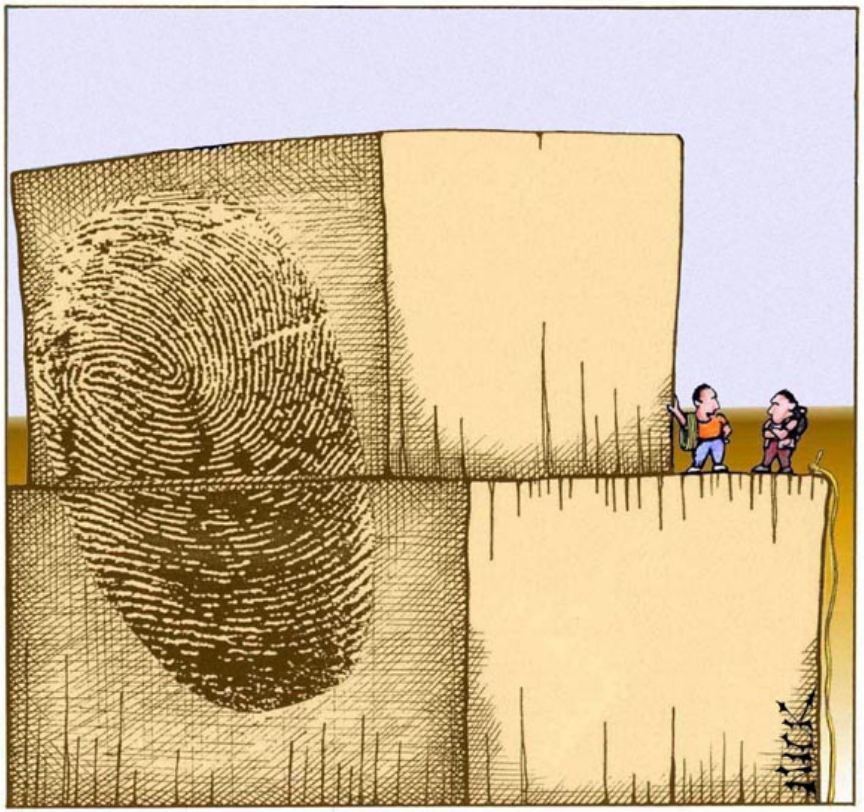
What We've Learned about Wine Spoilage...



"Of course, it's still a complete mystery as to how the ancients even managed to MOVE these massive stones..."

...and *Brettanomyces*

...and *Lactobacillus*

...and *Saccharomyces*

Charles G. Edwards

Washington State University

Viticulture/Enology Team

School of Food Science

What have we learned about controlling *Brettanomyces*?

Impact of SO_2?

Ethanol x Temperature?

Removal by filtration?

Growth in oak (barrels)?

Impact of SO_2

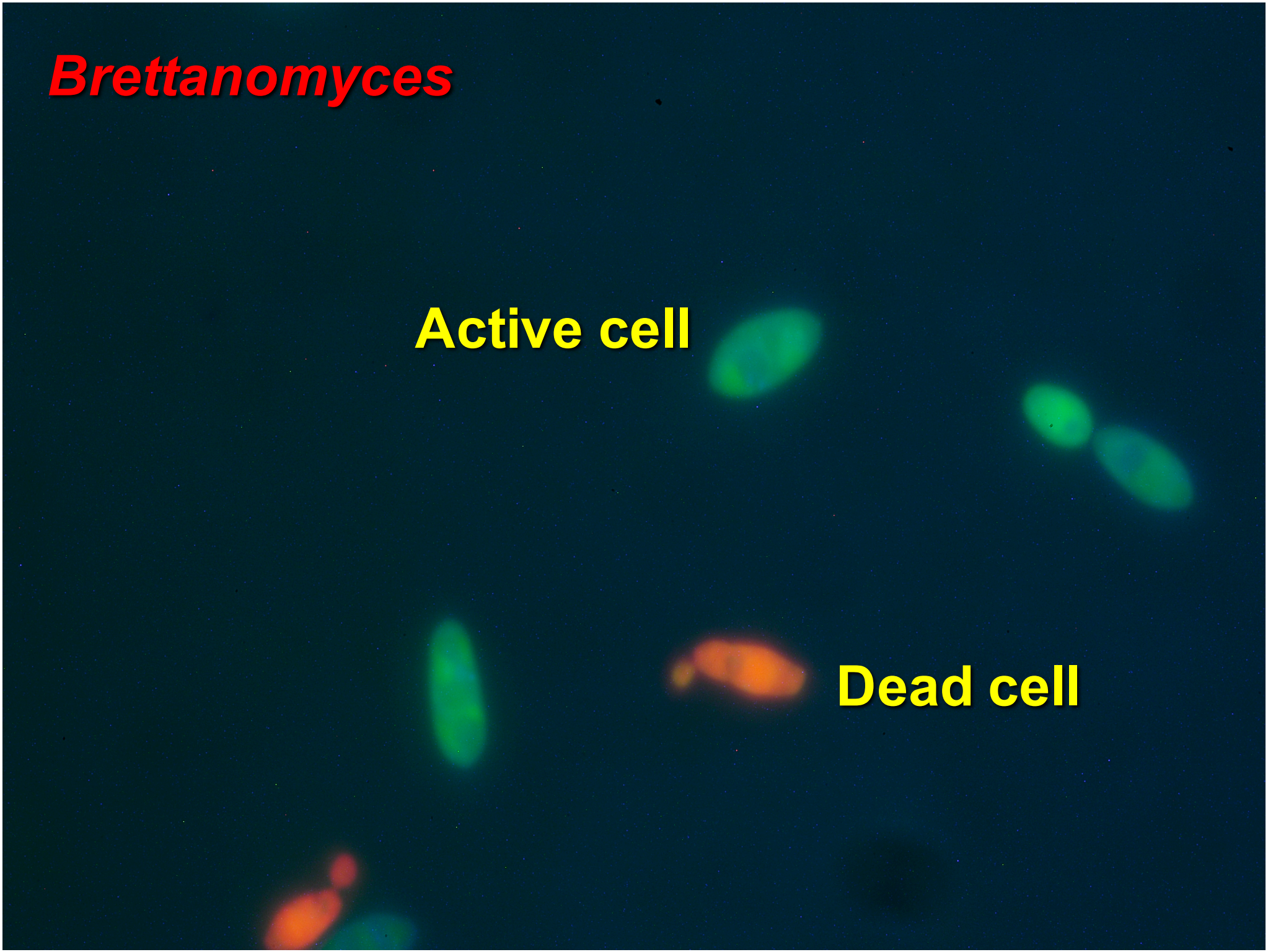
A. Can SO_2 cause "VBNC"?

1. "Viable-But-Not-Culturable."
2. Yeast alive but will not grow on media.
3. If valid → yeast could be undetected.

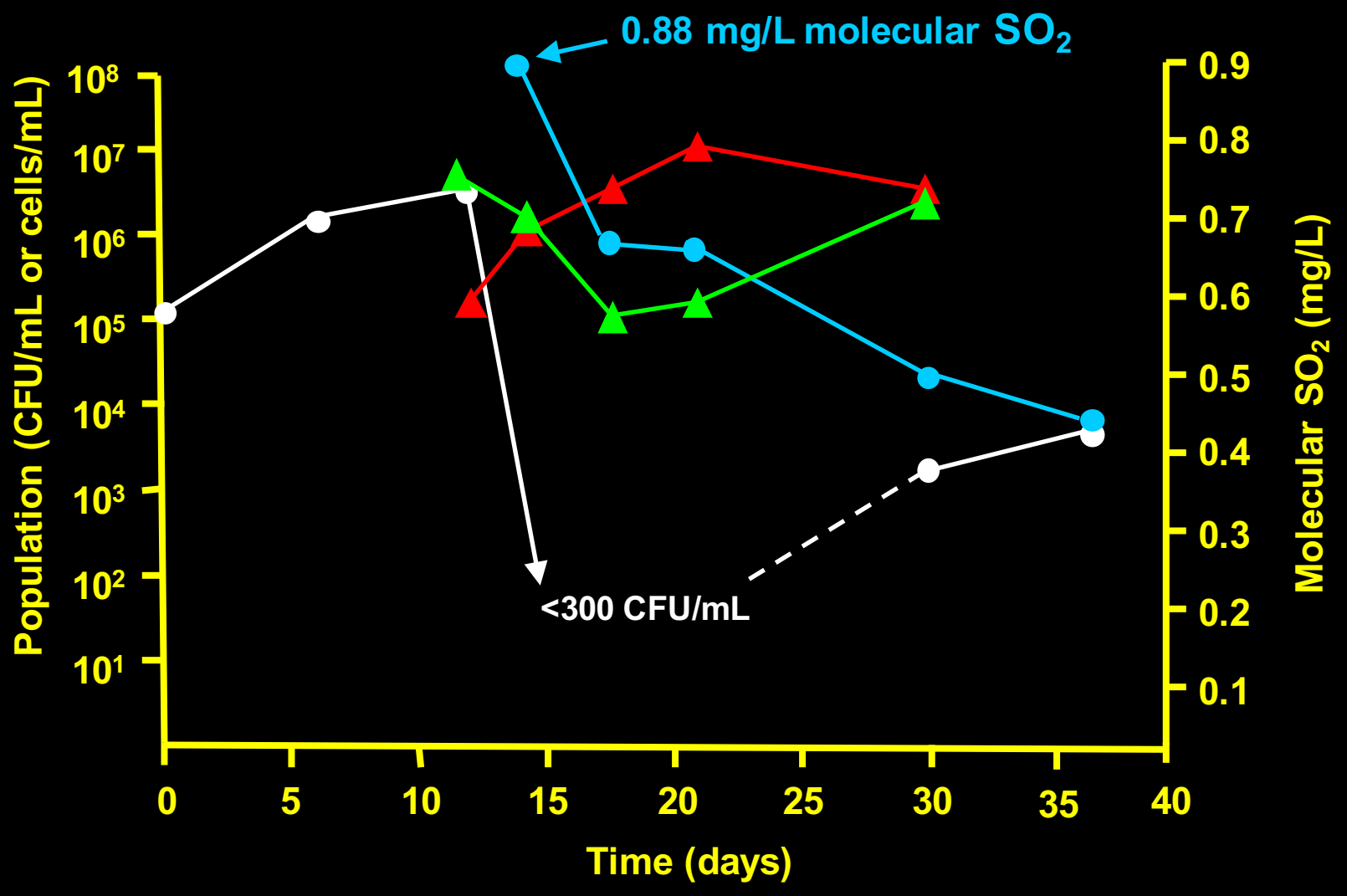
B. How to detect VBNC?

1. Measure metabolic activity or qPCR (?).
2. Fluorescence microscopy.

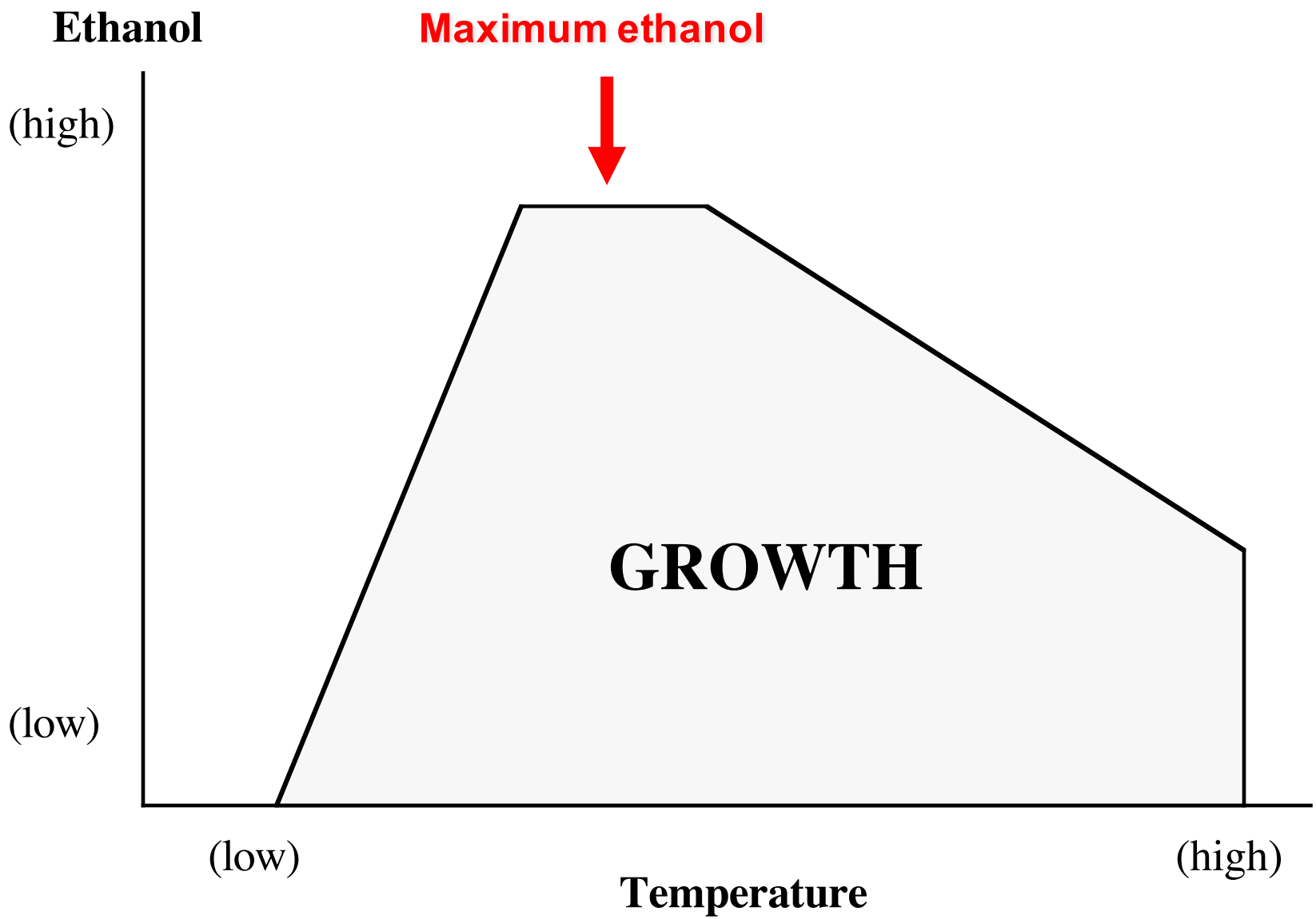
Green = viable Orange/Red = dead


Brettanomyces
Active cell
Dead cell

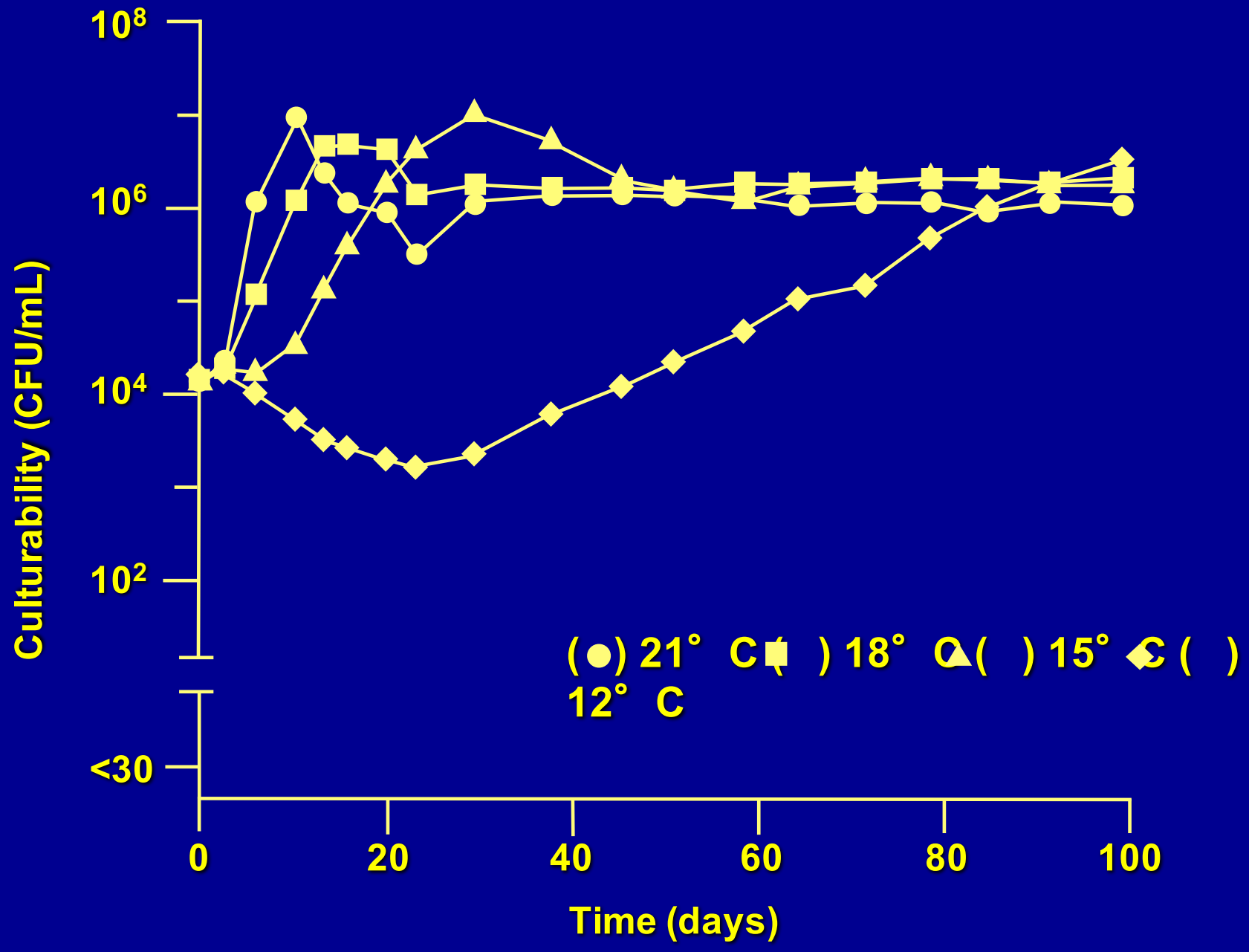
B. bruxellensis (B1b)

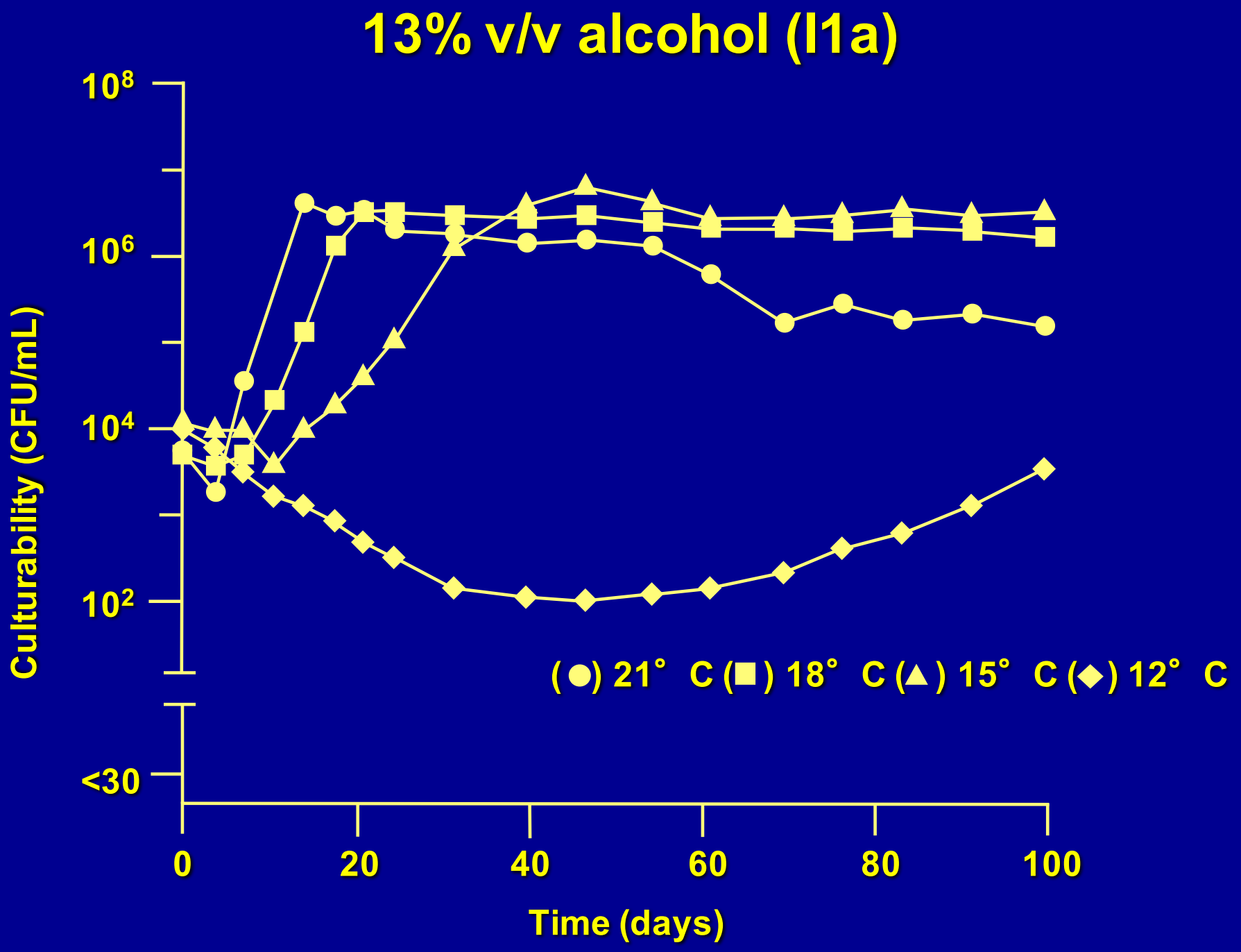


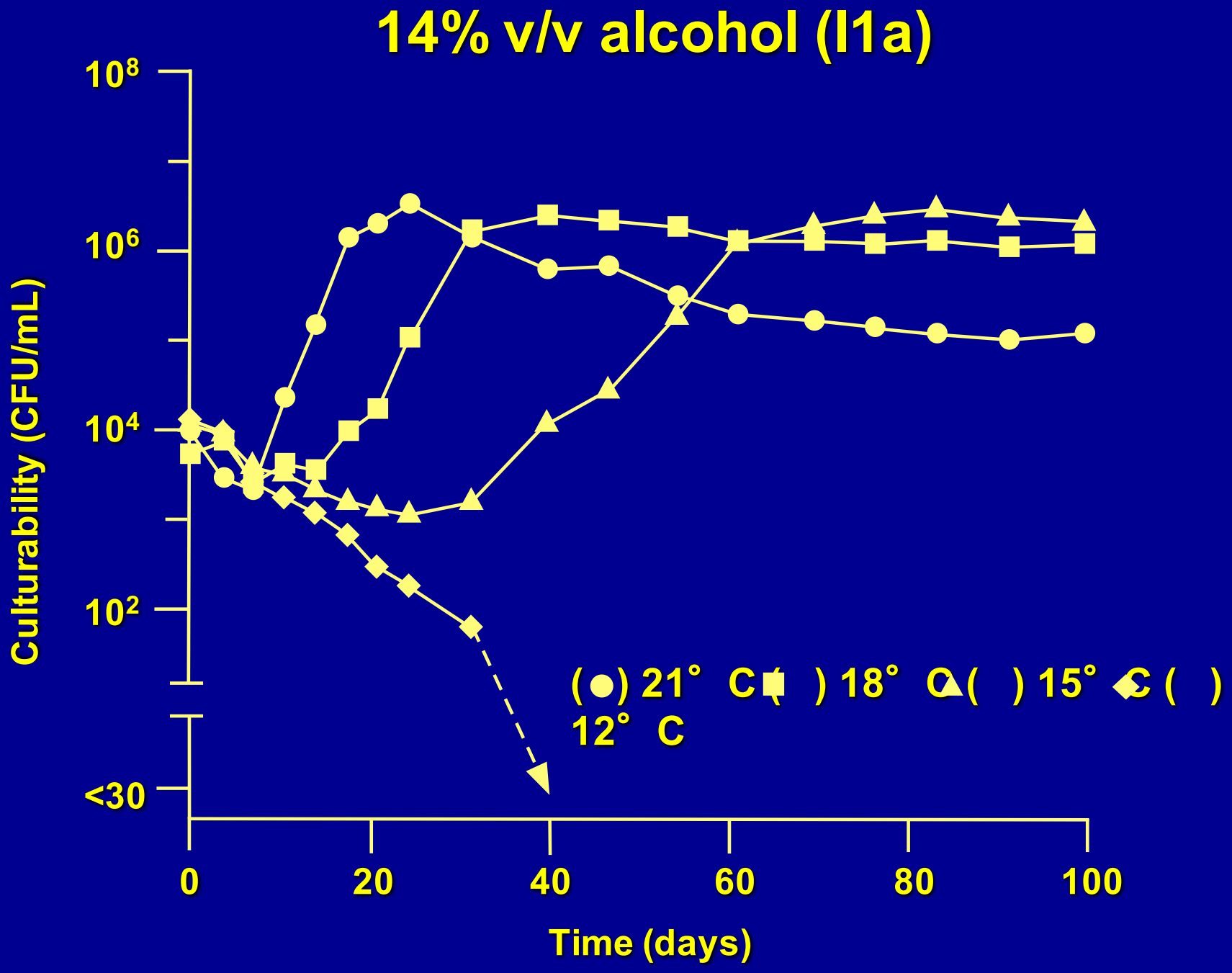
Is there a relationship between ethanol and temperature that could be useful to control *Brettanomyces*?


Ethanol
Maximum ethanol
(high)
(low)
GROWTH
(low)
(high)
Temperature

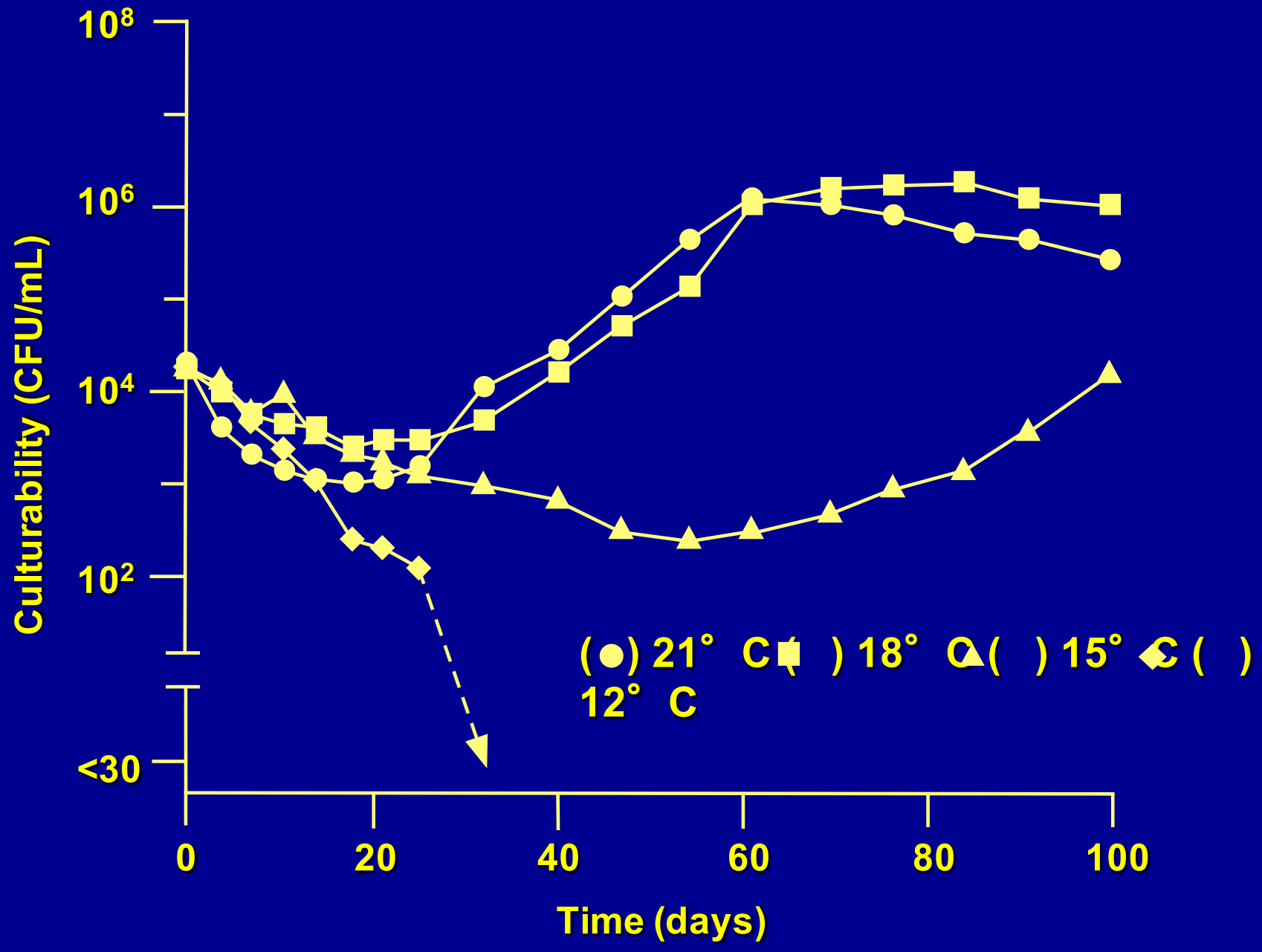
12% v/v alcohol (I1a)



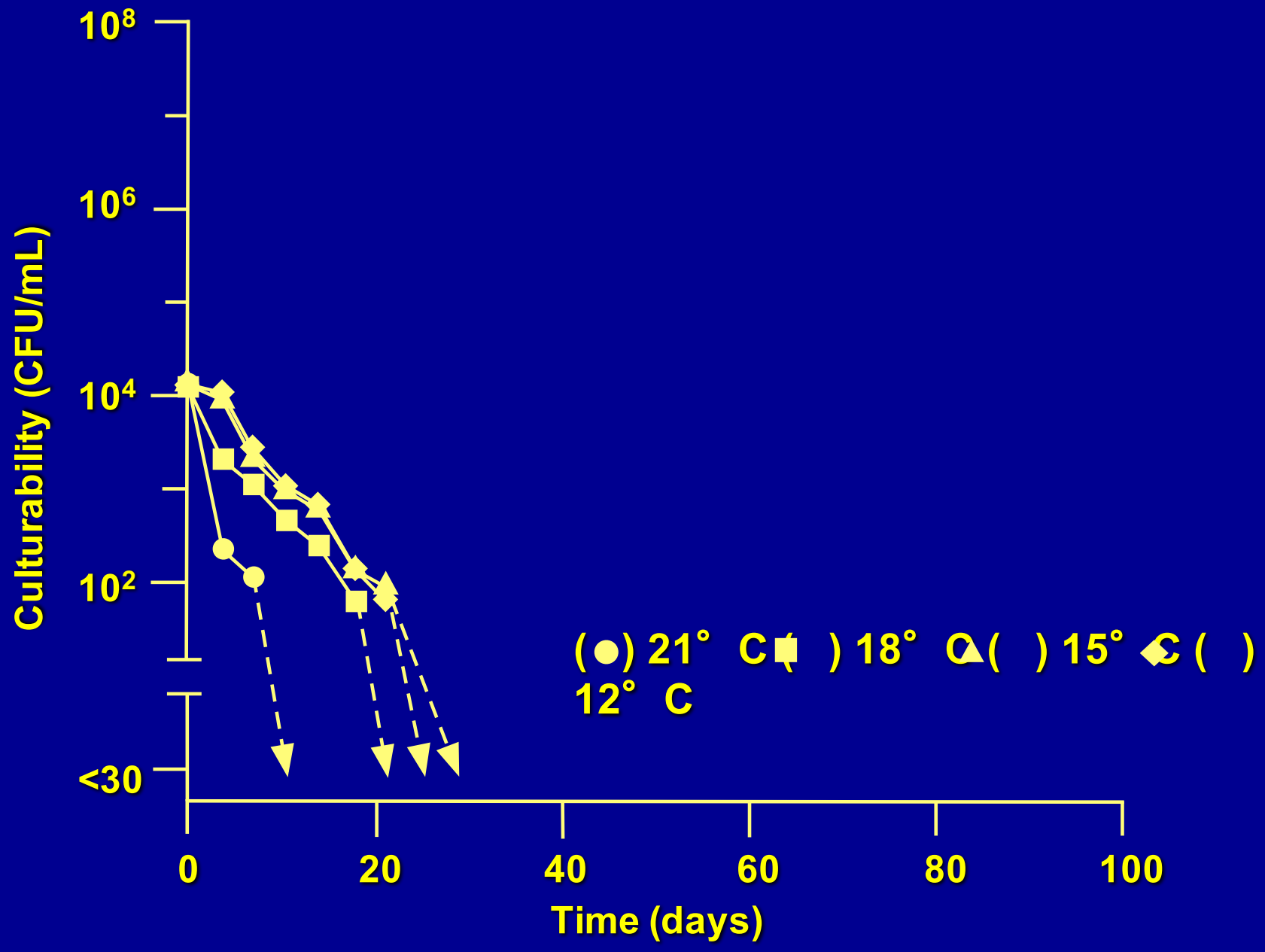

13% v/v alcohol (I1a)
Culturability (CFU/mL)
10^8
10^6
10^4
10^2
<30
0
20
40
60
80
100
Time (days)
(●) 21° C (■) 18° C (▲) 15° C (◆) 12° C


14% v/v alcohol (I1a)
Culturability (CFU/mL)
10^8
10^6
10^4
10^2
<30
0
20
40
60
80
100
Time (days)
(●) 21° C (■) 18° C (▲) 15° C (◆) 12° C

15% v/v alcohol (I1a)



16% v/v alcohol (I1a)



Production of 4-Ethylphenol (µg/L)

°C	Ethanol (%)	Strain F3	Strain I1a
21°	12	(+)	(+)
	13	(+)	(+)
	14	(+)	(+)
	15	(-)	(+)
	16	(-)	(-)
18°	12	(+)	(+)
	13	(+)	(+)
	14	(+)	(+)
	15	(-)	(+)
	16	(-)	(-)

(-) <30 µg/L; (±) 31 to 1000 µg/L; (+) >1300 µg/L

Production of 4-Ethylphenol (µg/L)

°C	Ethanol (%)	Strain F3	Strain I1a
15°	12	(+)	(+)
	13	(+)	(+)
	14	(+)	(+)
	15	(-)	(-)
	16	(-)	(-)
12°	12	(-)	(±)
	13	(-)	(-)
	14	(-)	(-)
	15	(-)	(-)
	16	(-)	(-)

(-) <30 µg/L; (±) 31 to 1000 µg/L; (+) >1300 µg/L

What about filtration?

Research Focus
(Filtration)

Red wines (0 or 0.5 mg/L mSO_2)

Filter Treatment Cartridge #1

Filter Treatment Cartridge #2

Filter Treatment Cartridge #3

Treatment Replicate = Filter cartridge

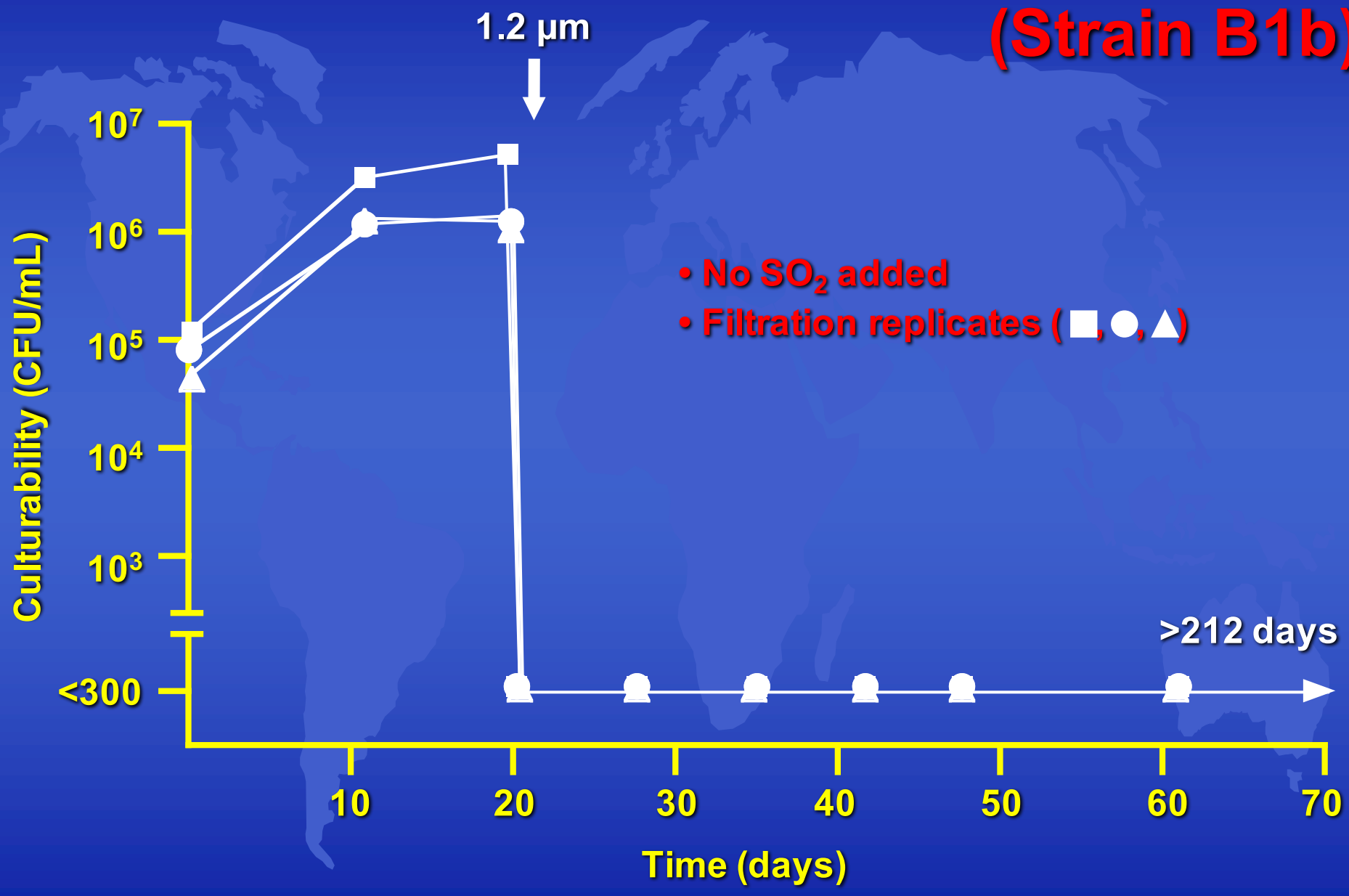
3 samples (No SO_2)
3 samples (SO_2)

3 samples (No SO_2)
3 samples (SO_2)

3 samples (No SO_2)
3 samples (SO_2)

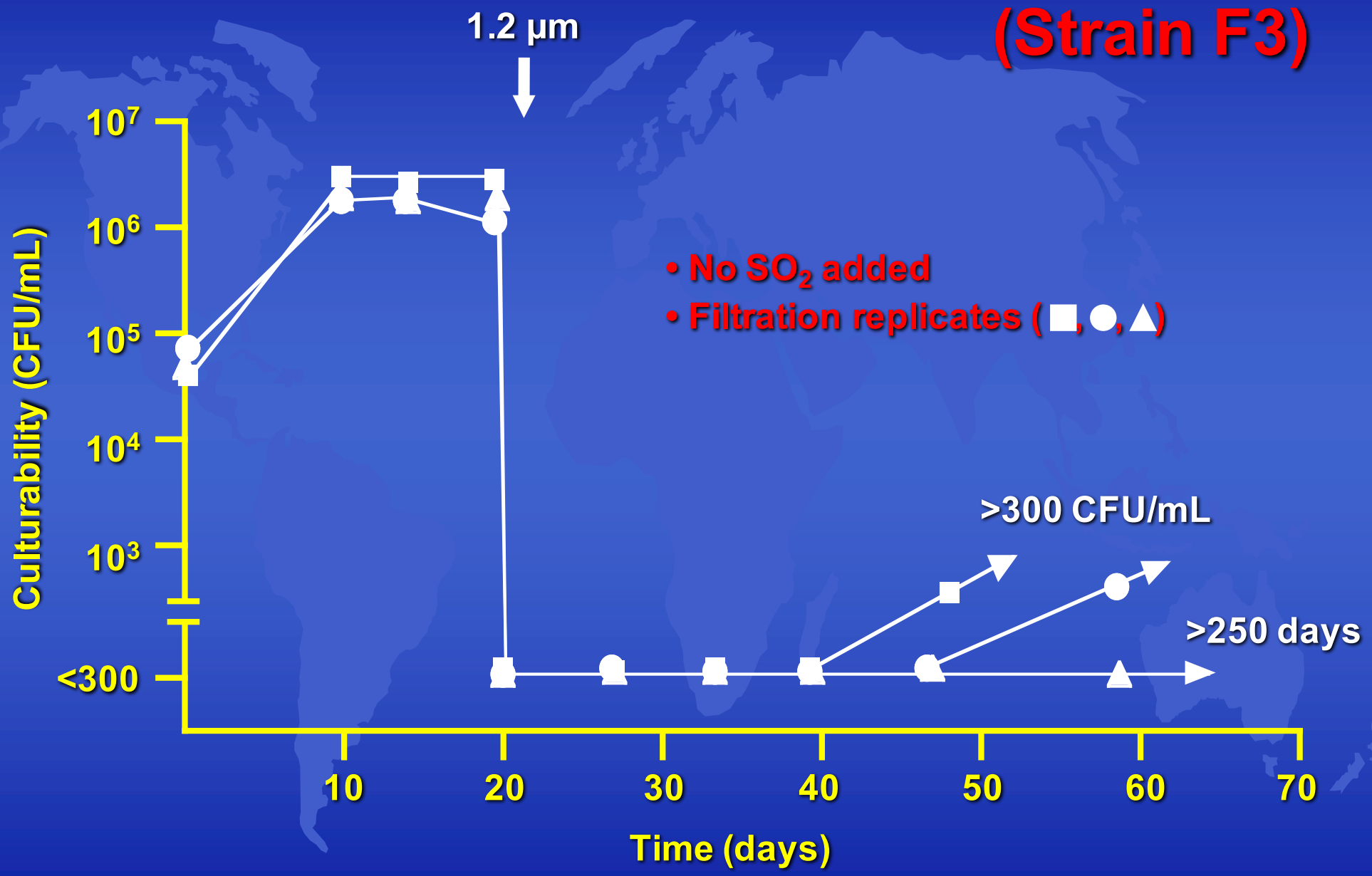
- Multiple ferments incubated >200 days.

Filtration
(Strain B1b)

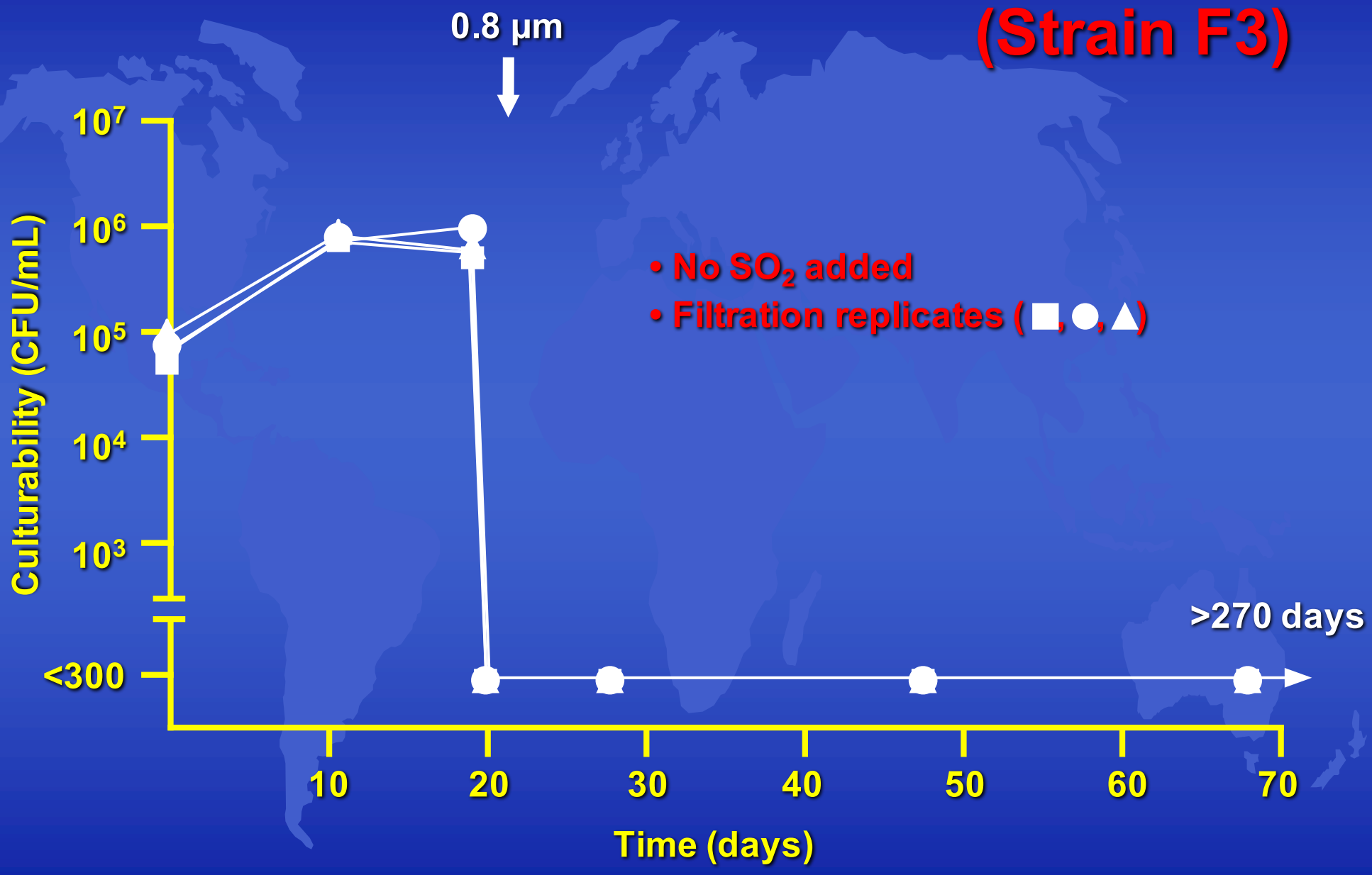


- No SO_2 added
- Filtration replicates (■, ●, ▲)

Filtration
(Strain F3)



Filtration
(Strain F3)



- No SO_2 added
- Filtration replicates (■, ●, ▲)

Growth in oak barrels?

Research Focus
(Growth in Oak Barrels)

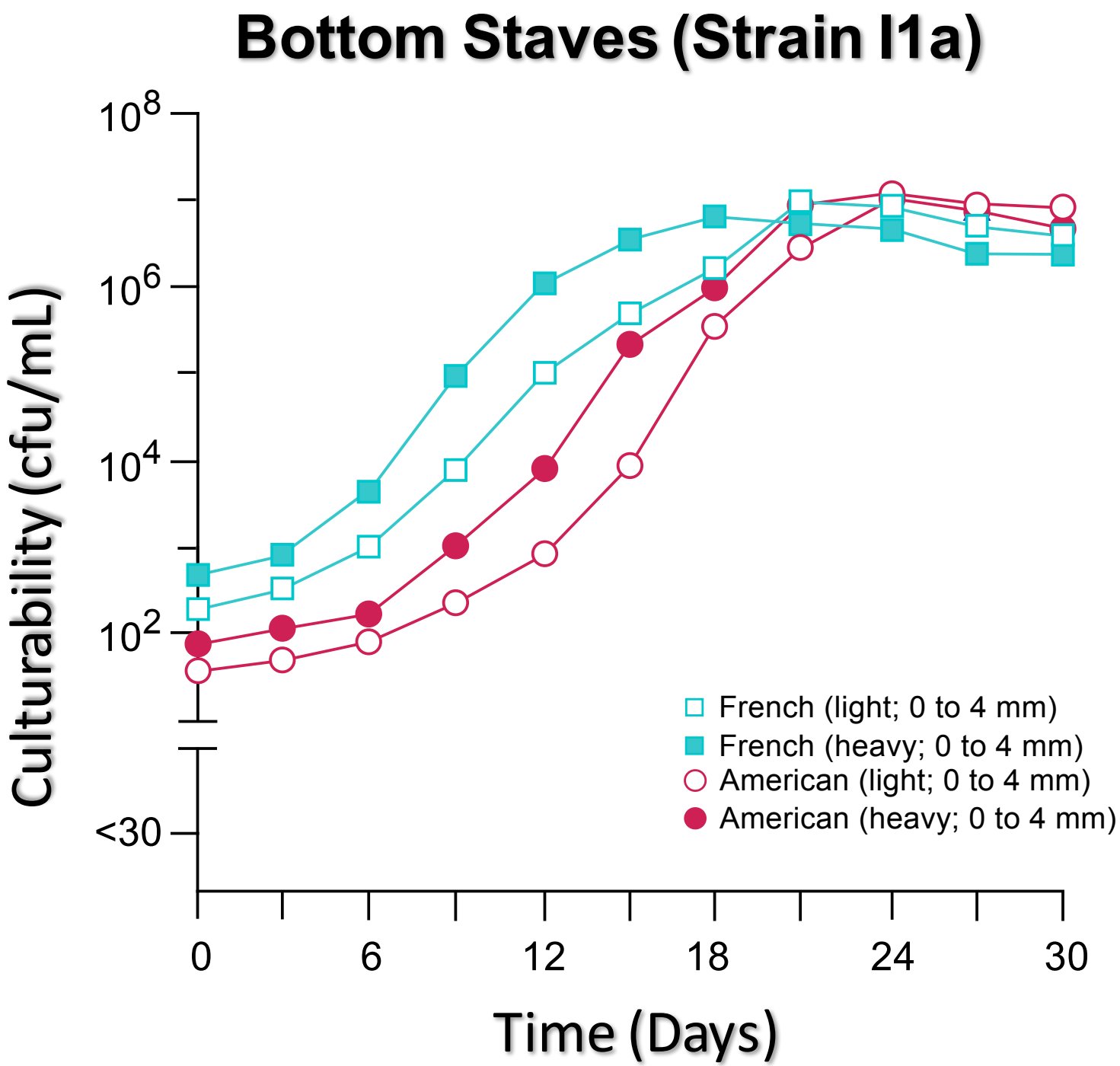
(E1 and I1a).

Research Focus
(Growth in Oak Barrels)

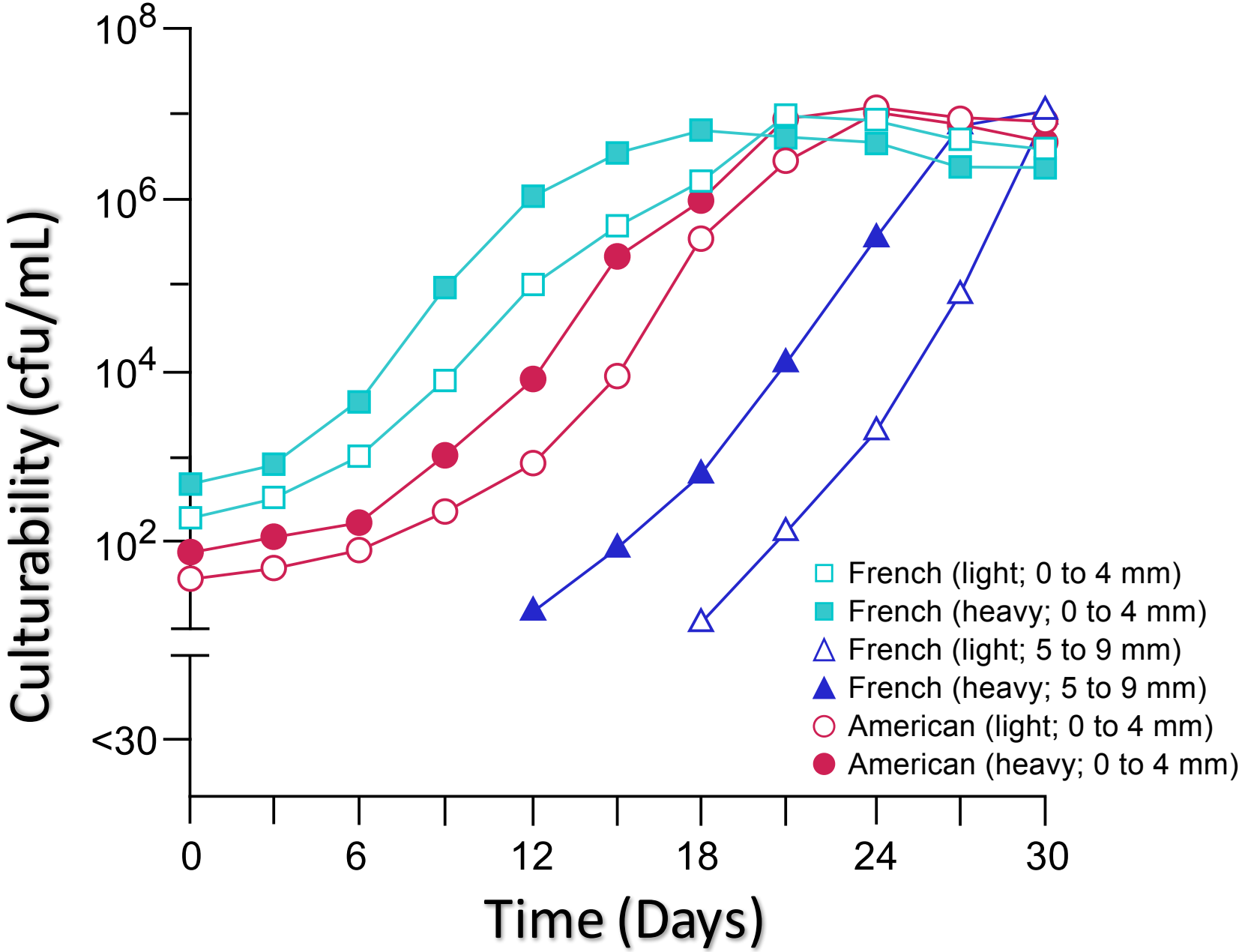
Cross-Sectional Layer
(transfer into sterile wine)

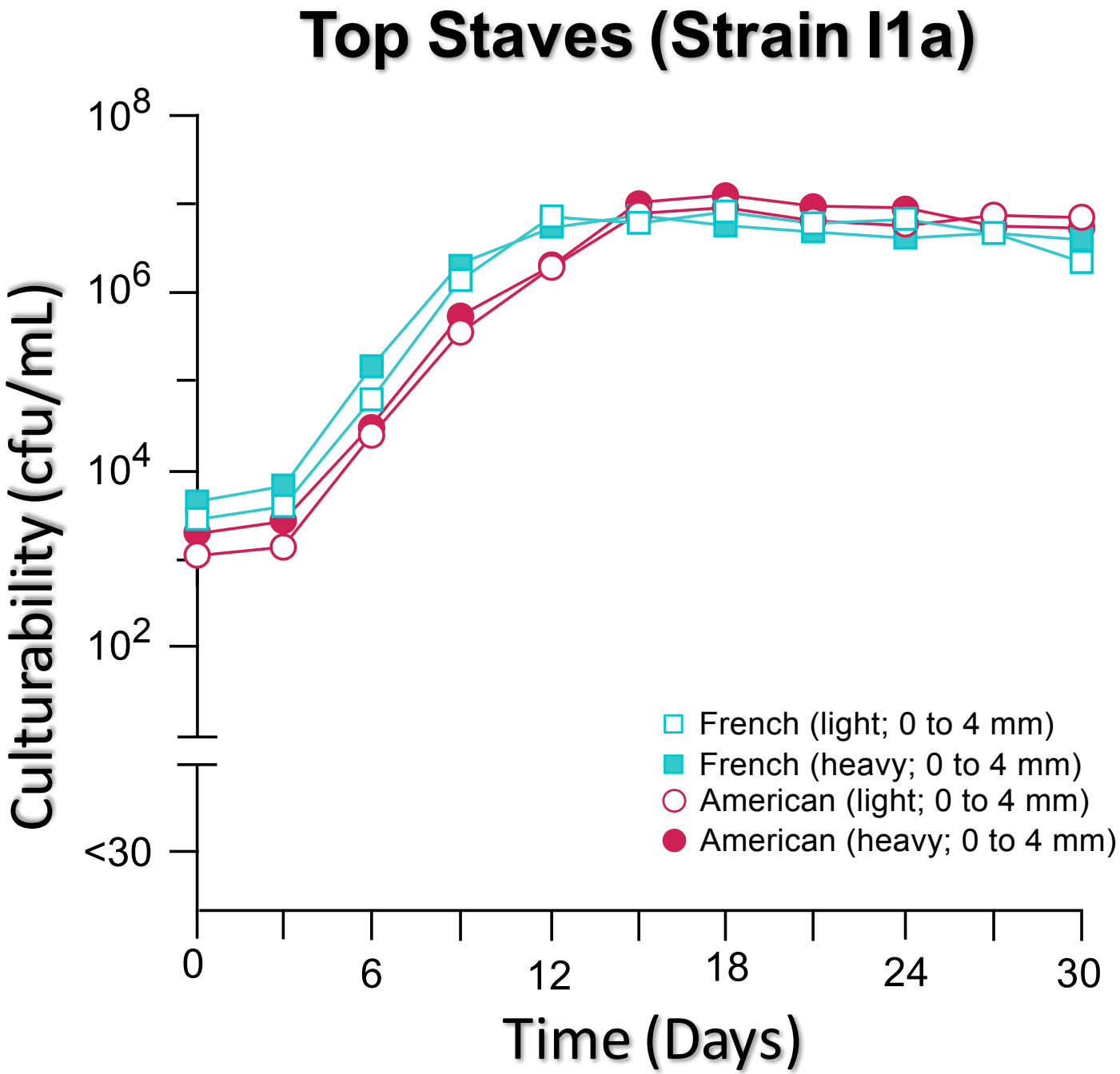
0 to 4 mm 10 to 14 mm

5 to 9 mm >15 mm


Bottom Staves (Strain I1a)
Culturability (cfu/mL)
10^8
10^6
10^4
10^2
<30
0
6
12
18
24
30
Time (Days)
French (light; 0 to 4 mm)
French (heavy; 0 to 4 mm)
American (light; 0 to 4 mm)
American (heavy; 0 to 4 mm)

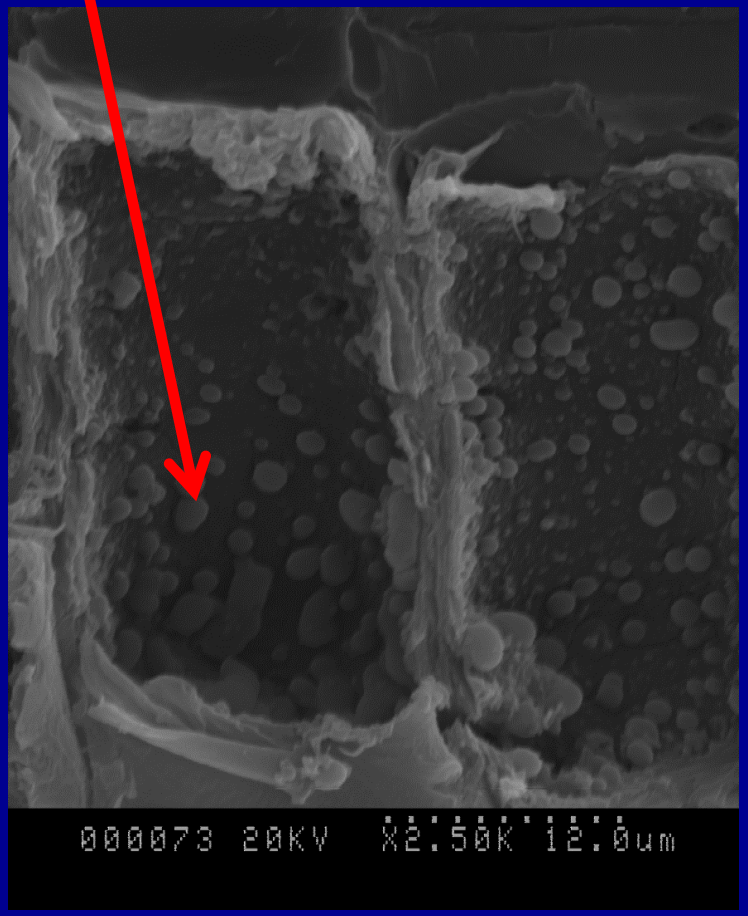
Bottom Staves (Strain I1a)

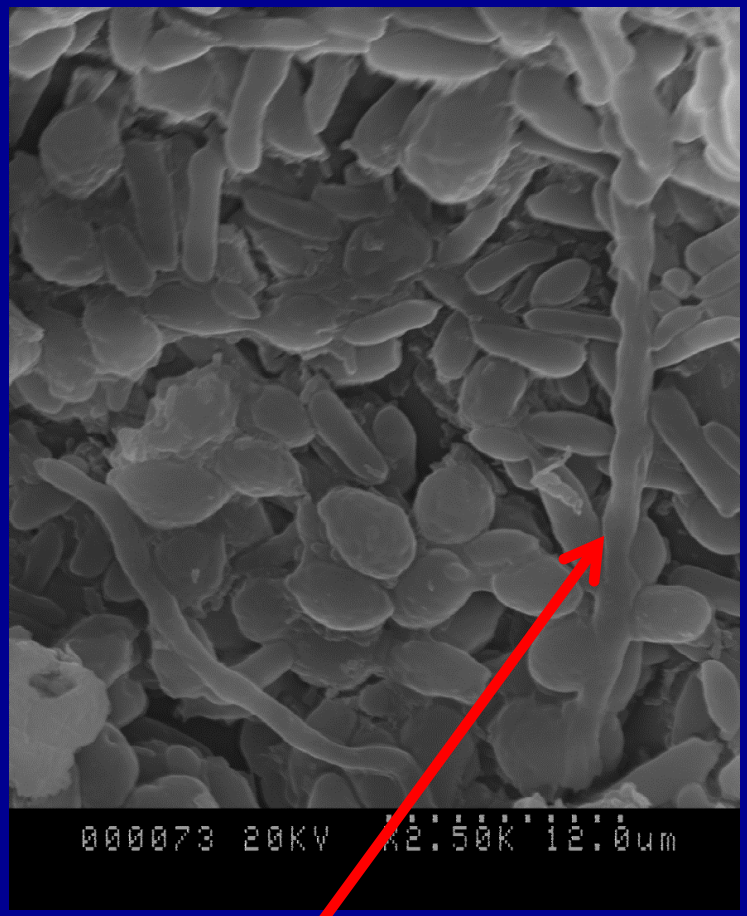



Top Staves (Strain I1a)
Culturability (cfu/mL)
10^8
10^6
10^4
10^2
<30
0
6
12
18
24
30
Time (Days)
French (light; 0 to 4 mm)
French (heavy; 0 to 4 mm)
American (light; 0 to 4 mm)
American (heavy; 0 to 4 mm)

Research Focus
(Growth in Oak Barrels)

Yeast





Possible pseudo-hyphae

Research Focus
(Growth in Oak Barrels)

A. Commercial barrels

1. Obtained four infected barrels.
2. Disassemble to determine yeast penetration.

B. Steam treatments

1. Determine heat penetration into staves.
2. Evaluate recovery of yeast.

Overview of Program

What other research projects have we worked with industry on?

Lactobacillus Infections

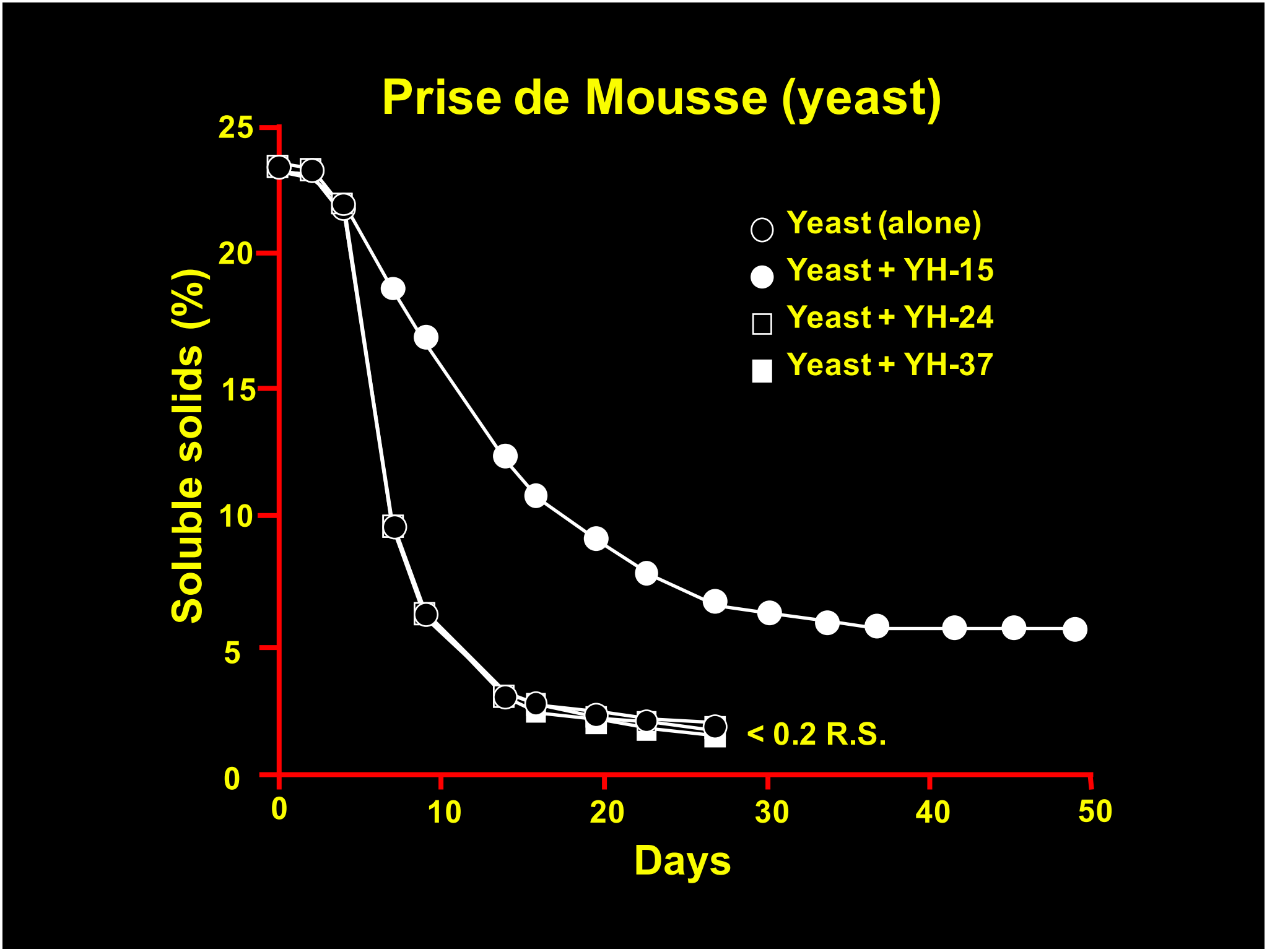
Wineries experimenting with low [SO_2].

- Marketing advantage?
- Importance of sanitation questioned?

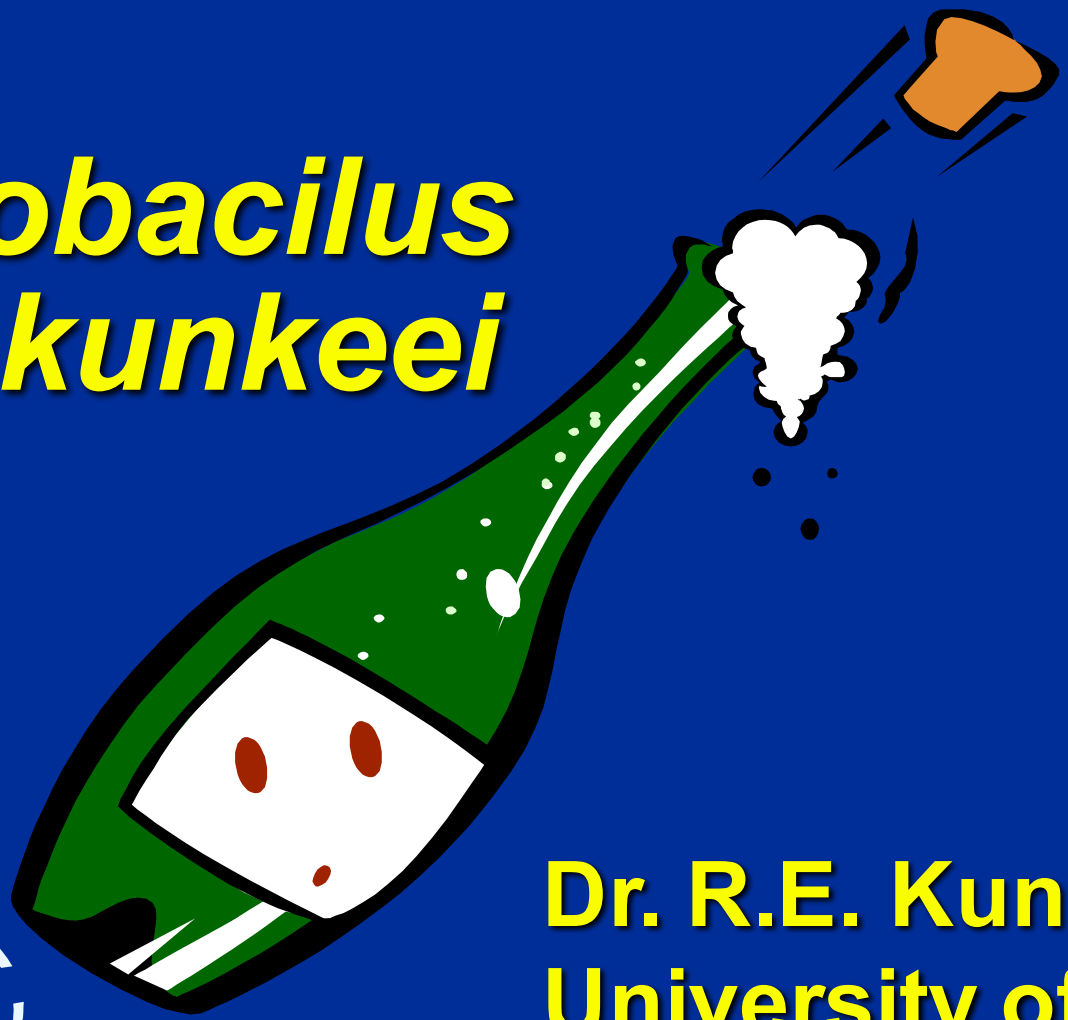
Some wineries began to have infections of unidentified bacteria.

- Led to stuck fermentations.

"Cause and effect" not established.

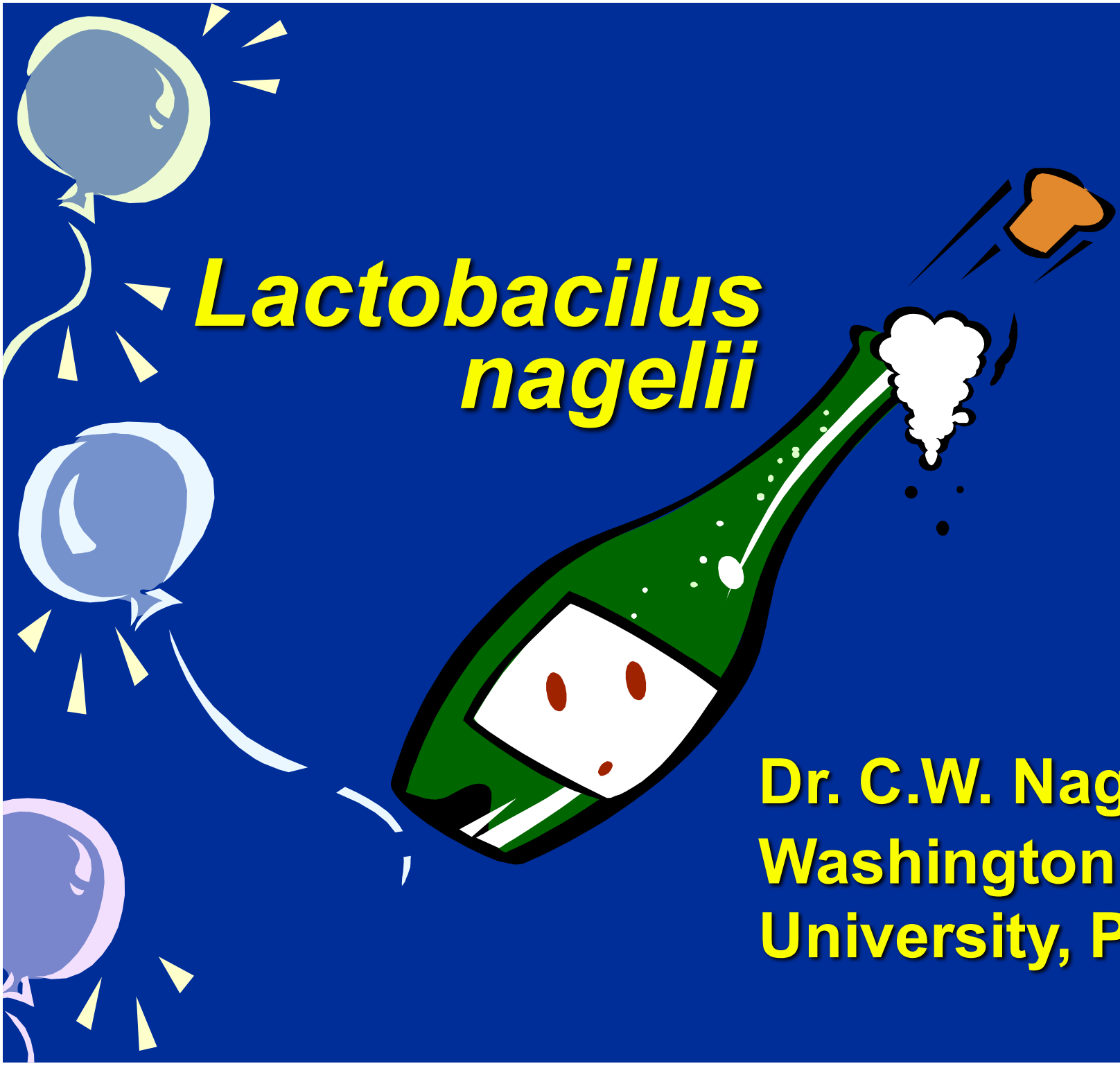

Prise de Mousse (yeast)
Soluble solids (%)
25
20
15
10
5
0
0
10
20
30
40
50
Days
Yeast (alone)
Yeast + YH-15
Yeast + YH-24
Yeast + YH-37
< 0.2 R.S.

Lactobacilus kunkeei



Dr. R.E. Kunkee
University of California, Davis

Lactobacilus nagelii



Dr. C.W. Nagel
Washington State University, Pullman

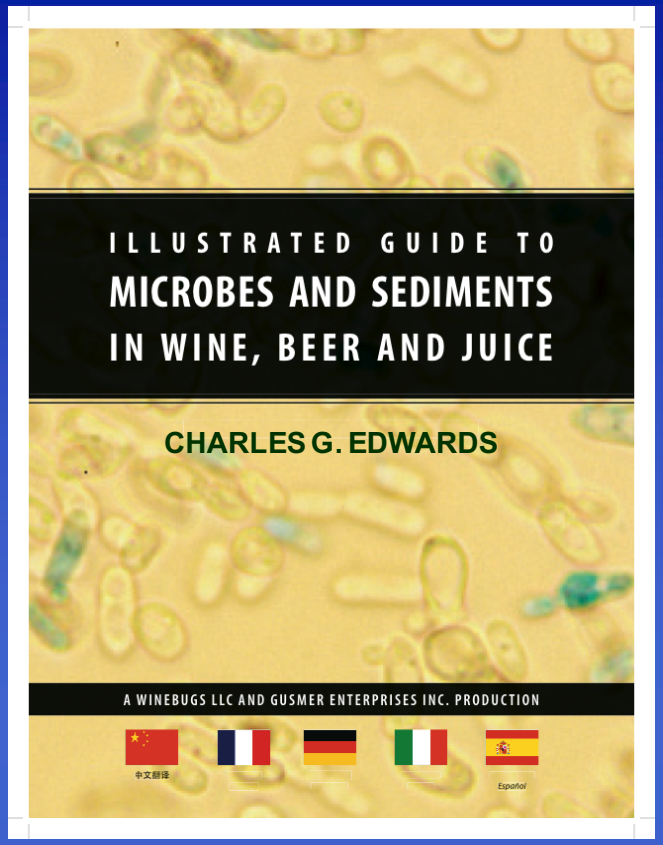
Nutritional Requirements of Yeast

A. Modified nutritional supplements

- Emphasis on certain vitamins.
- Change in US regulations.

B. Modified process

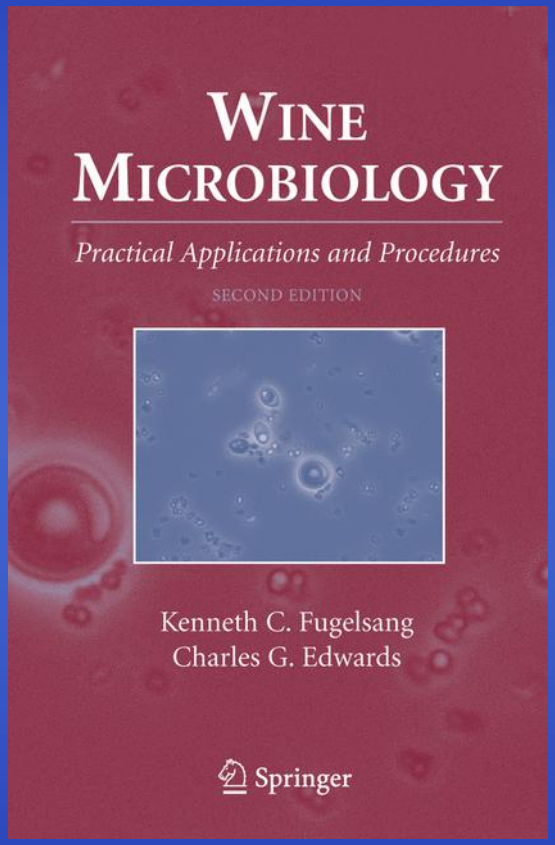
- Not a GMO-type product.
- Change in how commercial "active dry yeasts" are prepared based on nutritional research.



www.gusmerenterprises.com

OIV

Best Enology Work in 2007



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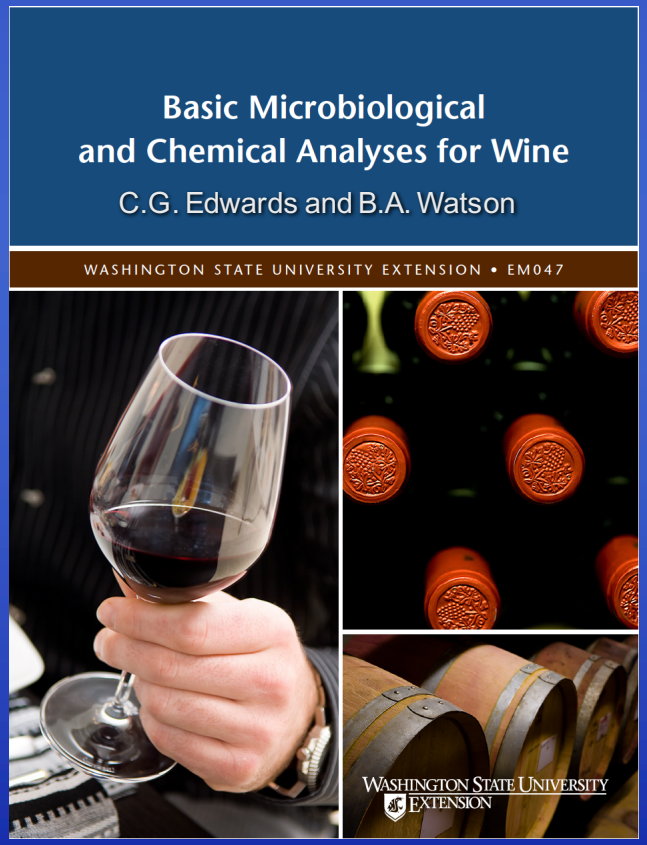

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References

Thank you for your support!

- Washington Wine Advisory Committee
- Ste. Michelle Wine Est.
- Washington wineries
- Vinotec Napa
- Lallemand Inc.
- GusmerEnterprises
- WSU students

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Felton Road Winery, Central Otago, NZ