26.1
Y-17524	29.70	26.9	7.0	52.3
Y-17534	31.26	19.0	8.5	52
D. anomala				
Y-17521	29.00	23.0	18.6	29.8
Y-17520	26.66	24.4	10.2	24.4
Y-17522	29.19	20.4	7.7	58.8

Brett. and acid production

- *Brett* CAN produce acid

BUT

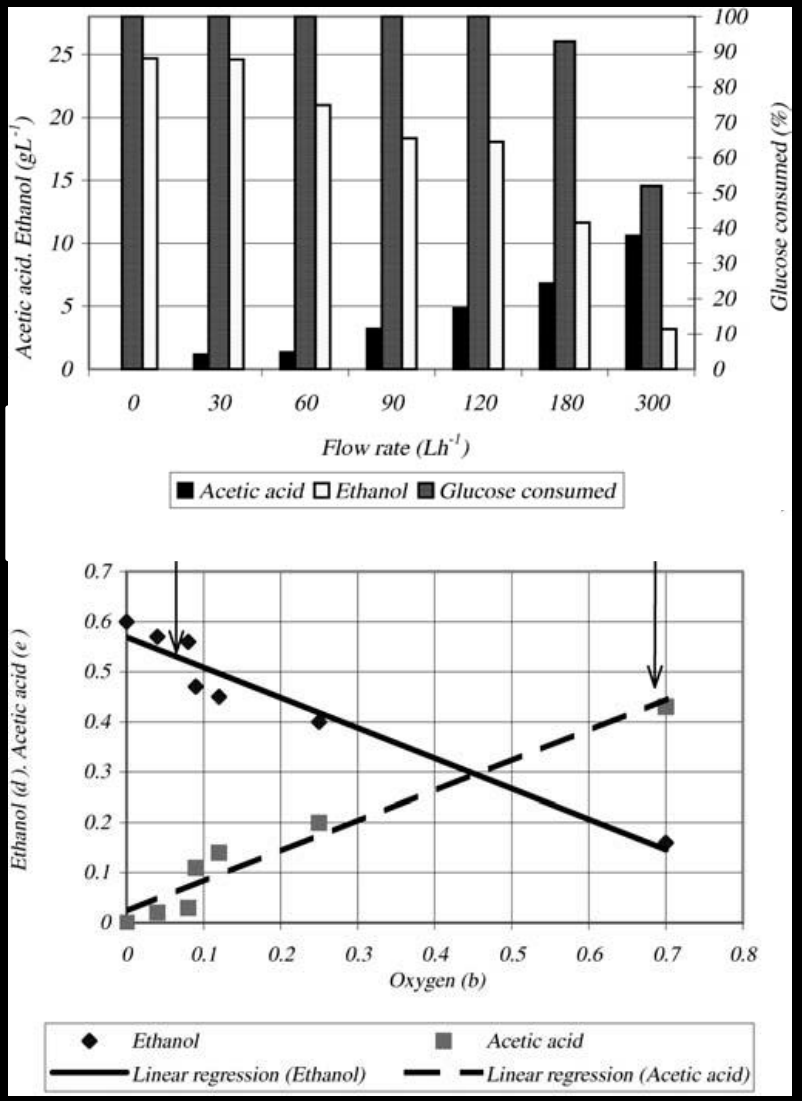
- Only in the presence of oxygen or other electron acceptors

AND

- It's acetic acid

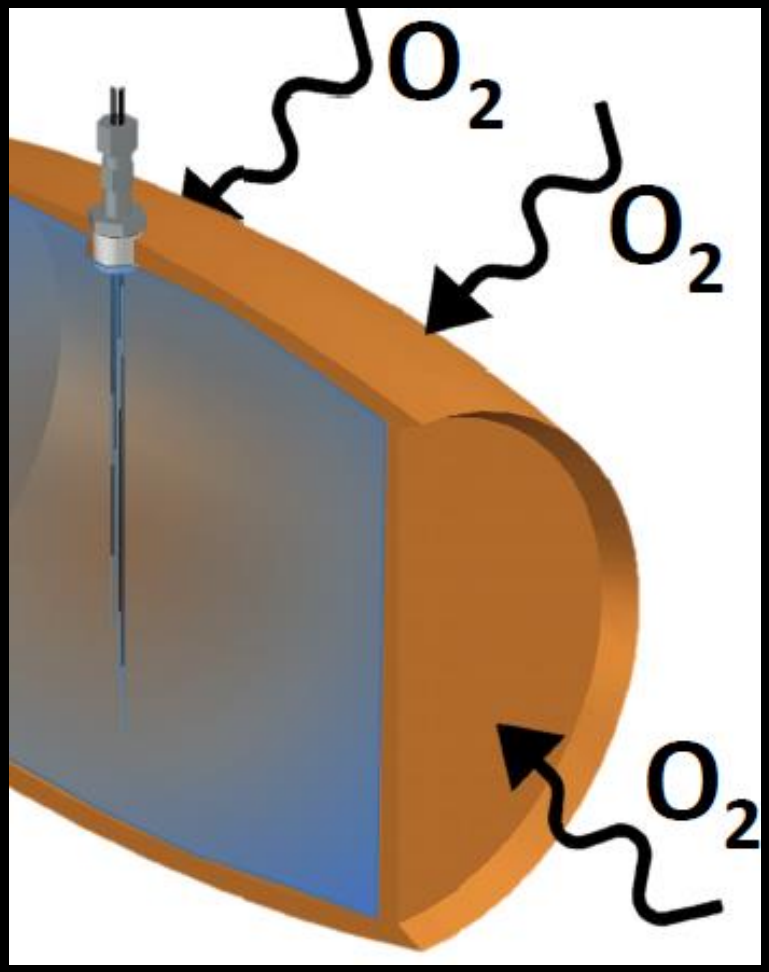
AND

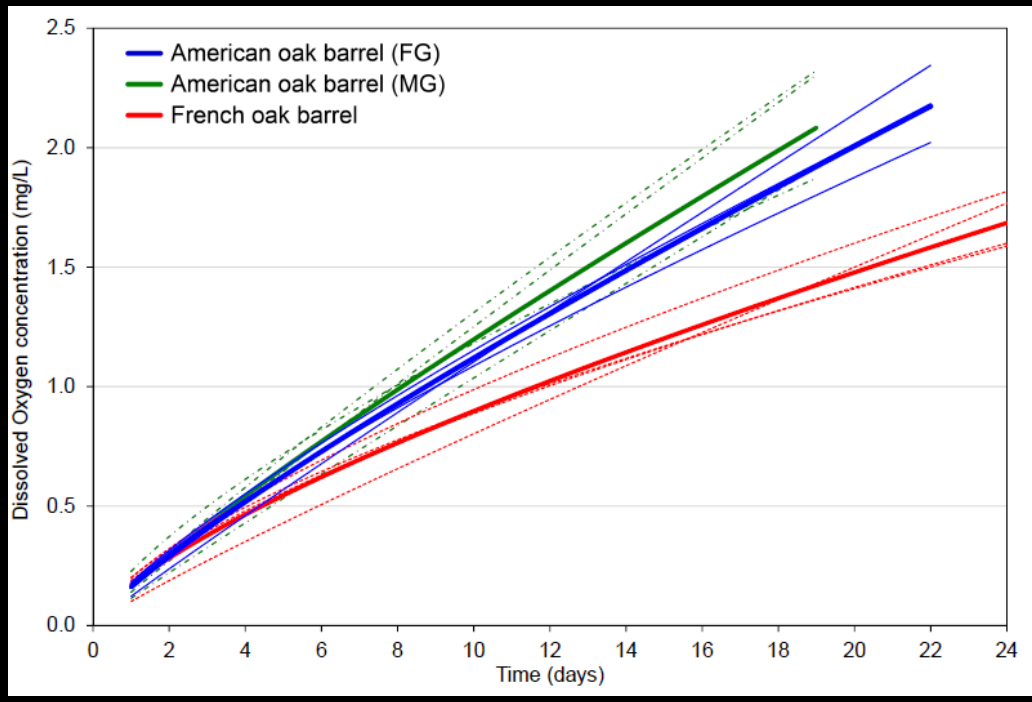
- Costs EtOH and AA

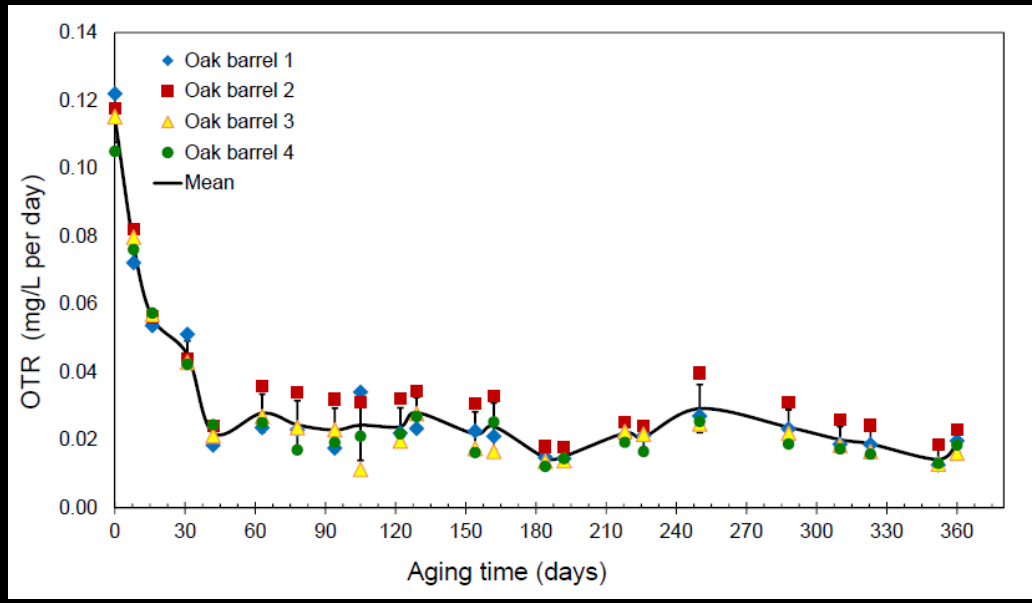


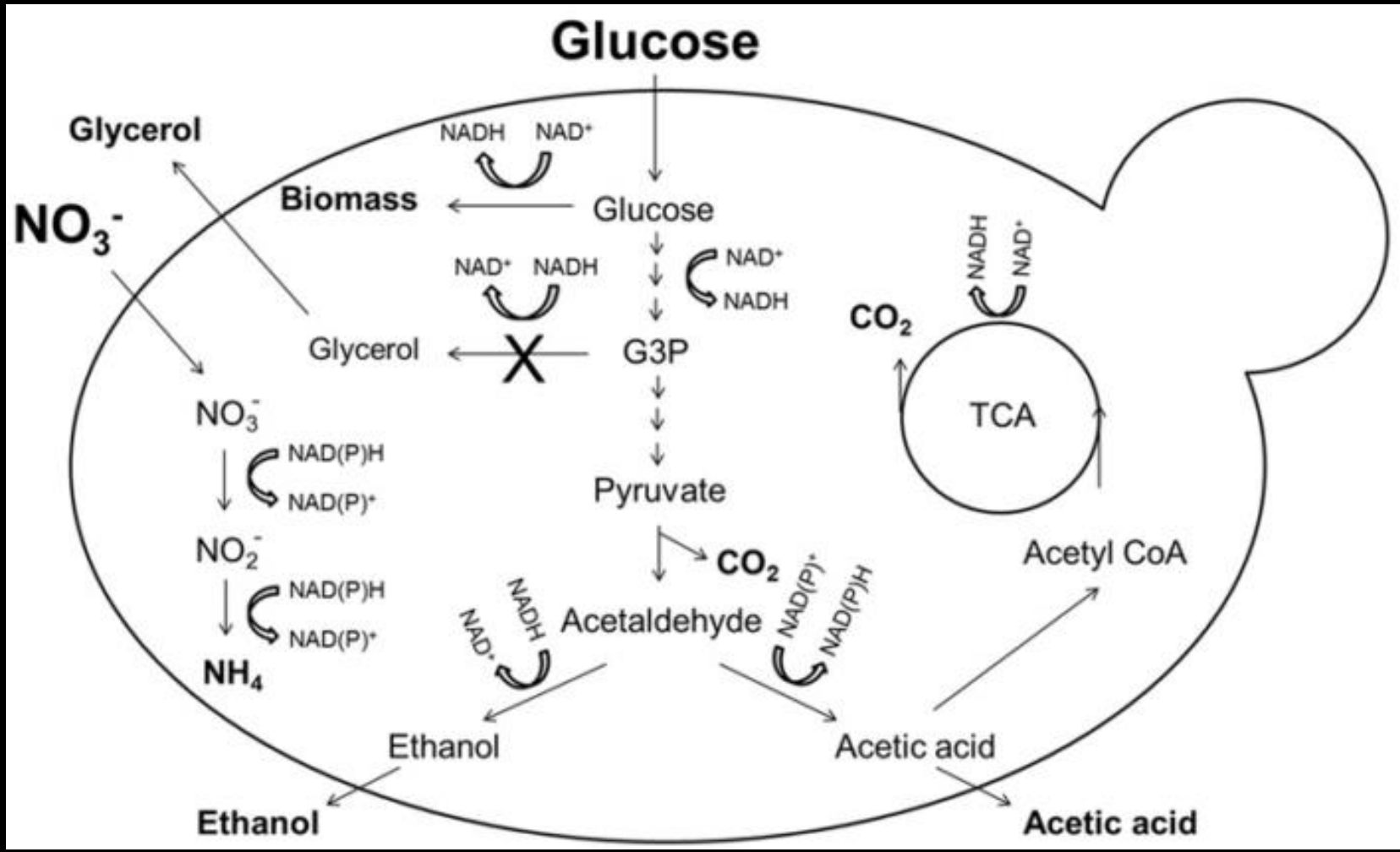
Uscanga et al, 2003

Test	Oxygen Transfer Rate	Citation
Theoretical Max, Semipermeable membrane	37mg/L/year	Kelly and Wollan, 2003
Measured, New French Oak	20mg/L/year	Vivas and Glories, 1997
Measured, Fine Grain American Oak,	11.6mg/L/year	Alamo-Sanza and Nevares, 2014


O2
O2
O2


American oak barrel (FG)
American oak barrel (MG)
French oak barrel
Dissolved Oxygen concentration (mg/L)
Time (days)
0.0
0.5
1.0
1.5
2.0
2.5
0
2
4
6
8
10
12
14
16
18
20
22
24


Oak barrel 1
Oak barrel 2
Oak barrel 3
Oak barrel 4
Mean
OTR (mg/L per day)
Aging time (days)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0
30
60
90
120
150
180
210
240
270
300
330
360


Glucose
Glycerol
NADH
NAD+
Biomass
Glucose
NO3-
NAD+
NADH
NAD+
NADH
CO2
NADH
NAD+
Glycerol
G3P
TCA
NO3-
NAD(P)H
NAD(P)+
Pyruvate
NO2-
NAD(P)H
NAD(P)+
CO2
Acetyl CoA
NADH
NAD+
Acetaldehyde
NAD(P)+
NAD(P)H
NH4
Ethanol
Acetic acid
Ethanol
Acetic acid