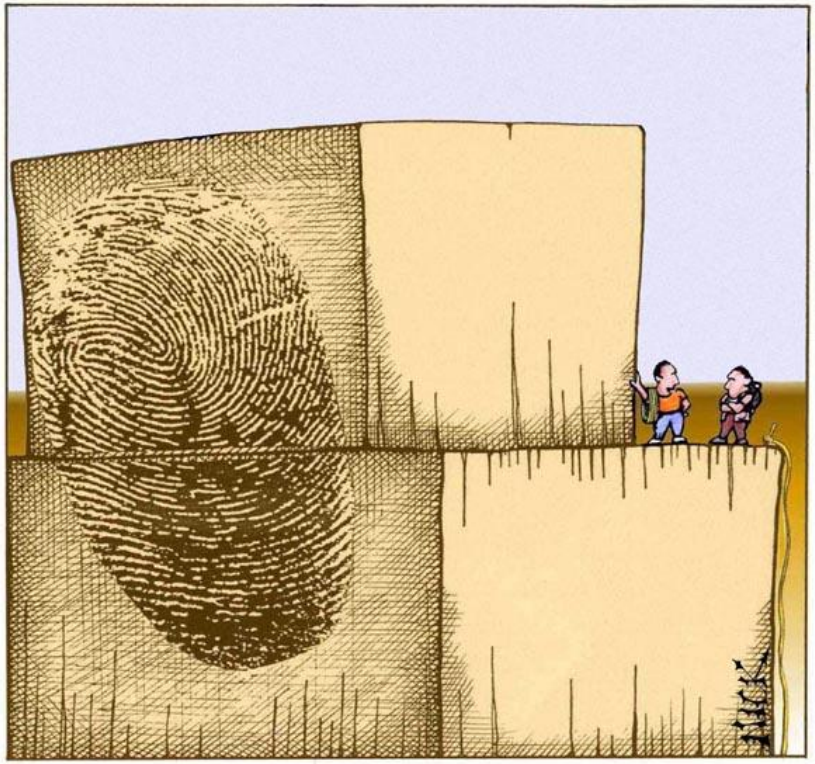
New insights into preventing *Brettanomyces* spoilage



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

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 must nutrition?

Impact of SO_2

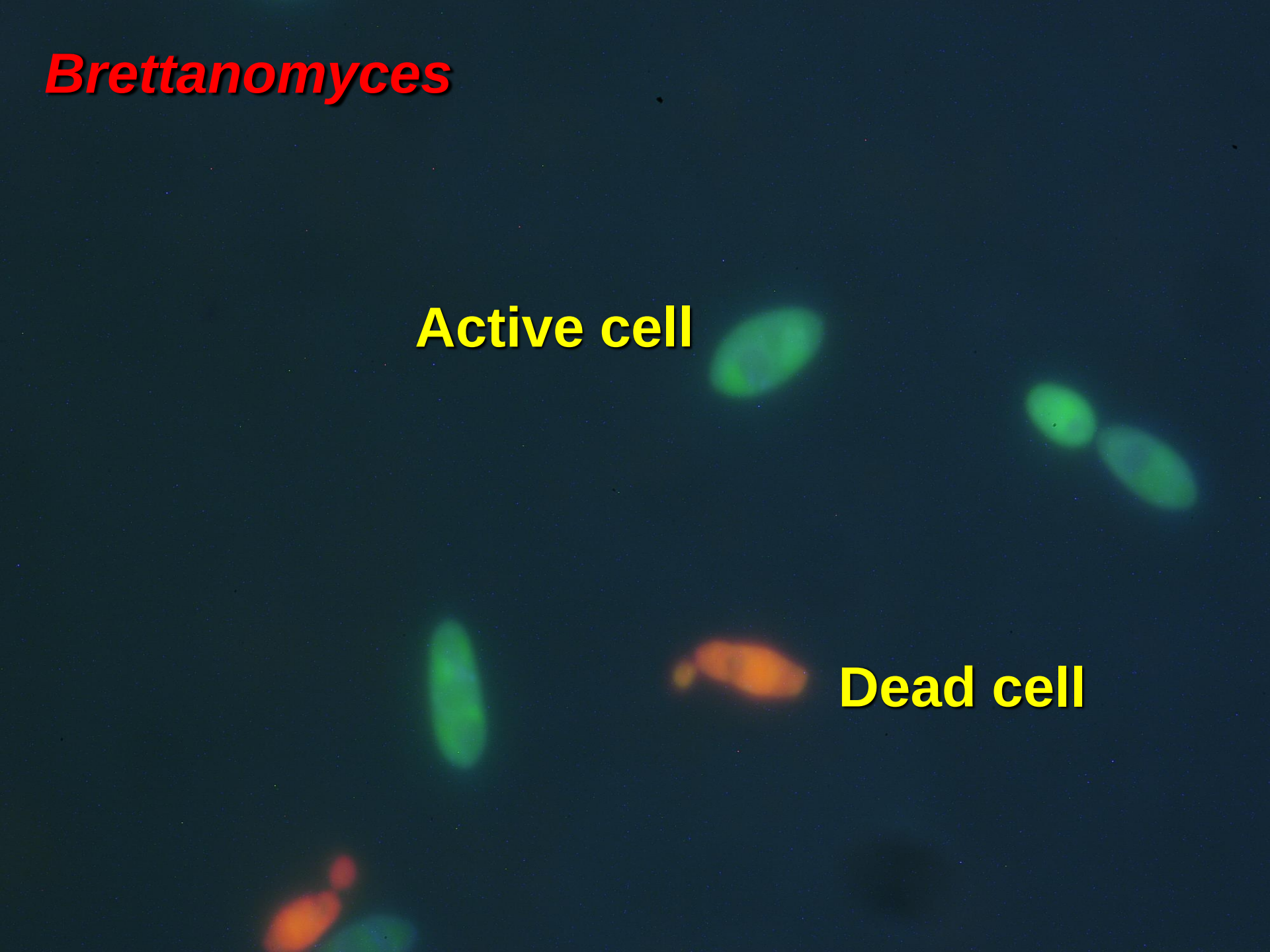
A. Can SO_2 cause "VBNC"?

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

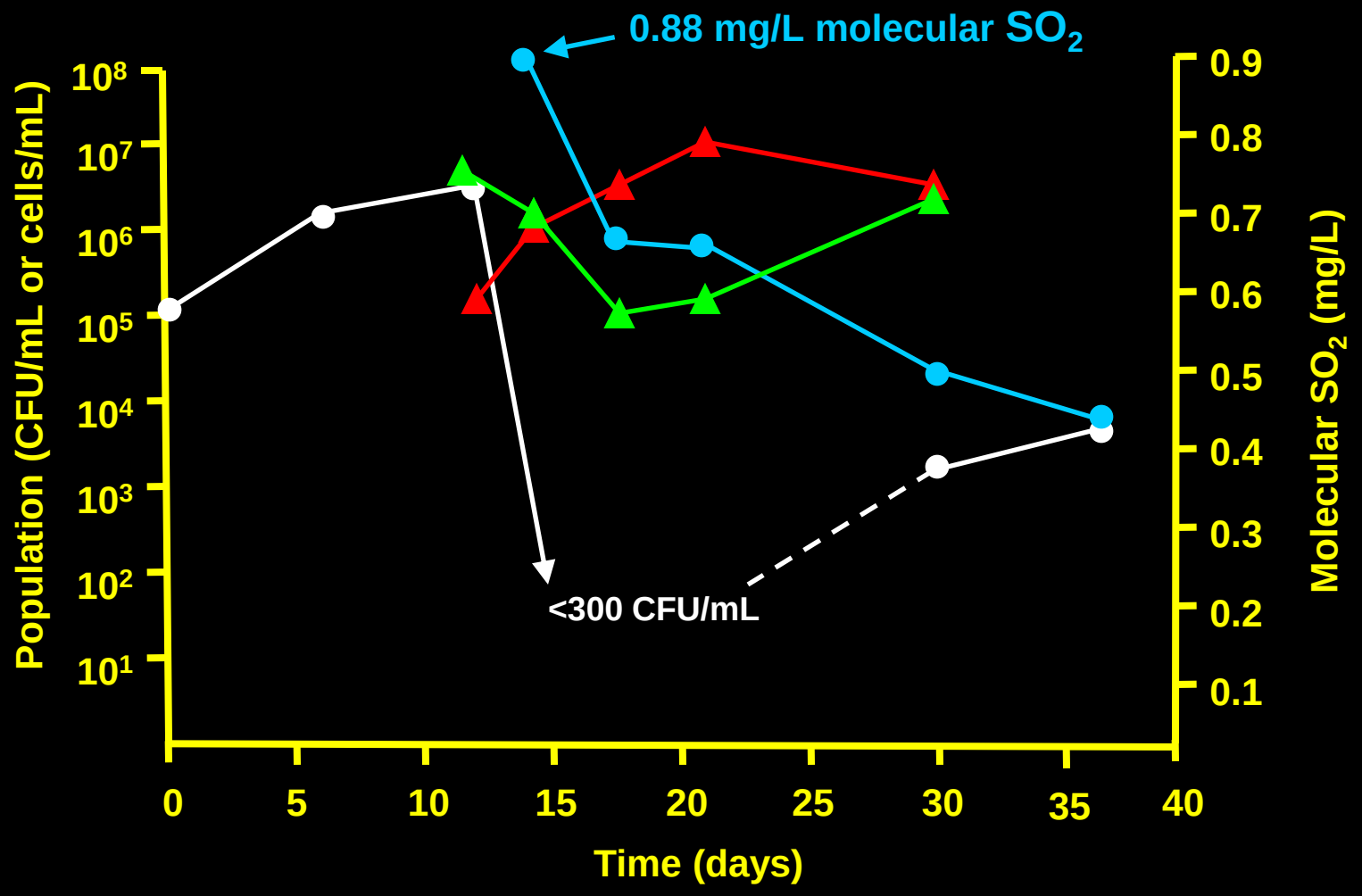
B. How to detect VBNC?

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

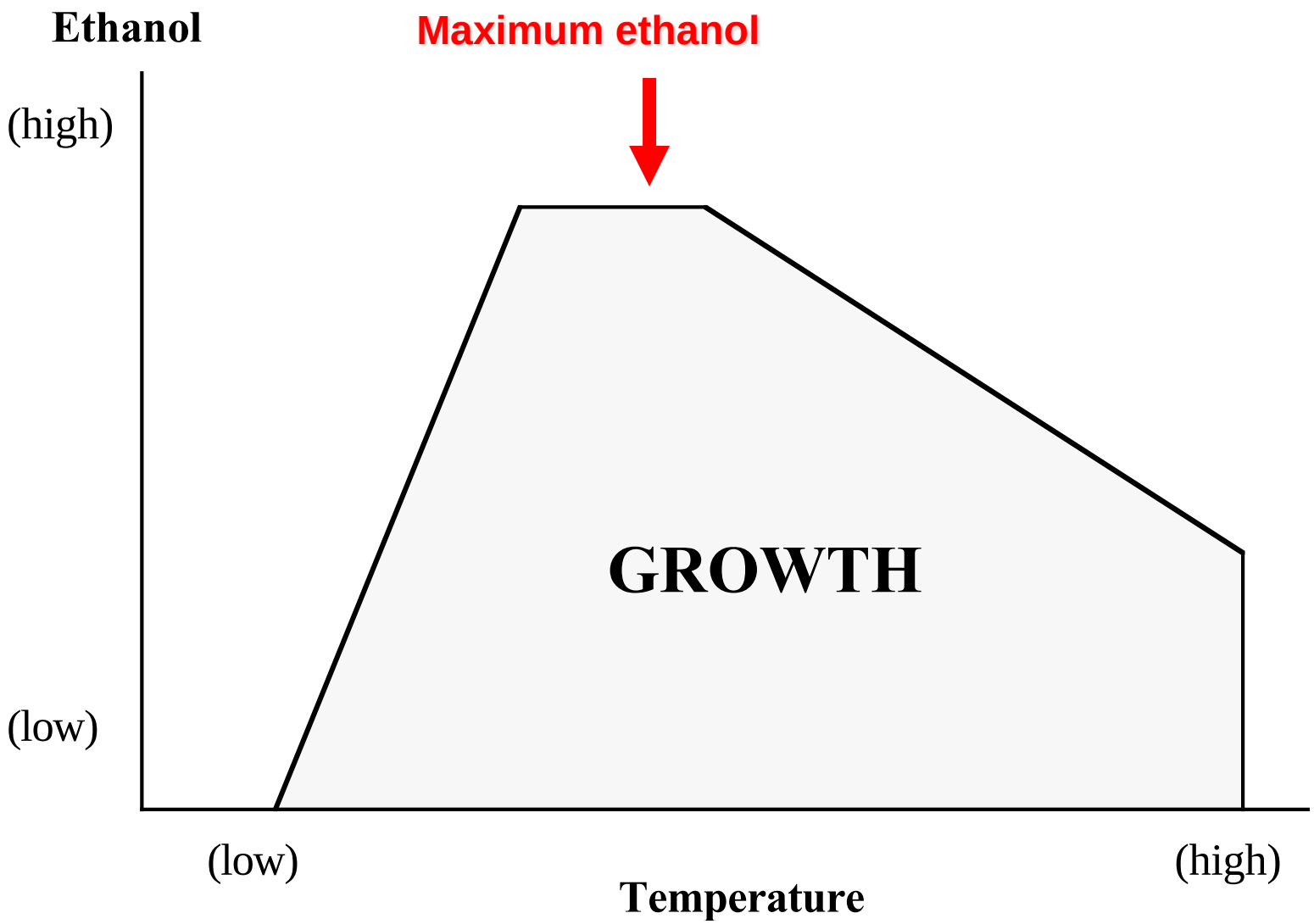
Green = viable Orange/Red = dead


Brettanomyces
Active cell
Dead cell

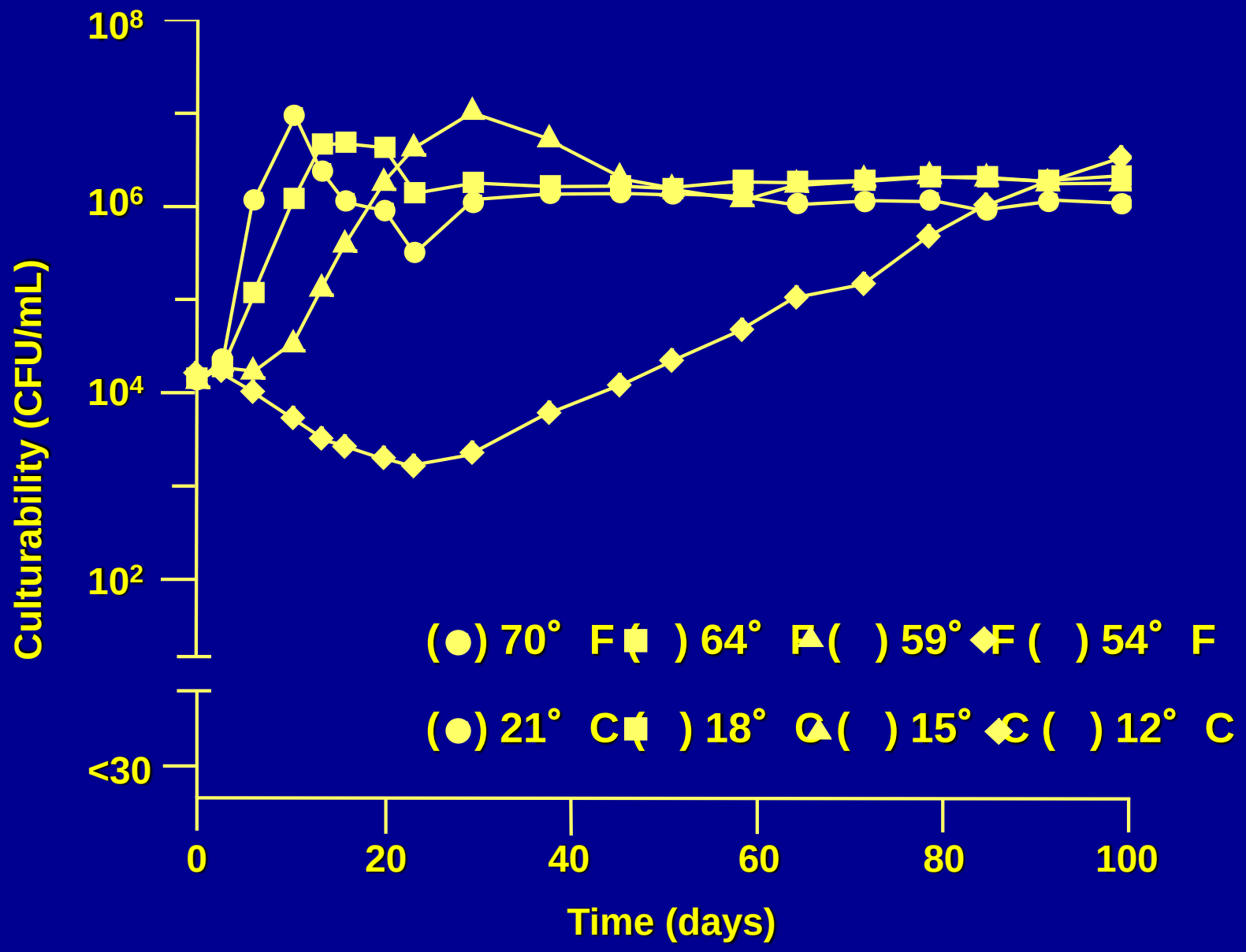
B. bruxellensis (B1b)



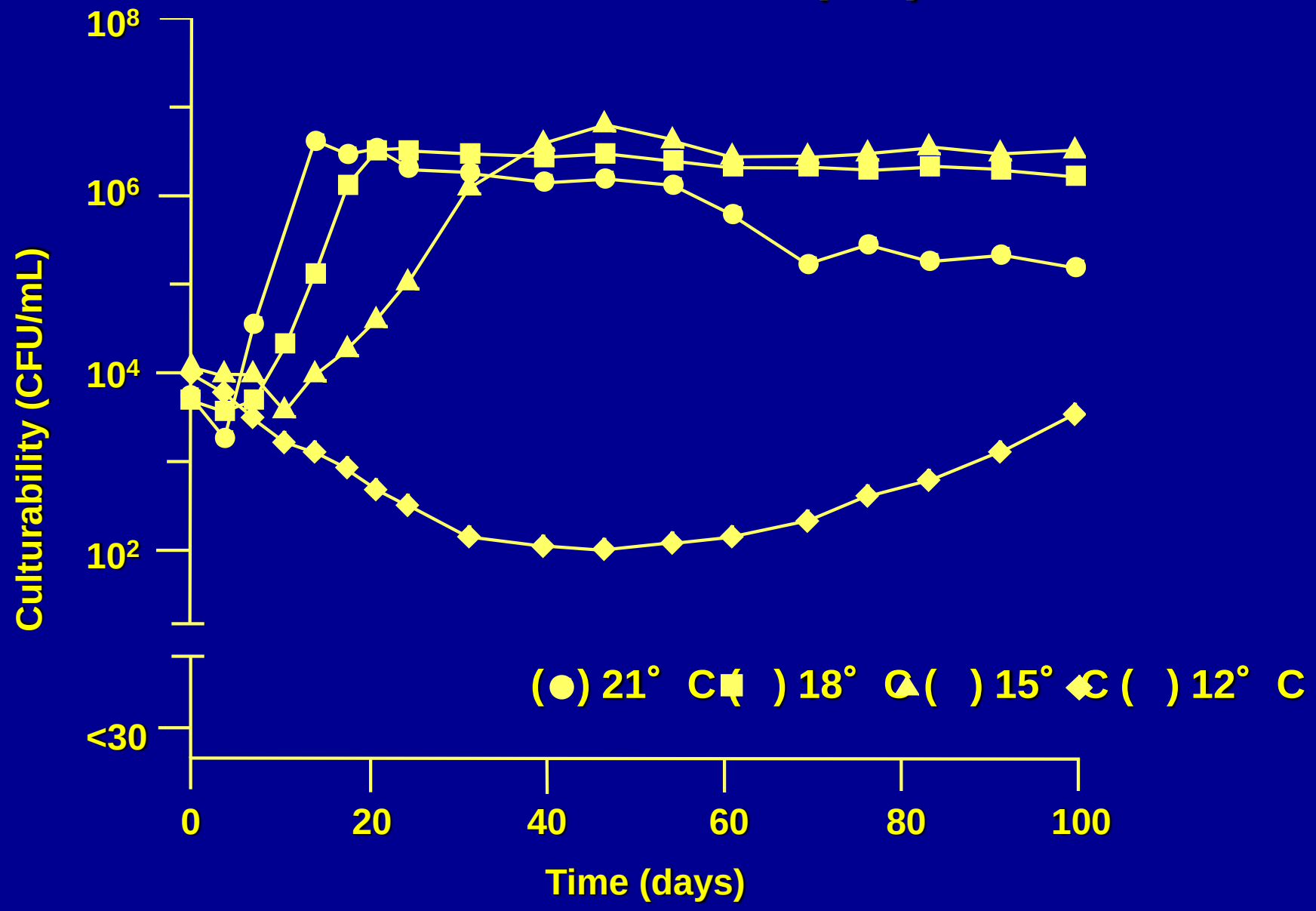
Is there a relationship between ethanol and temperature that could be useful to control *Brettanomyces*?


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

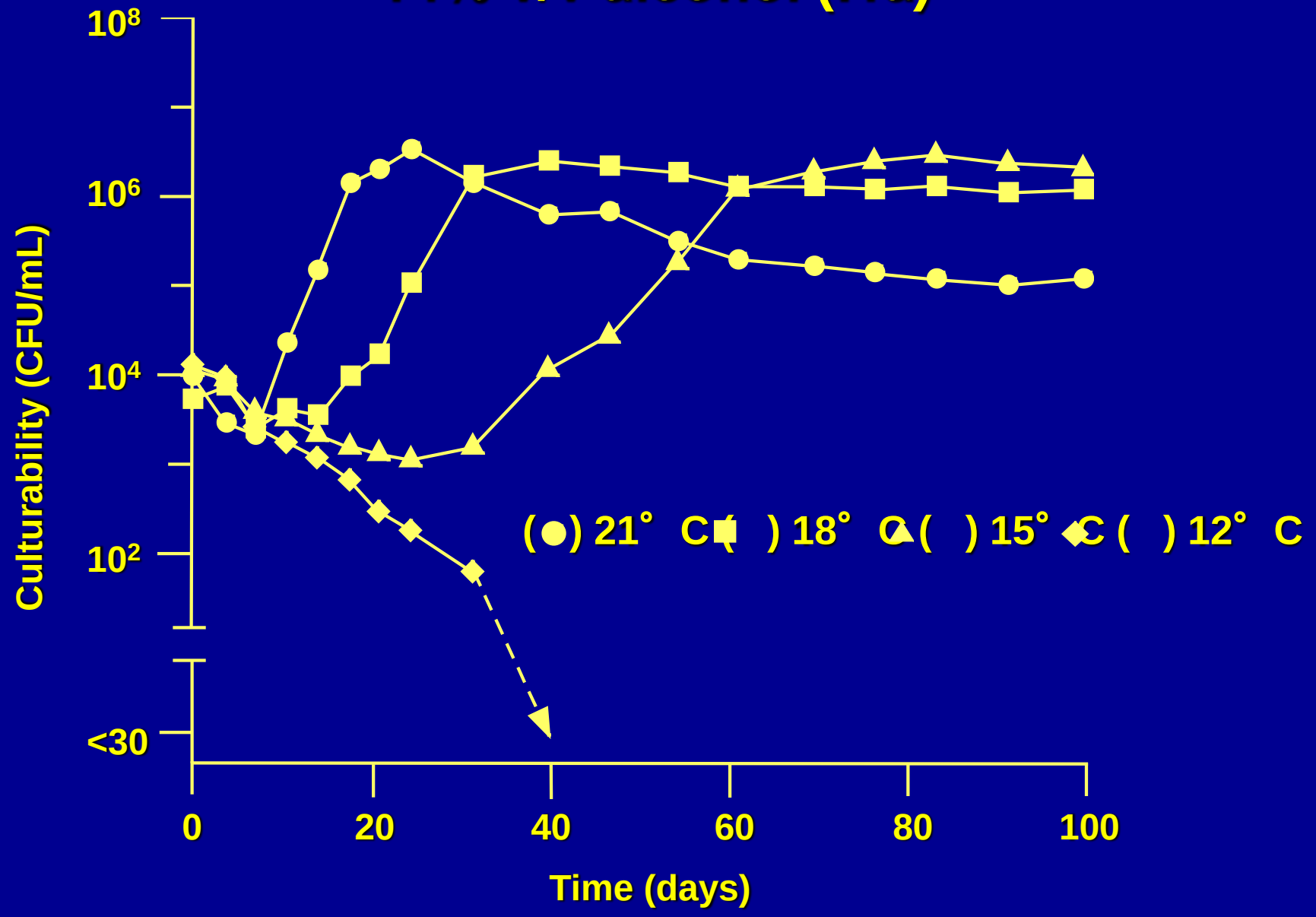
12% v/v alcohol (I1a)



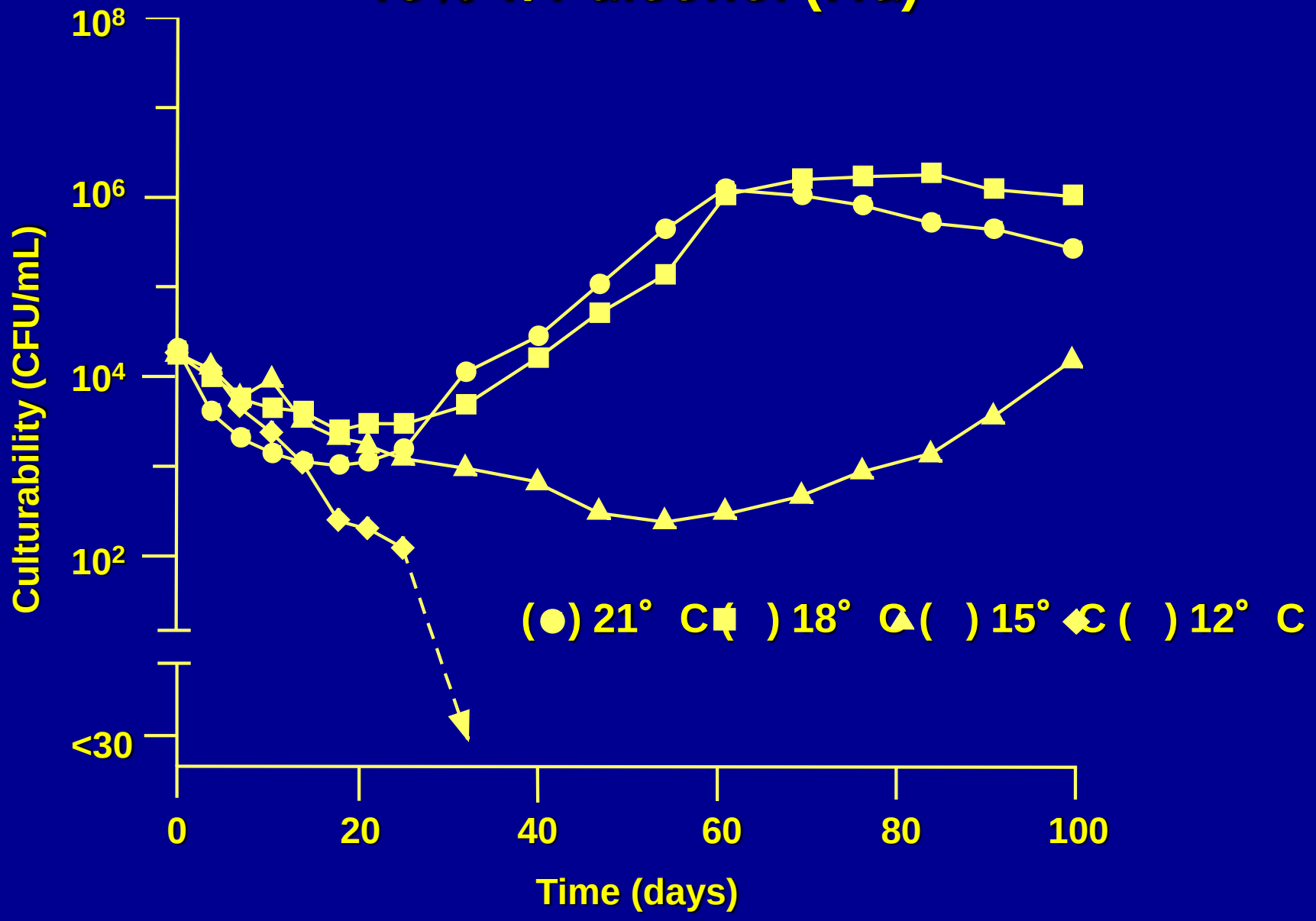
13% v/v alcohol (I1a)



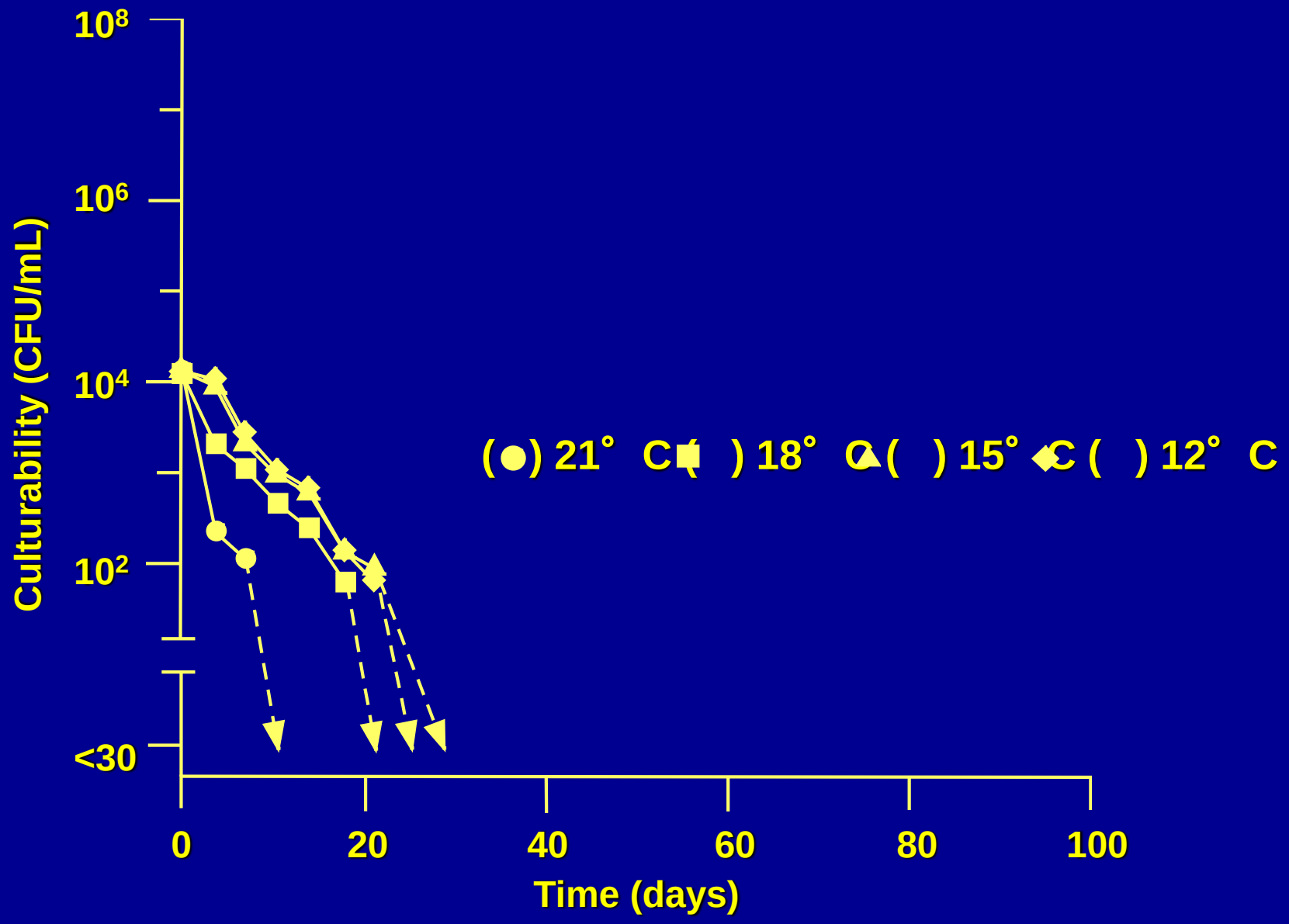
14% v/v alcohol (I1a)



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)

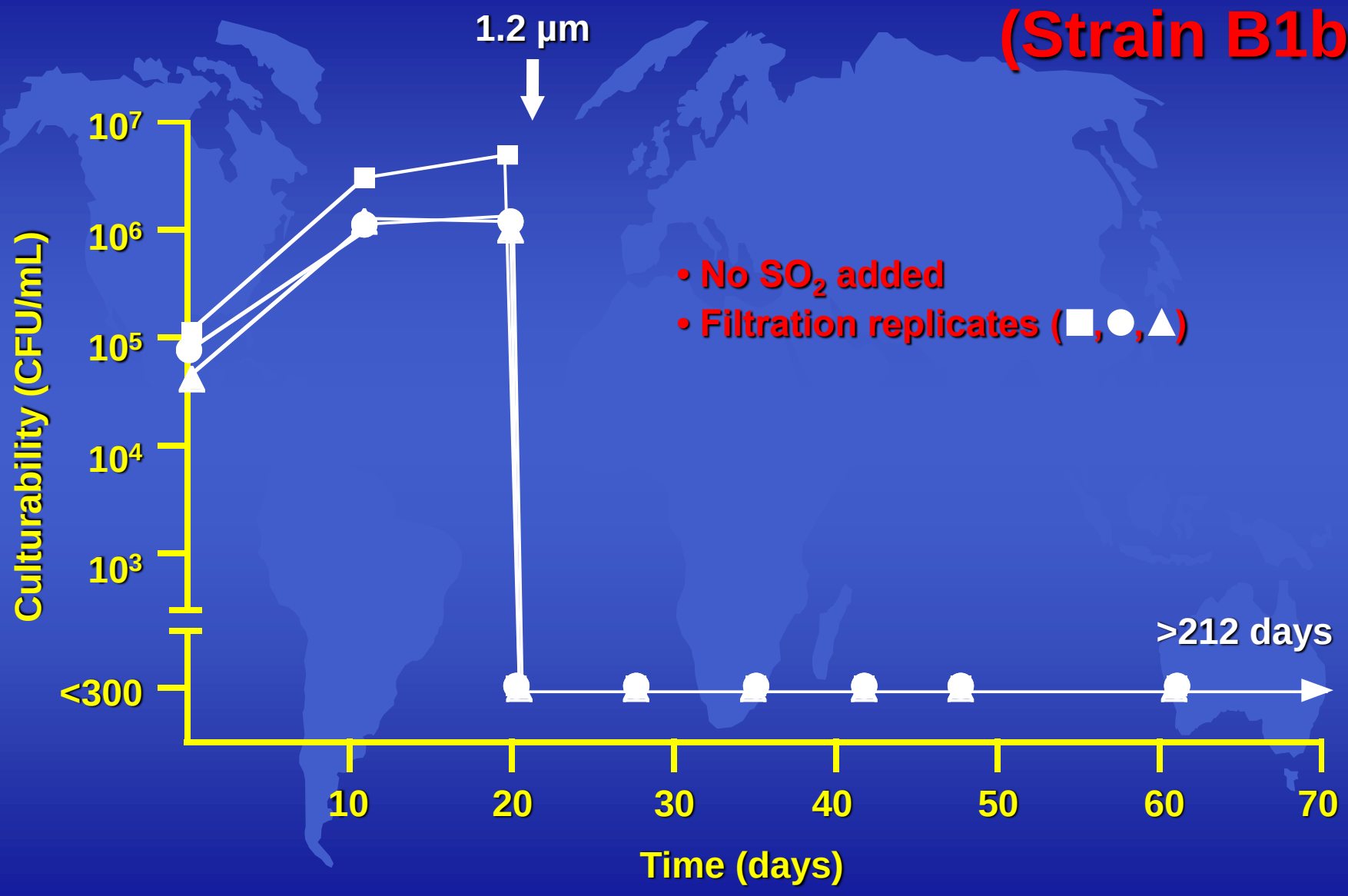
Red wines (0 or 0.5 mg/L mSO_2)

Treatment Filter Cartridge #1	Treatment Filter Cartridge #2	Treatment Filter Cartridge #3
3 samples (No SO_2)	3 samples (No SO_2)	3 samples (No SO_2)
3 samples (SO_2)	3 samples (SO_2)	3 samples (SO_2)

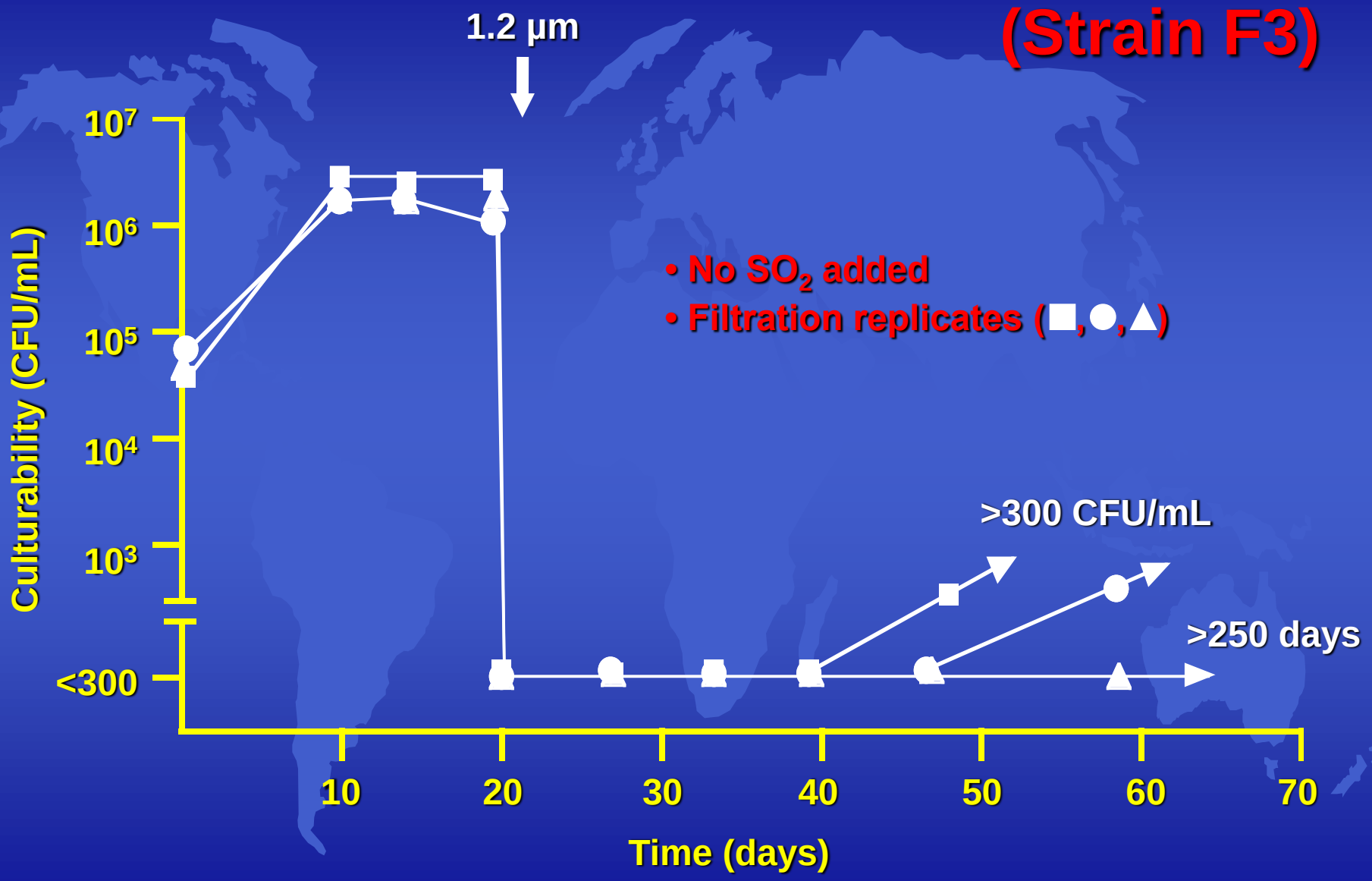
Treatment Replicate = Filter cartridge

- Multiple ferments incubated >200 days.

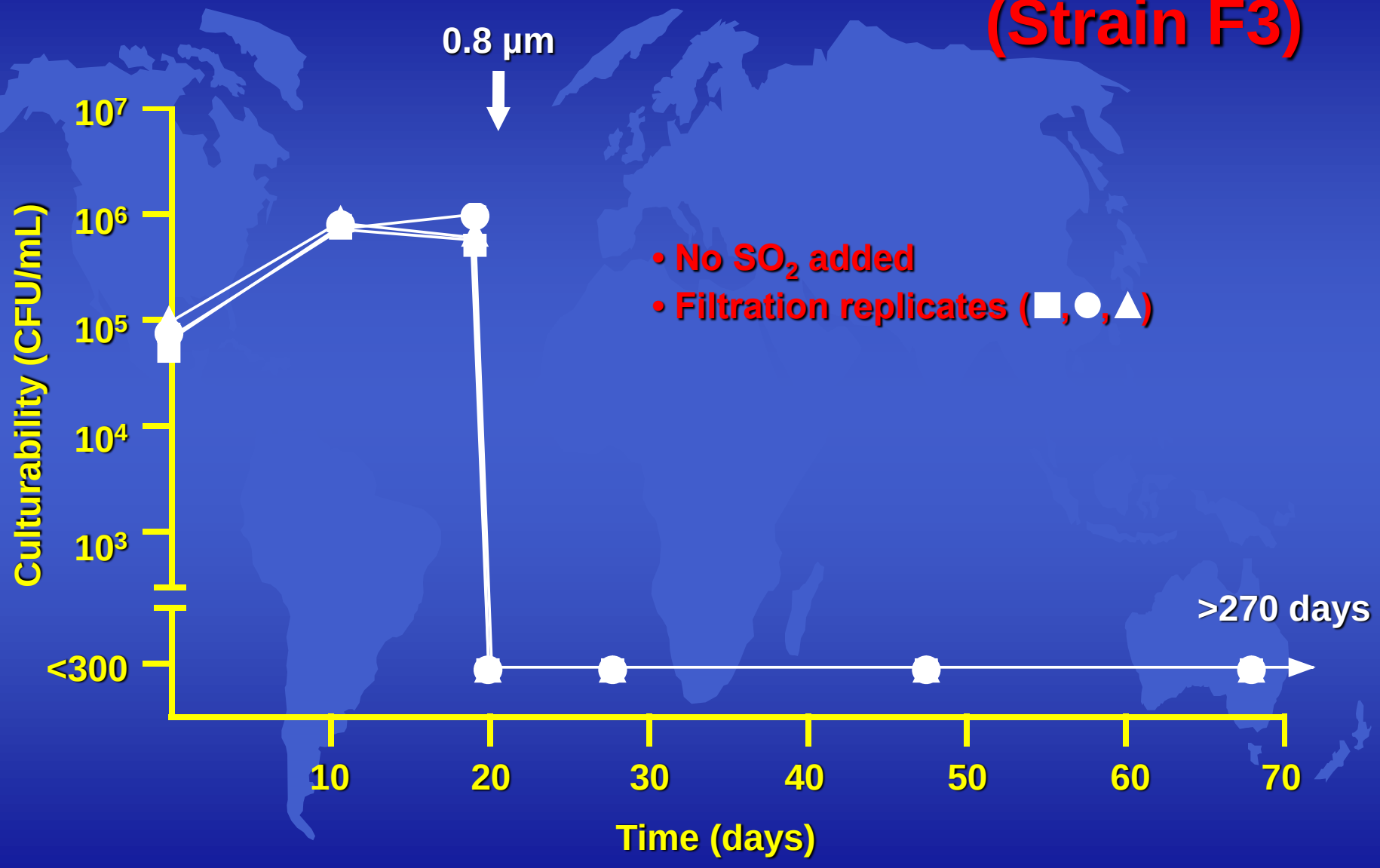
Filtration
(Strain B1b)



Filtration
(Strain F3)



Filtration
(Strain F3)



Growth in oak barrels?

Research Focus
(Growth in Oak Barrels)

- **American and French oaks (16L and 225L).**
- **Various toasted levels.**
- **D value = time for 90% reduction**
  (*Brettanomyces* → 1 minute at 55° C)

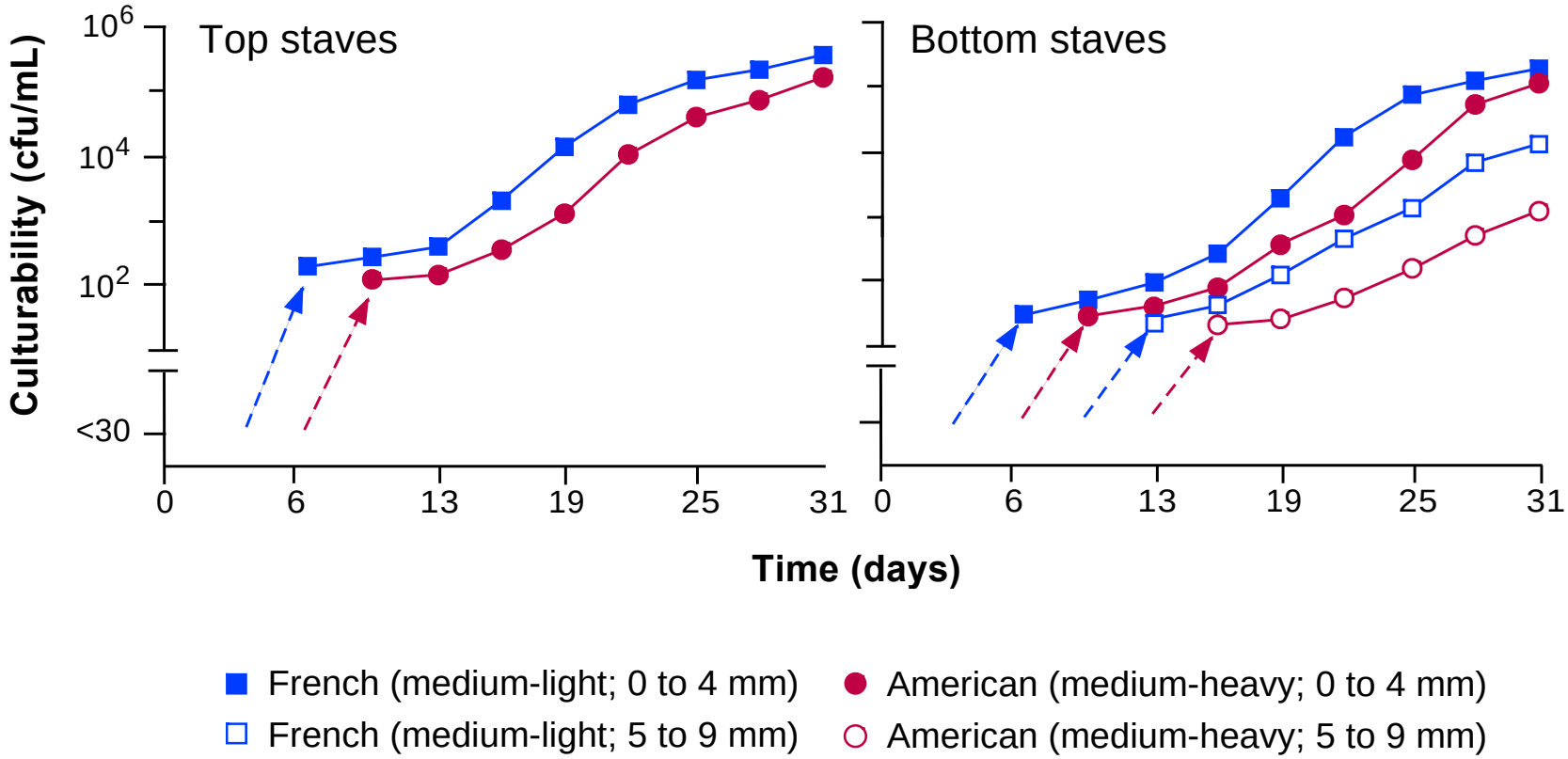
Research Focus
(Growth in Oak Barrels)

Cross-Sectional Layer
(transfer into sterile wine)

0 to 4 mm 10 to 14 mm

5 to 9 mm >15 mm

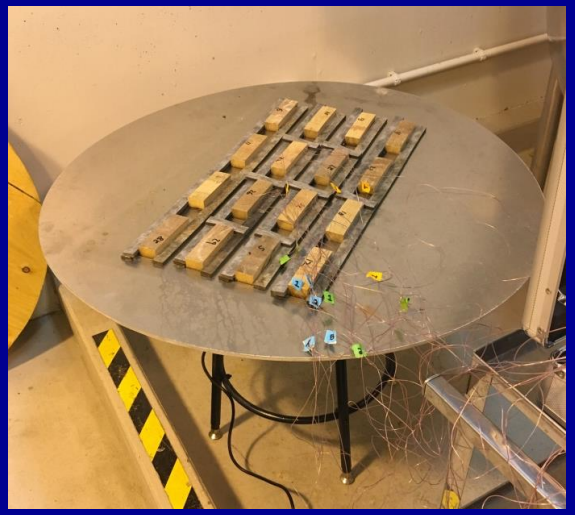
Recovery of *Brettanomyces* (225L barrels)



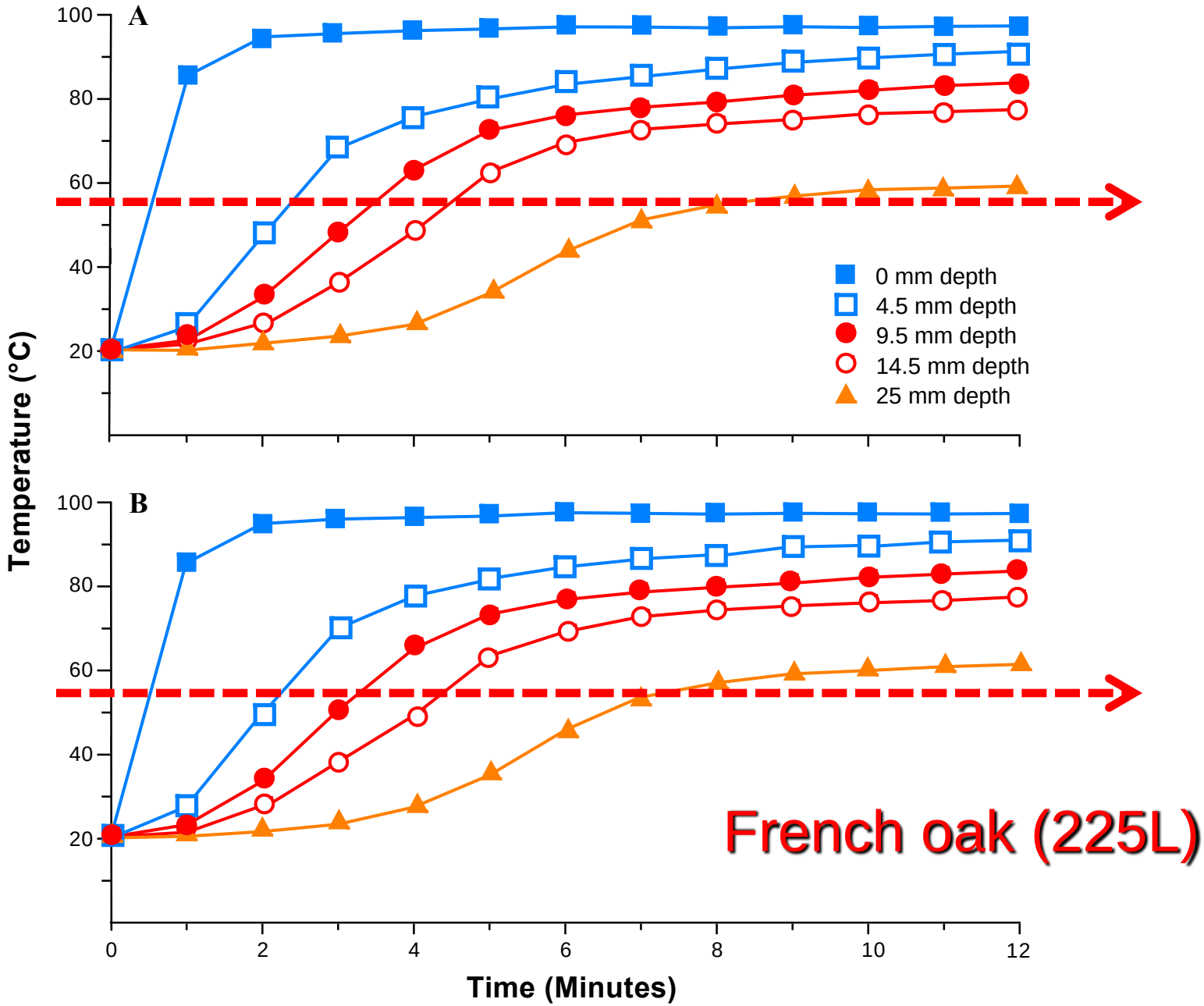
Research Focus

(Use of heat to reduce populations)

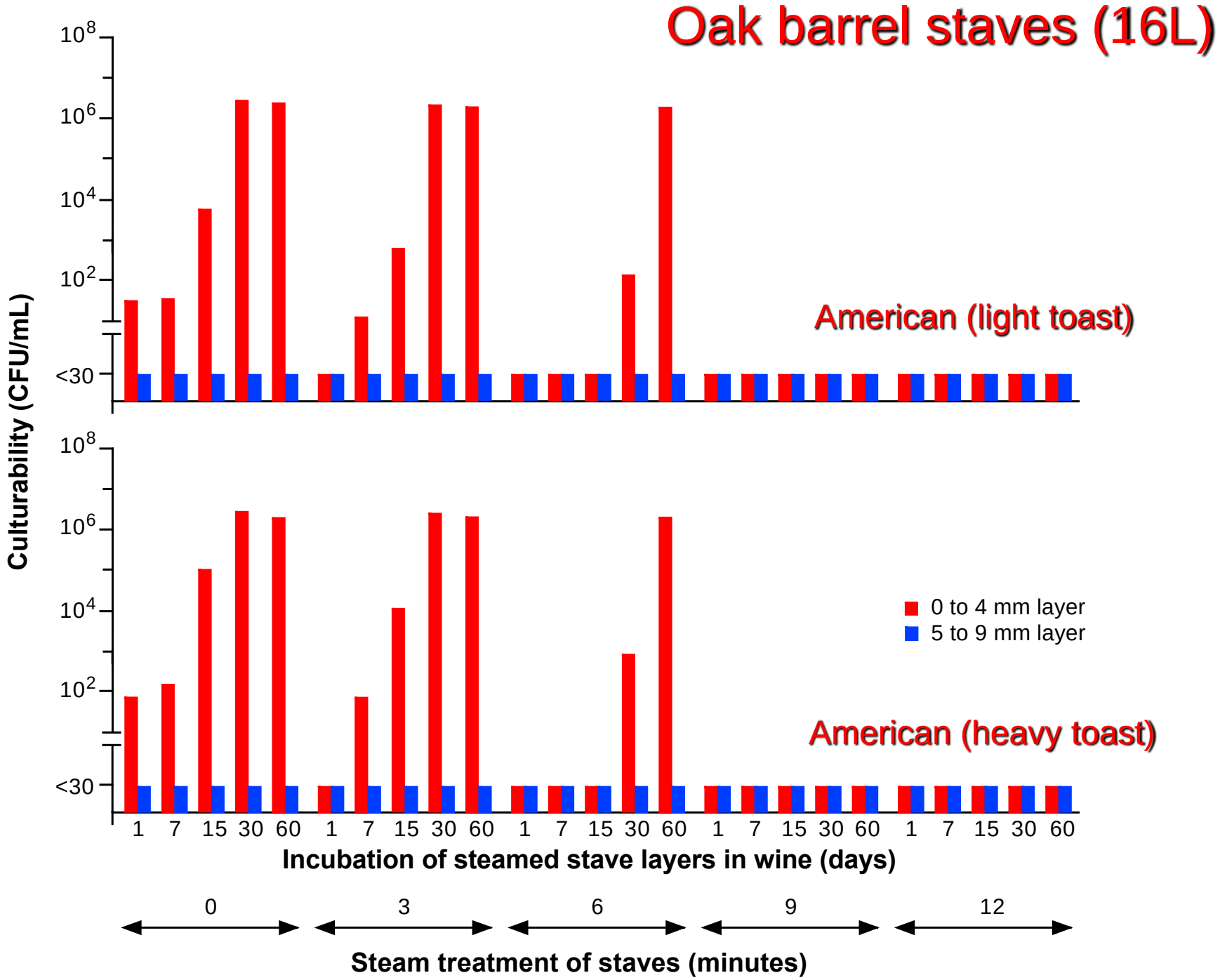
- Cut staves into 3 x 10 cm blocks/place into SS plate
- Thermocouples inserted every 4 mm
- Place plate over a steam kettle to record °C
- Periodically remove blocks; determine yeast recovery



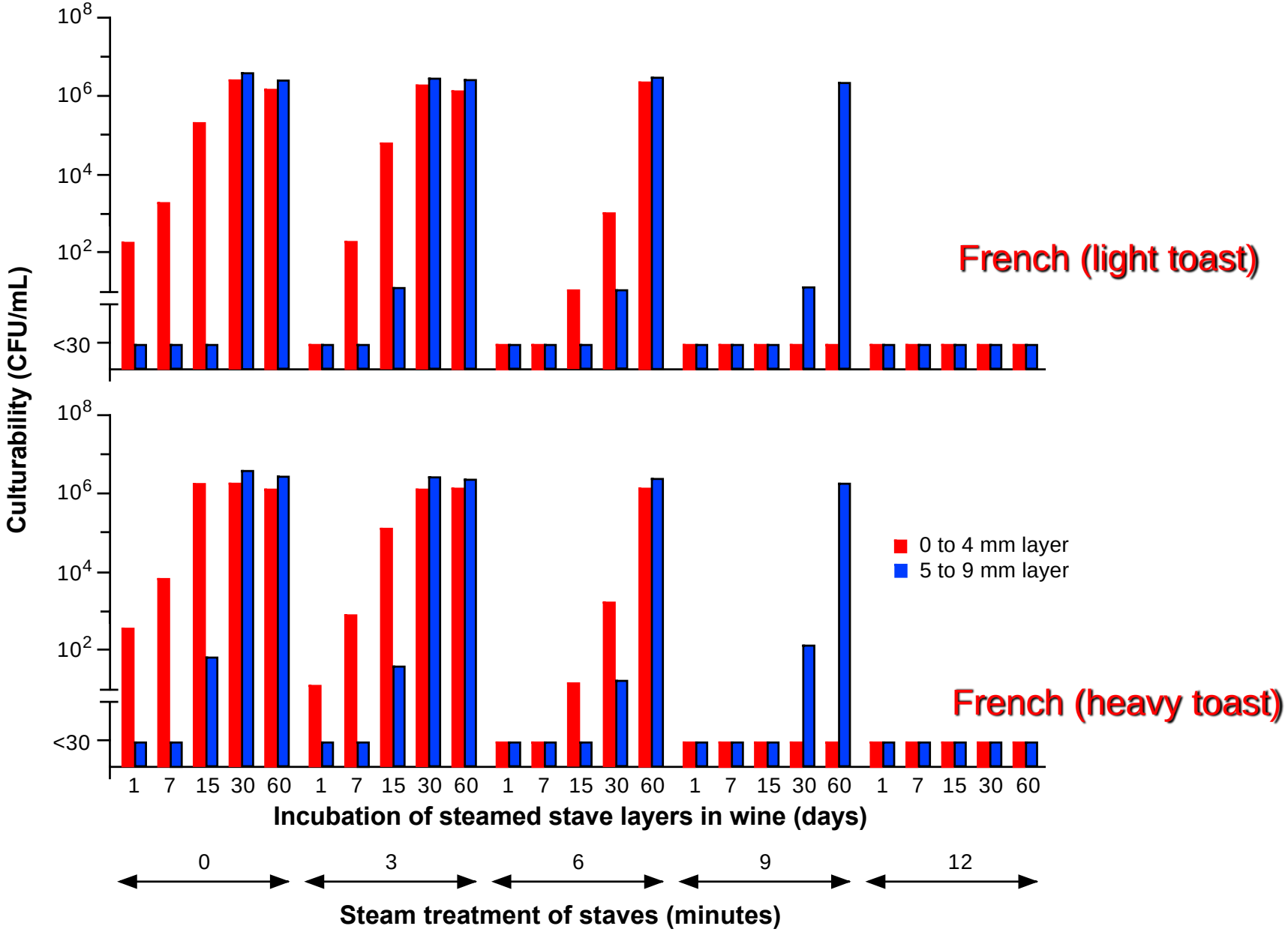
American oak (225L)



French oak (225L)


Oak barrel staves (16L)
American (light toast)
American (heavy toast)
0 to 4 mm layer
5 to 9 mm layer
Culturability (CFU/mL)
10^8
10^6
10^4
10^2
<30
1 7 15 30 60 1 7 15 30 60 1 7 15 30 60 1 7 15 30 60 1 7 15 30 60
Incubation of steamed stave layers in wine (days)
0
3
6
9
12
Steam treatment of staves (minutes)

Oak barrel staves (16L)



Impact of Must Nutrition
(*Brettanomyces*)

1. If more N is added to grape must →
   is more N present in resultant wines?

2. Does higher N in wines encourage infections?

   - What amount of N is needed to support growth?

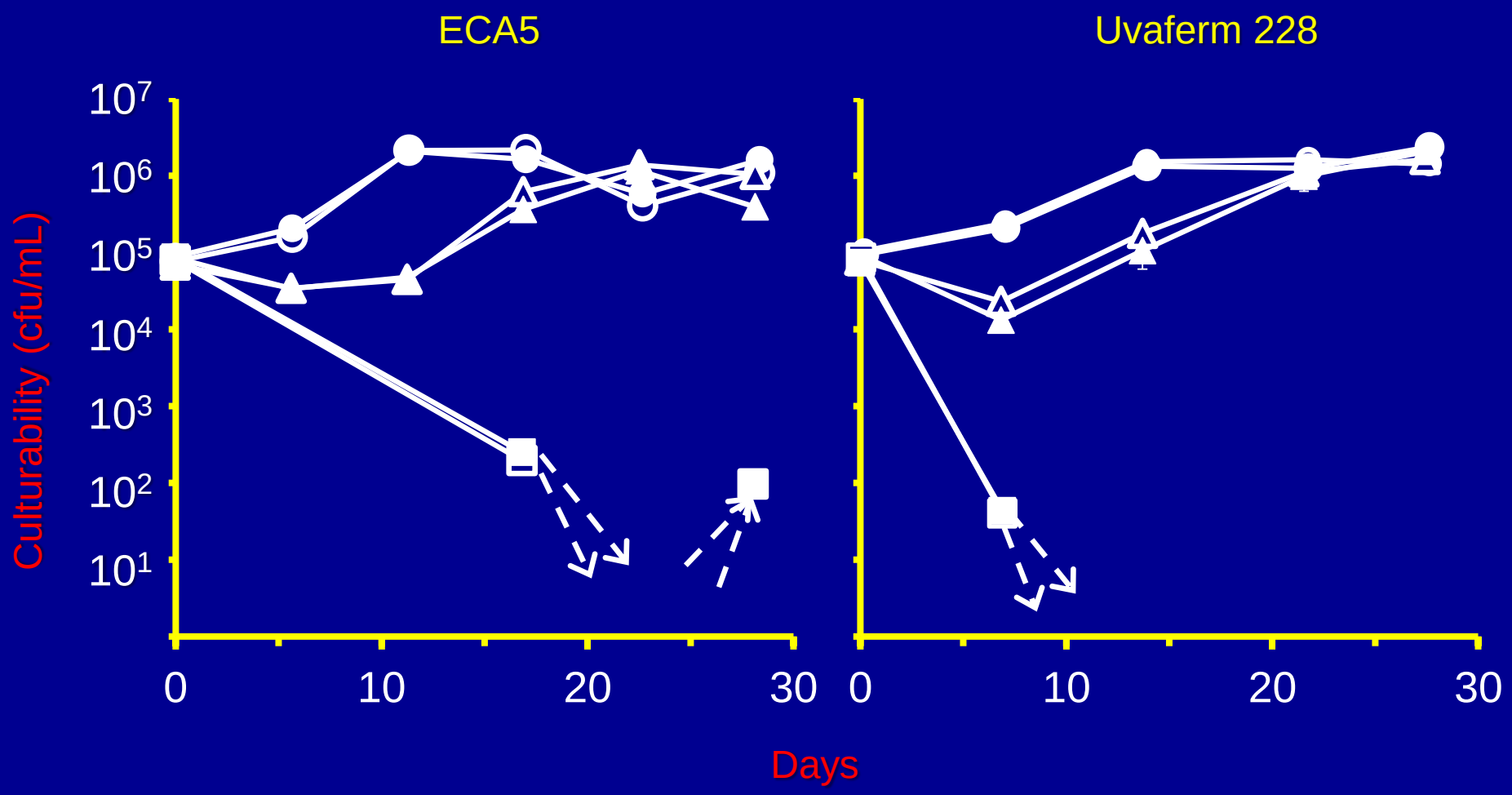
Residual nitrogen in “synthetic” wines

	Must treatment		Wine	
Yeast strain	YAN (mg N/L)	Sugar (g/L)	α-Amino nitrogen (mg N/L)	Ammonia nitrogen (mg N/L)
ECA5	150	230	50^{b}	1^{b}
		250	48^{bc}	1^{b}
		270	45^{b}	1^{b}
	250	230	85^{a}	60^{b}
		250	84^{a}	60^{b}
		270	86^{a}	60^{b}

Ethanol concentrations in wines

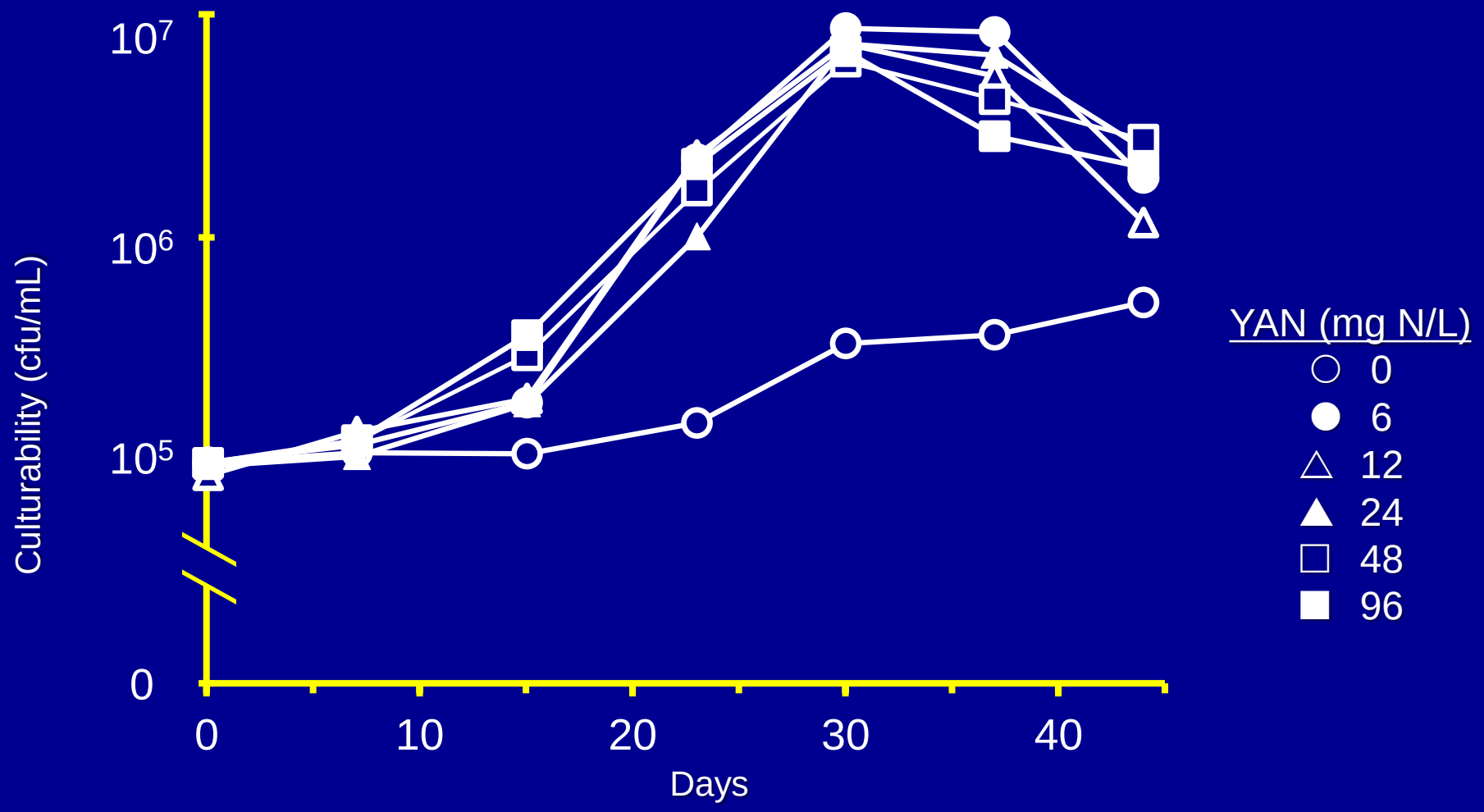
Sugar in musts (g/L)	Ethanol in wines (% v/v)	
230	13.5 to 14.1	“low”
250	14.7 to 14.9	“medium”
270	15.6 to 15.9	“high”

Brettanomyces E1 in wines



○ Low YAN, Low EtOH
●High YAN, Low EtOH
△ Low YAN, Med EtOH
▲ High YAN, Med EtOH
□ Low YAN, High EtOH
■ High YAN, High EtOH

Brettanomyces E1 in synthetic wine (13% v/v alcohol)



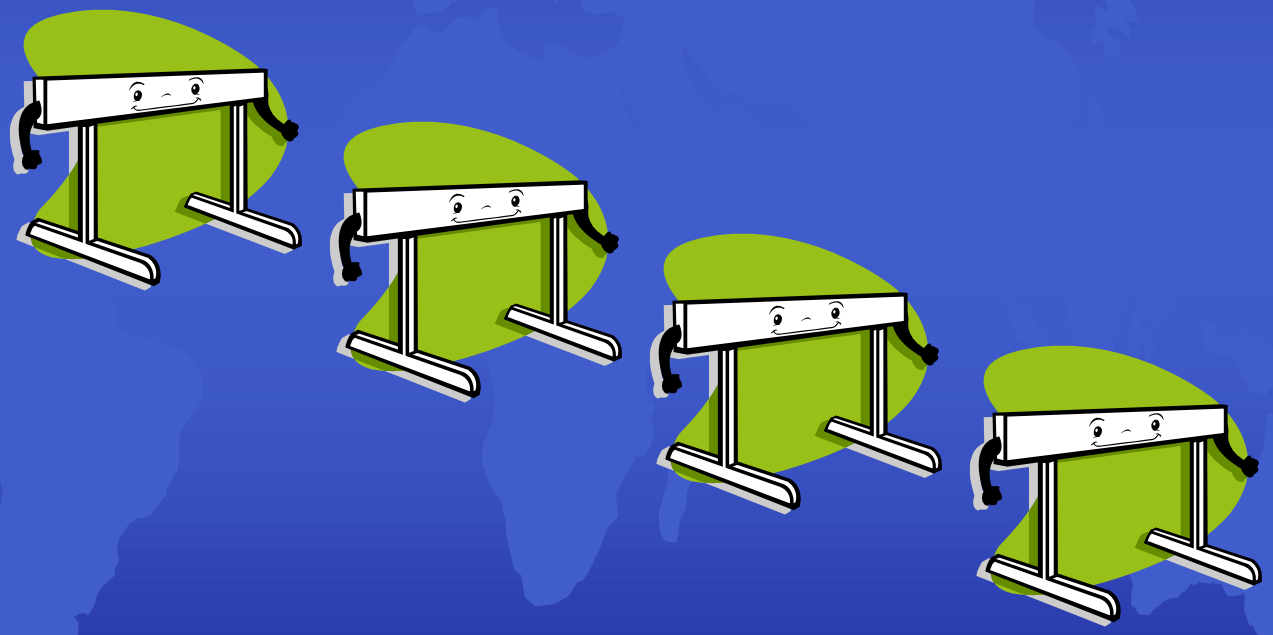
Microbe Management

OR really.....

minimizing the risk of spoilage

Control Spoilage By Using Multiple "Hurdles"

Initial infection



Spoilage

Use of "Hurdles"

- Avoid importing unfiltered wine
- Avoid used barrels
- SO_2, ethanol, and low temperature
- Filtration (0.8 micron absolute)
- Cleaning / sanitizing program

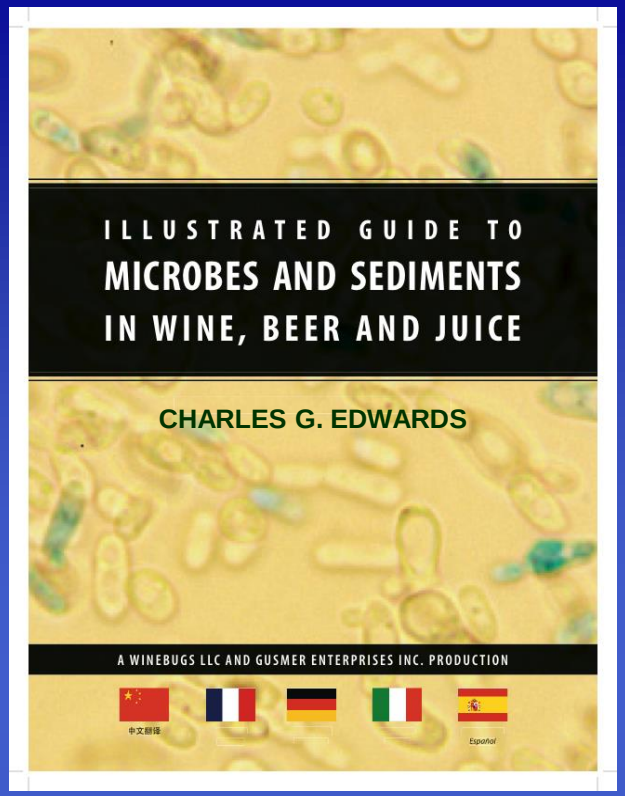
Conclusions

Incorporate as many hurdles as is REASONABLE for the winery

Too few hurdles =
increased risk of spoilage.

Too many hurdles =
expense higher than benefit.

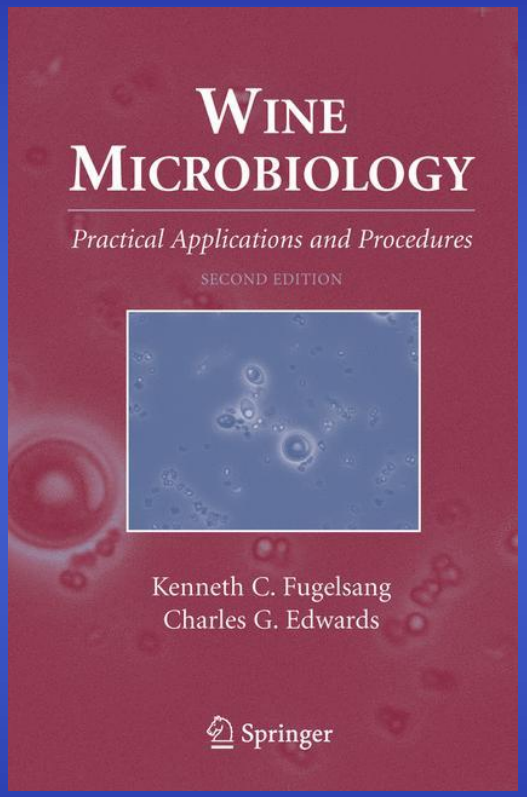
KEY → *Hurdle interactions exist!*



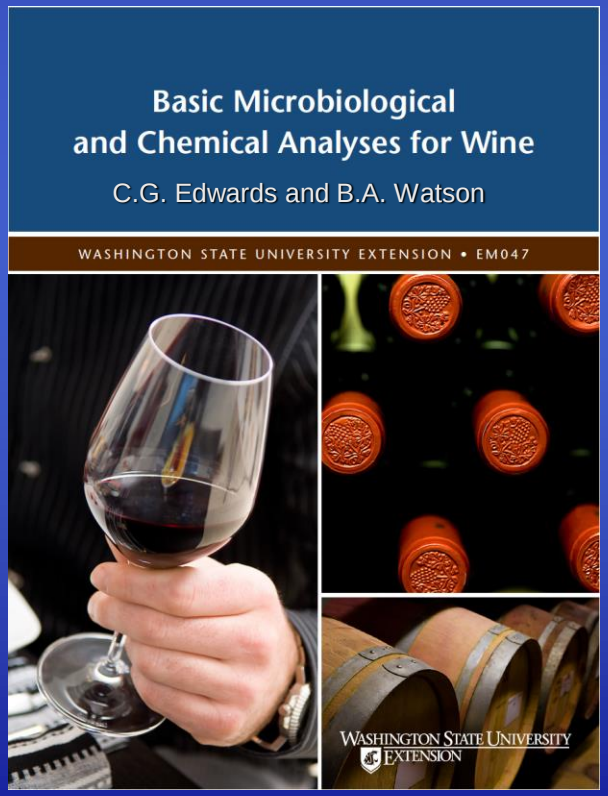
www.gusmerenterprises.com

OIV

Best Enology Work in 2007



www.amazon.com



pubs.wsu.edu

References

Thank you for your support!

Washington Wine Advisory Committee

GusmerEnterprises

ETS Laboratories

Scott Laboratories

Ste Michelle Wine Estates

Vinotech Napa

Undergraduate/graduate students

Lallemand Inc.

Washington Association of Wine Grape Growers

Northwest Center for Small Fruits Research

Washington and regional wineries

Oregon Wine Board

WASHINGTON STATE UNIVERSITY

World Class. Face to Face.

Seminar Dedication

Ken Fugelsang (CSUF)