BCAWA Winemaker Conference

Preventing and Fixing a Stuck Fermentation

Sigrid Gertsen-Briand
Lallemand/ Scott Labs

May, 2010

Who is Lallemand?

- Privately owned Canadian company
- Established in Montréal in 1915
- We are approx. 2200 + people
- Invest a great deal in research around the world
- « Selection, research, production and marketing of micro-organisms and their by-products. »

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Oenology Product Range

- Active Dry Wine Yeast Strains
  - ~150 *Saccharomyces* (>1000 in collection)
  - Brands include Lalvin, Enoferm, Uvaferm, VI-A-DRY
- Encapsulted Wine Yeast
  - 4 winemaking applications
- Malolactic Bacteria
  - 10 *Oenococcus* Strains
  - Brands include Lalvin, Enoferm
- Enzymes
  - 10 different pectinases
  - Lallzyme Brand
- Nutrients
  - Yeast – Servomyces, Fermaid, Go-Ferm
  - Malolactic – OptiMalo Plus, ActiML
- Specific Yeast Derivatives
  - OptiRed, OptiWhite & BoosterRouge, Booster Blanc, Noblesse

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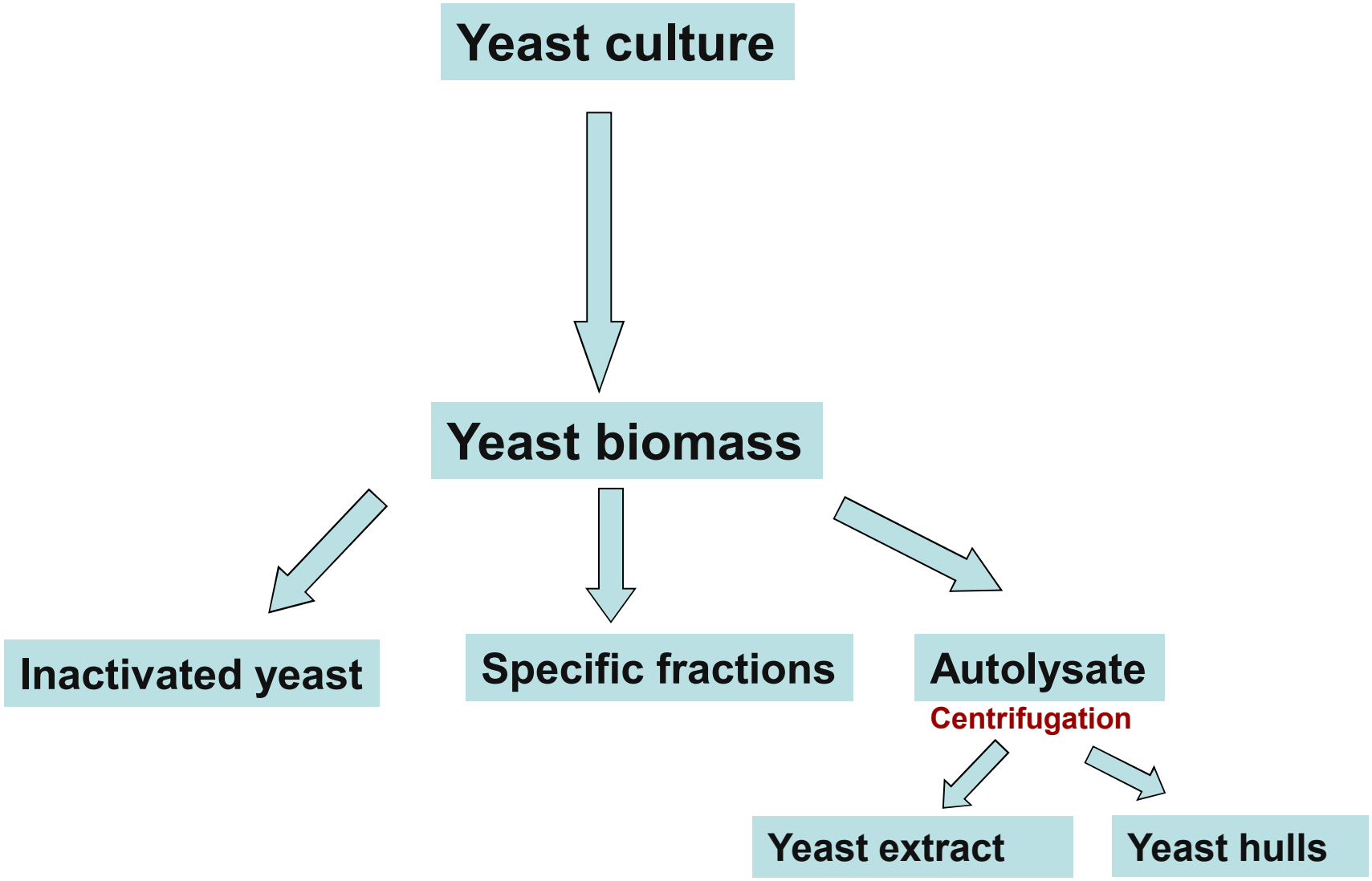
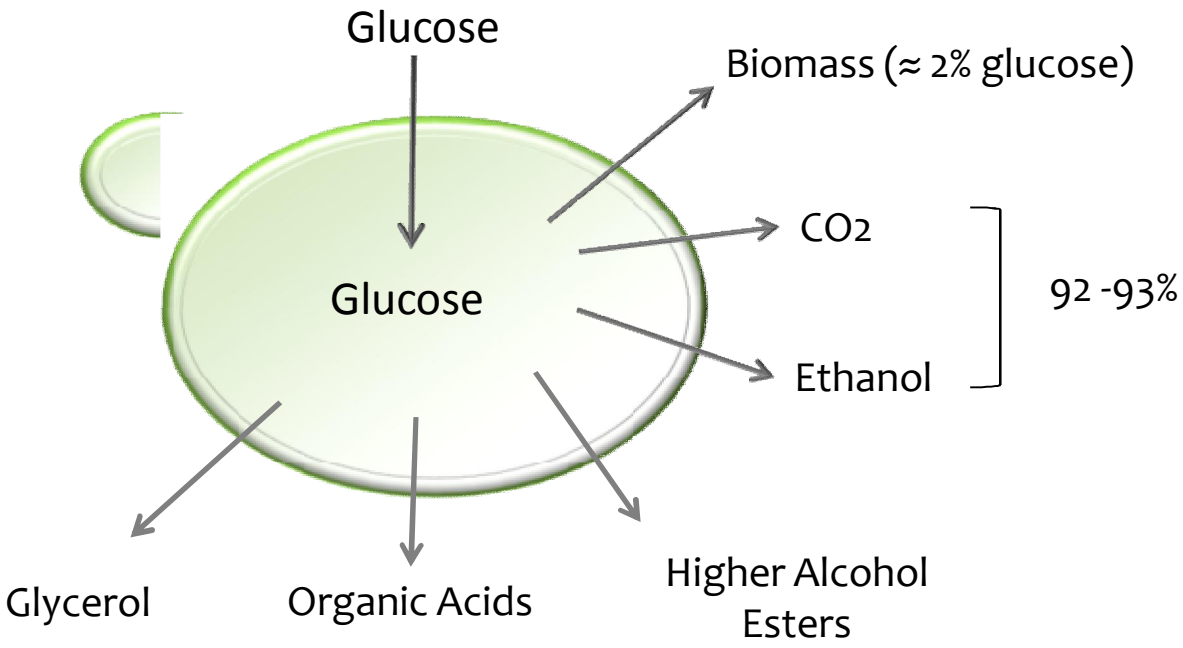
rivatives production – General steps



Prevention

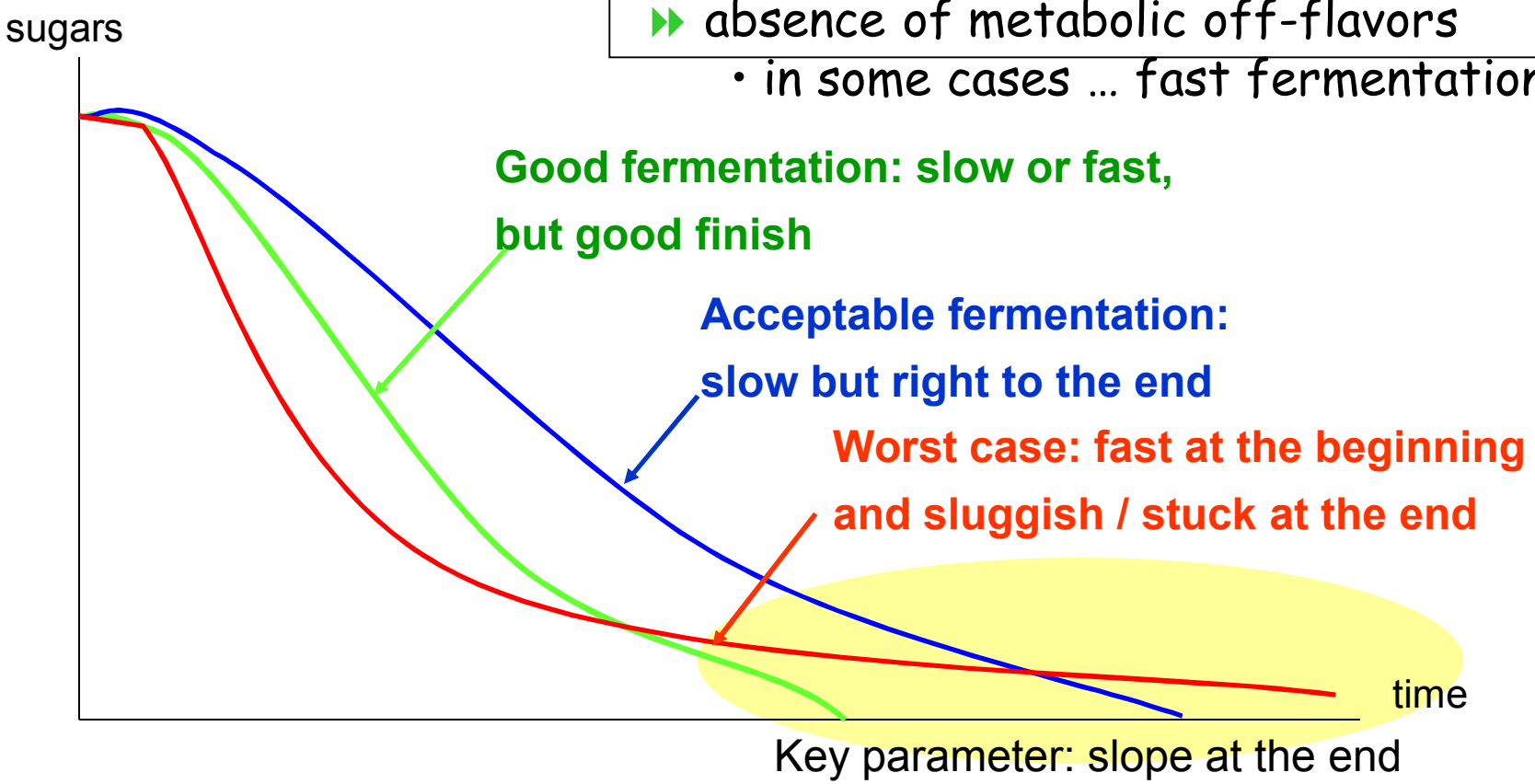
Alcoholic fermentation



1° alcohol ←→ 16,8 g/l of glucose

SECURE FERMENTS

- regular fermentation = easy finish
- absence of metabolic off-flavors
  - in some cases … fast fermentation



Defining Good Fermentation Practices

Good Fermentation Practices are considered options that will optimize:

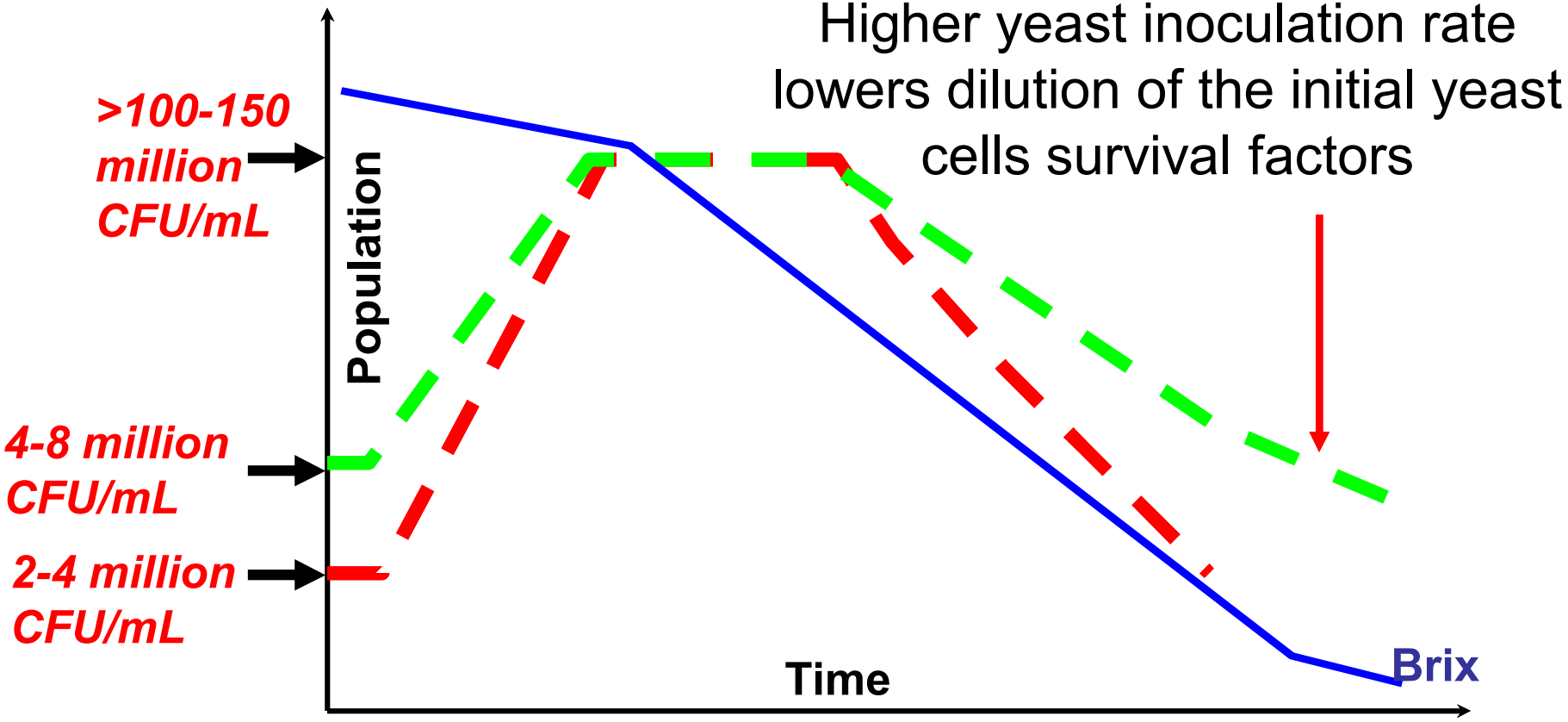
- **A complete and regular fermentation**
- **Achieving analytical and sensorial goals**

To have the most efficient results

using the least input,

added at the right moment.

Normal Fermentation Curve



Survival factors are important to ensuring the proper working of the cellular membrane: poly-unsaturated fatty acids and sterols

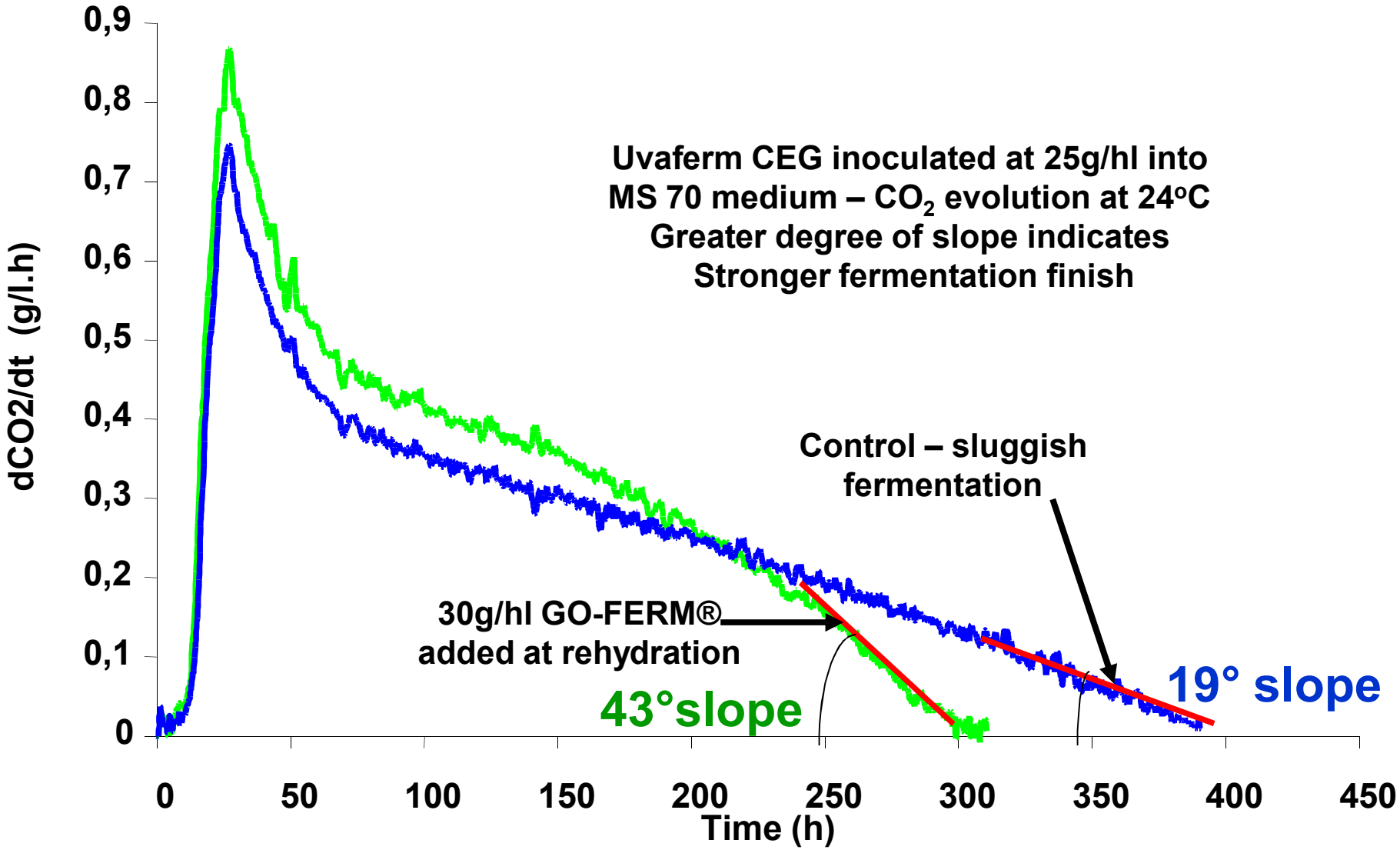
Yeast PROTECTION is essential

&

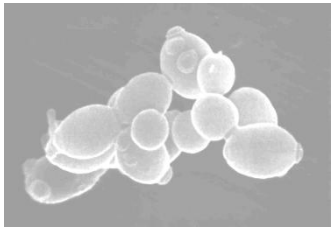
Yeast NUTRITION is vital.

ation Kinetics of GO-FERM® Micronutrient Addition During Yeast Rehydration

A. Julien, J. Sablayrolles - INRA Montpellier 2001



NS IN REHYDRATATION



- **UNSATURATED FATTY ACIDS & STEROLS**
- **MICRONUTRIENTS (vitamins and minerals)**

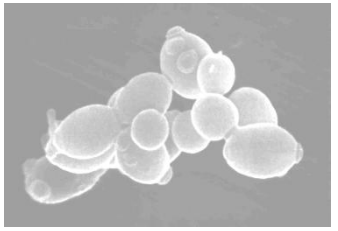
PROTECTION

INACTIVE YEASTS AS SOURCE

Benefit of using Rehydration nutrients

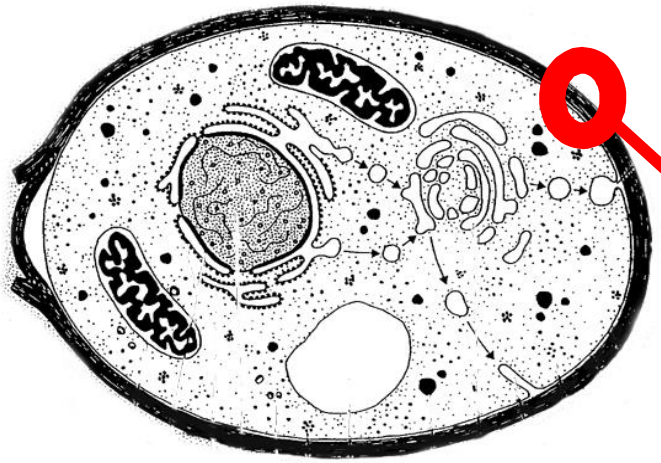
- No competition from other organisms (bacteria or other wild yeast)
- Biologically available
  - Either used initially
  - Stored in the cell until required
- Higher cell viability, More secure fermentation
- Better acclimatized yeast

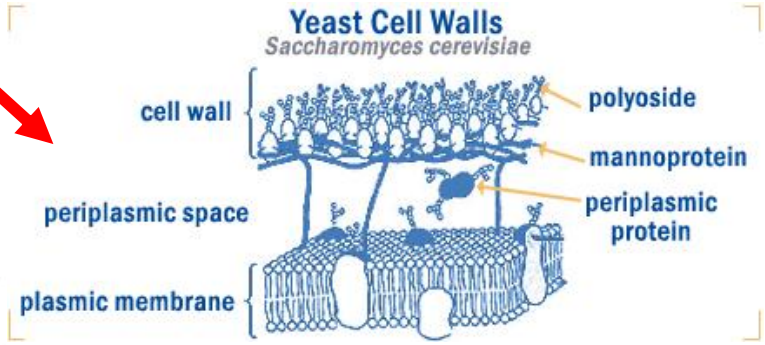
EHYDRATATION

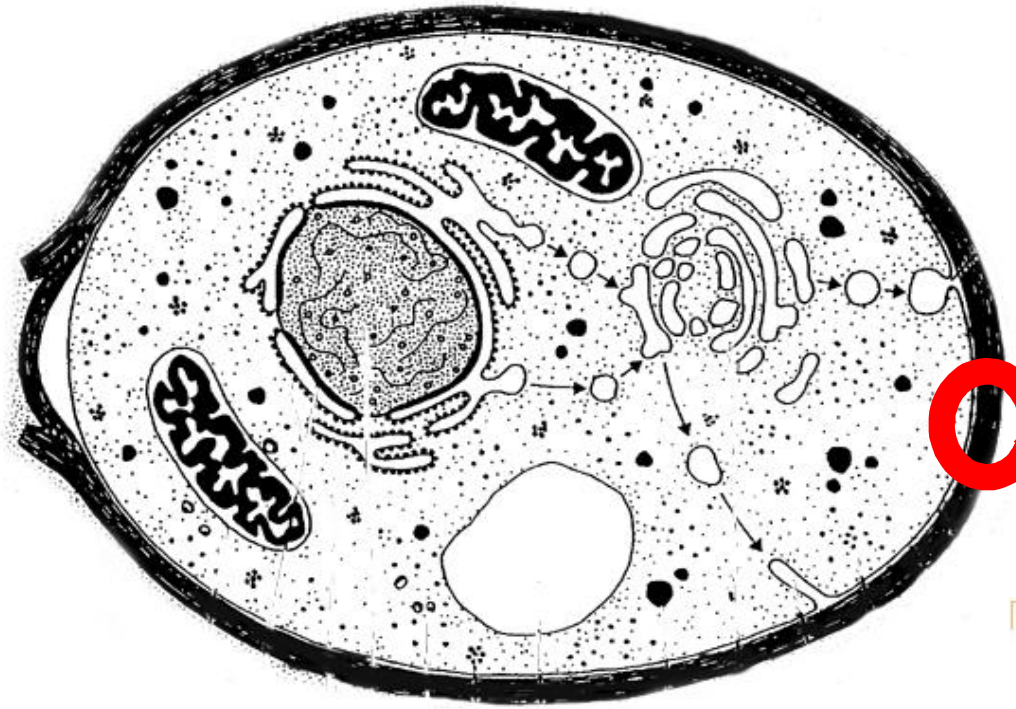


VERY IMPORTANT for YEAST LIFE

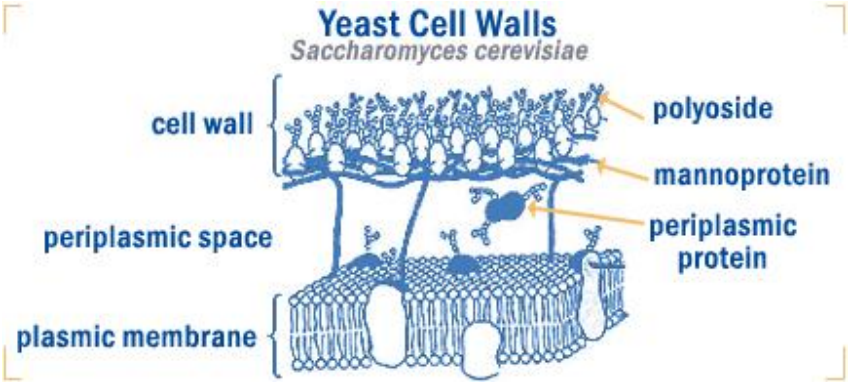
- Protect yeast against initial osmotic shock – lower V.A.
- Build-up yeast cell wall content of yeast stress resistant factors – protect against ethanol toxicity
- Adding minerals and Vitamins- bioavailable



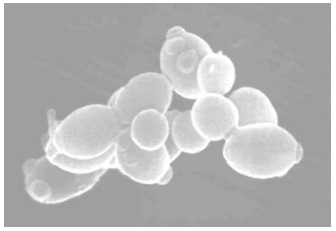


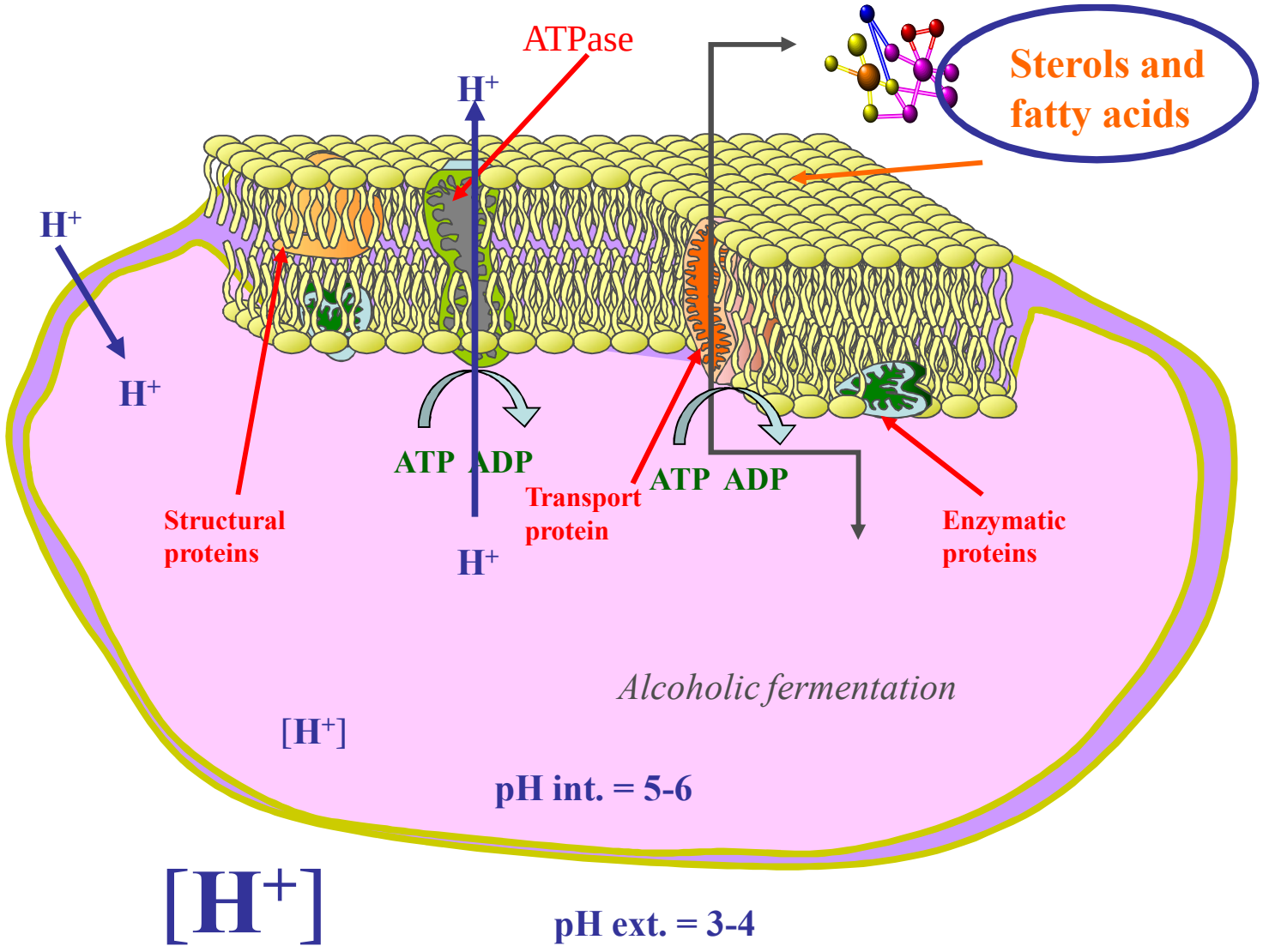


Yeast Cell Wall Cross section…

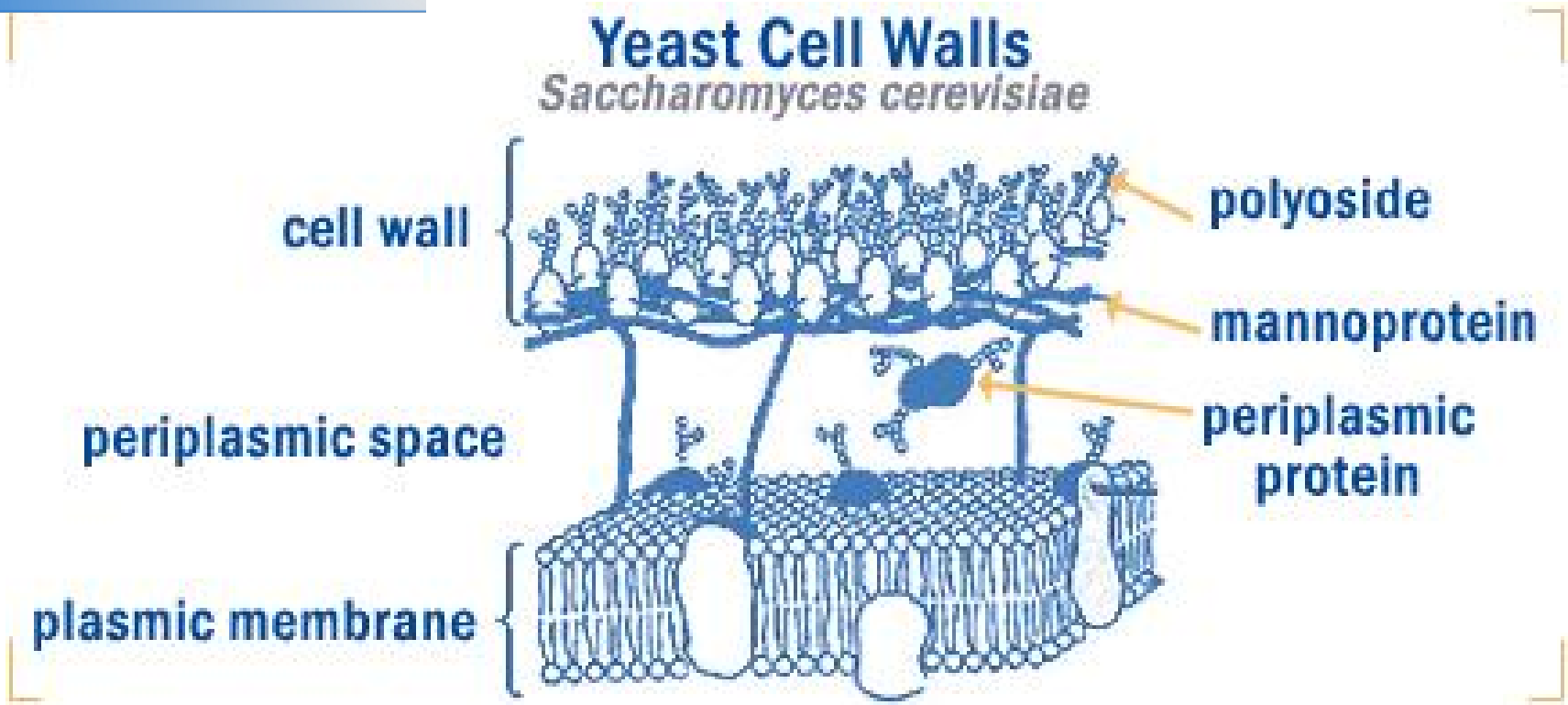


N- PLASMA MEMBRANE



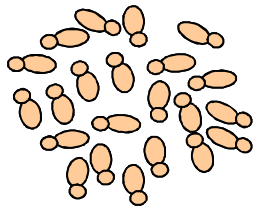


composition:



Plasma Membrane is ~5% lipids (sterols & unsaturated fatty acids)

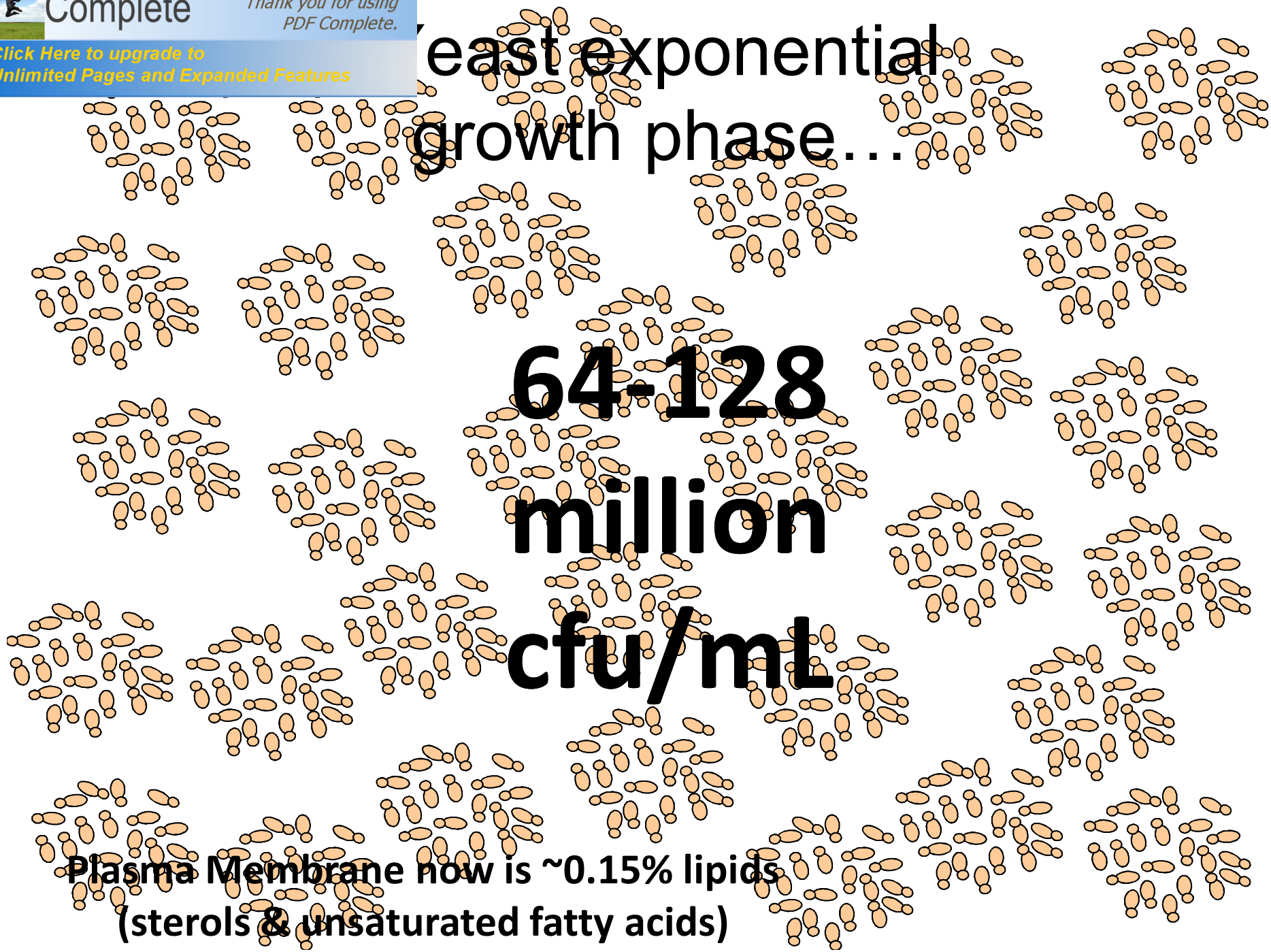
After yeast inoculation and lag phase begins yeast exponential growth phase…



2-4 million cfu/mL

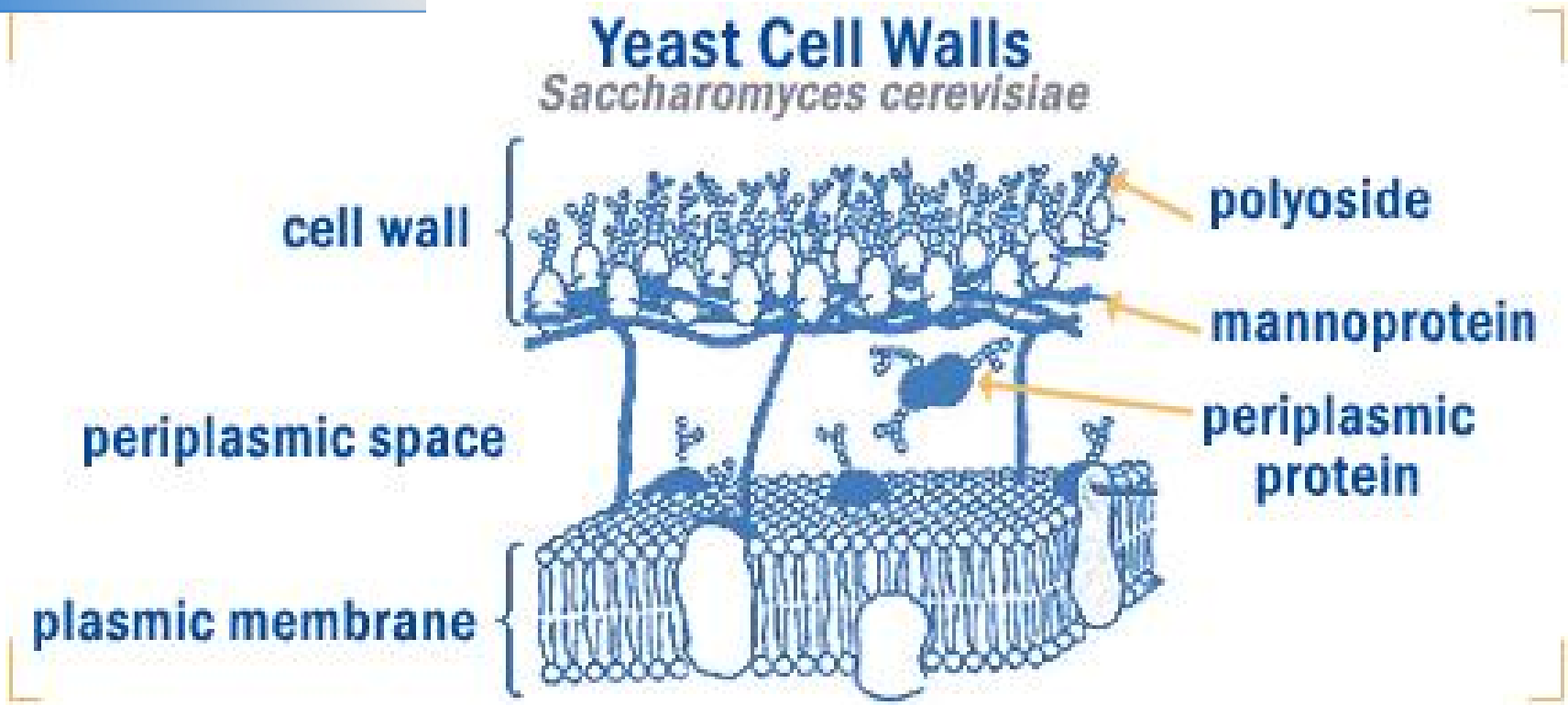
Inoculation rate 2 lbs. per 1000 gallons (25g/hL)

Yeast exponential growth phase…



Plasma Membrane now is ~0.15% lipids
(sterols & unsaturated fatty acids)

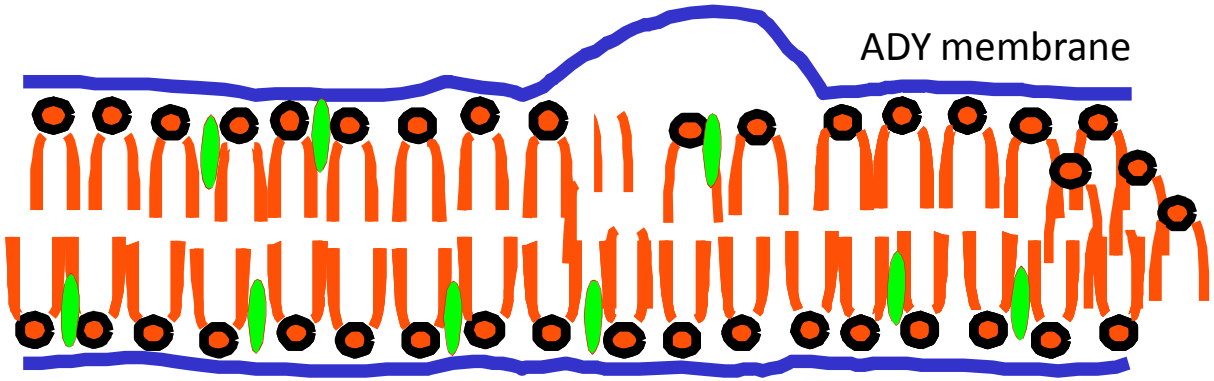
composition:



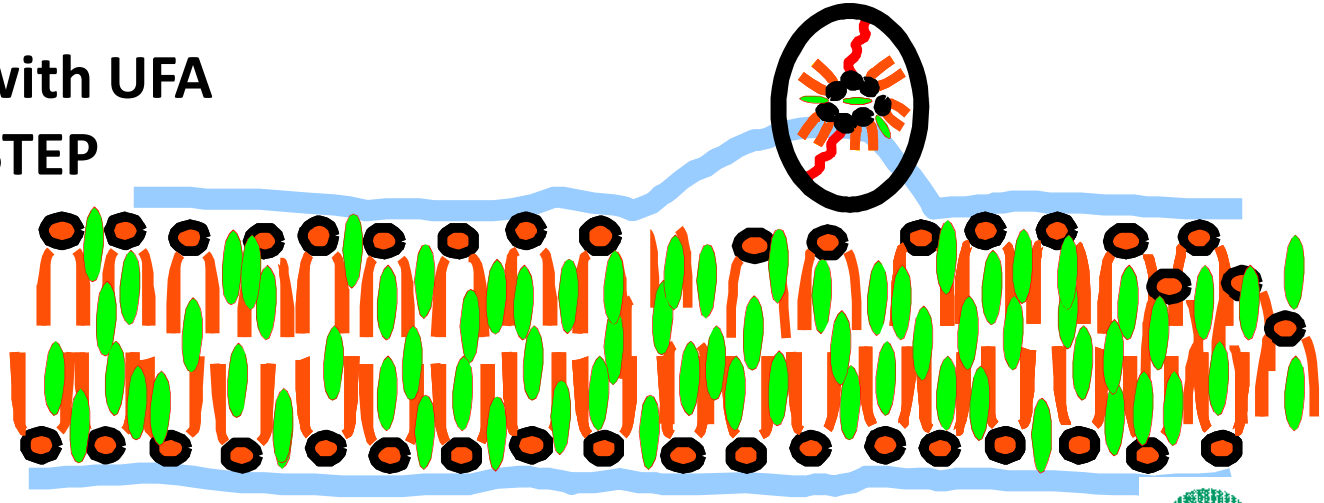
Plasma Membrane is now <0.2% lipids (sterols & unsaturated fatty acids) A critically low level!

d lipid depletion, add them
t rehydration

Rehydration Without Protection



Rehydration with UFA & Sterol NATSTEP Protection



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CENTRE DE MONTPELLIER

cronutrient needs (10^{-3}M)

Nutrient	Function
Carbon	Structural element, energy source
Nitrogen	Proteins and enzymes
Oxygen	Fatty acid and sterol production
Hydrogen	Transmembrane proton motive force
Phosphorus	Energy transduction, membrane structure and nucleic acids
Potassium	Ionic balance, enzyme activity
Magnesium	Cell structure, enzyme activity
Sulfur	Sulphydryl amino acids, vitamins

MICRONUTRIENTS: Minerals

Magnesium — better alcohol, temperature and osmotic resistance, ratio Ca:Mg < 1,

Zinc — cofactor of glycolysis enzymes, increase alcohol tolerance regulation of by-products (esters, alcohols, fatty acids),

Manganese — synergistic effect with Zn, shorter generation time

Copper — essential element, but toxic above 1-2 mg/l

Potassium — must be > 300 mg/l at low pH's

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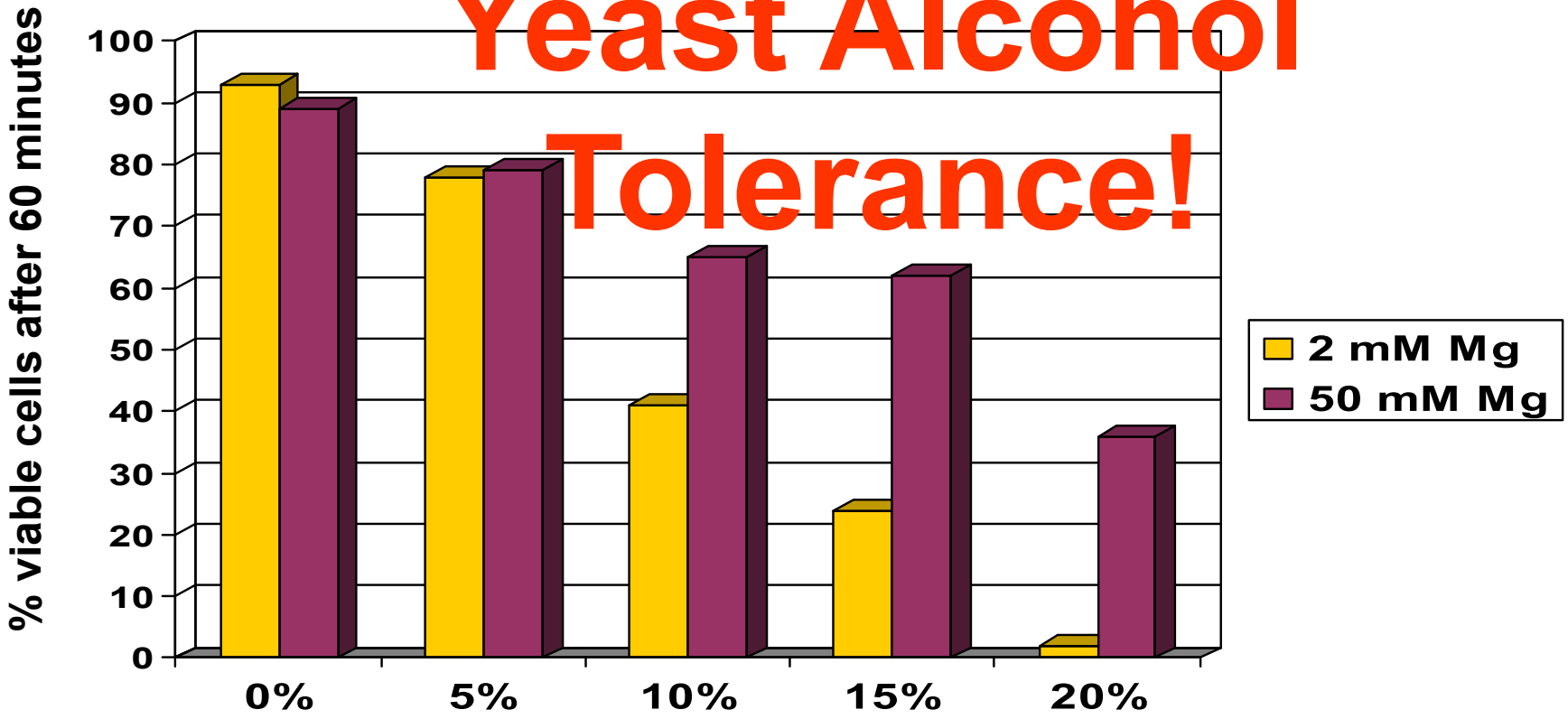
Why is Mg so Important?

Yeast Alcohol Tolerance!



Viability of *S. cerevisiae* after 60 min of Ethanol level at different concentrations of Mg^{2+} (Birch and Walker, 2000)

ENTS: VITAMINS

Pantothenate	avoids H_2S and VA formation, better kinetics, less acetaldehyde, strain sensitivity
Biotin	better kinetics, synergic effect with N, increases ester production, higher yeast viability at end AF
Thiamine	better cell growth, less acetaldehyde and VA
Inositol	essential for membrane phospholipid synthesis

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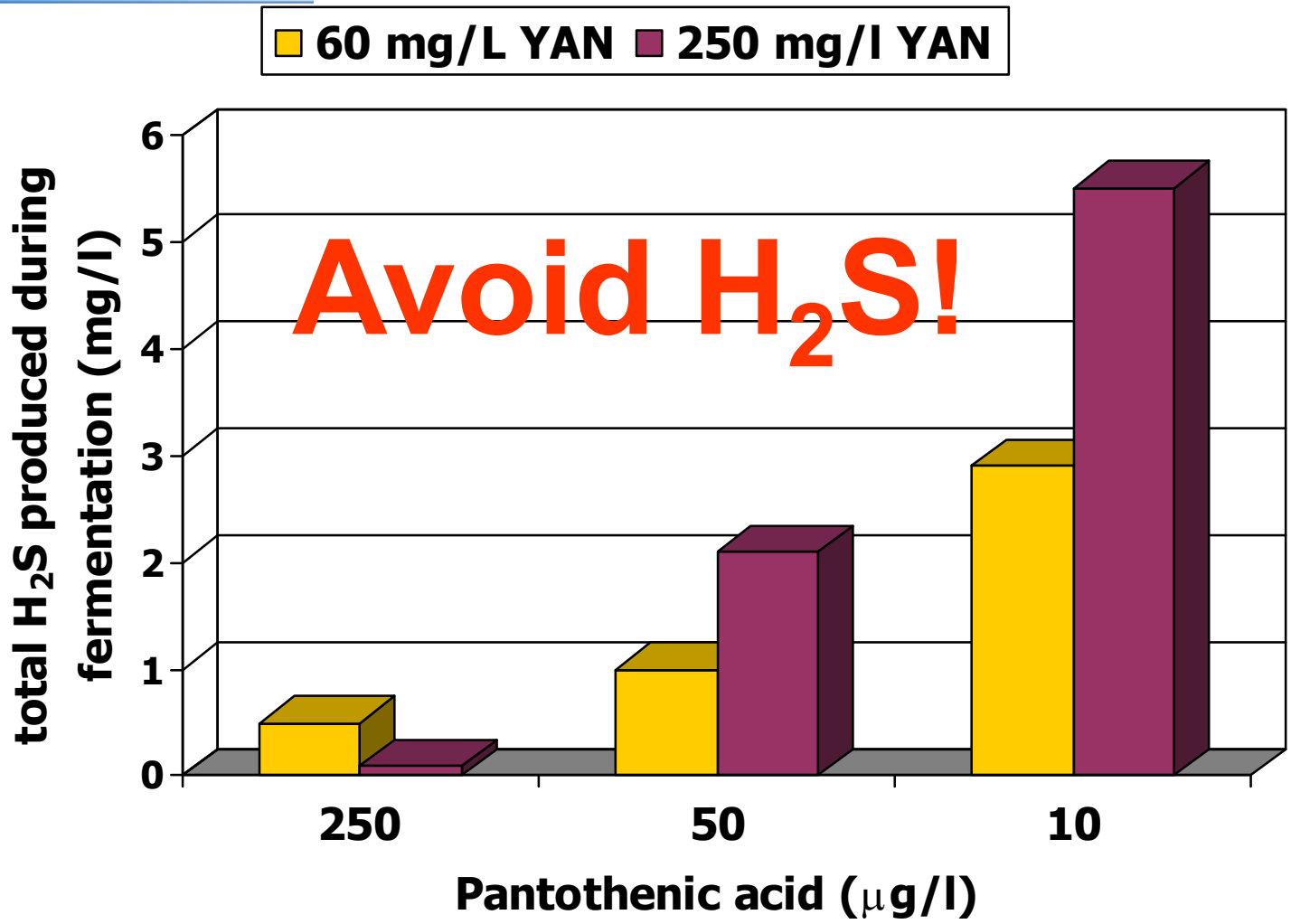
Pantothenic Acid Important?



Production of hydrogen sulphide by *S. cerevisiae* in a synthetic juice at different concentrations of Yeast Assimilable Nitrogen and Panthotenate (WSU, C. Edwards 2001)

Nitrogen

YANC OR YAN

Yeast Available Nitrogen Content

- sum of assimilable nitrogen from Free Ammonia Nitrogen (FAN) and alpha amino acids .
- low levels associated with production of undesirable sulfide compounds and stuck fermentations

Recommended levels:

- 250 ppm-350 ppm or higher depending on the initial BRIX level.

Nitrogen determination

Formol titration	NOPA
Simple titration Hazardous waste NH4 and FAN (including Proline) Good estimation	Measures FAN (excluding proline) Measure Ammonia separately (ISE Probe) No waste Spectrometry

ctors influencing accumulation

- pH
- Ethanol toxicity
- Temperature
- Degree of aeration
- Plasma membrane composition
- Strain of yeast
- Native microflora

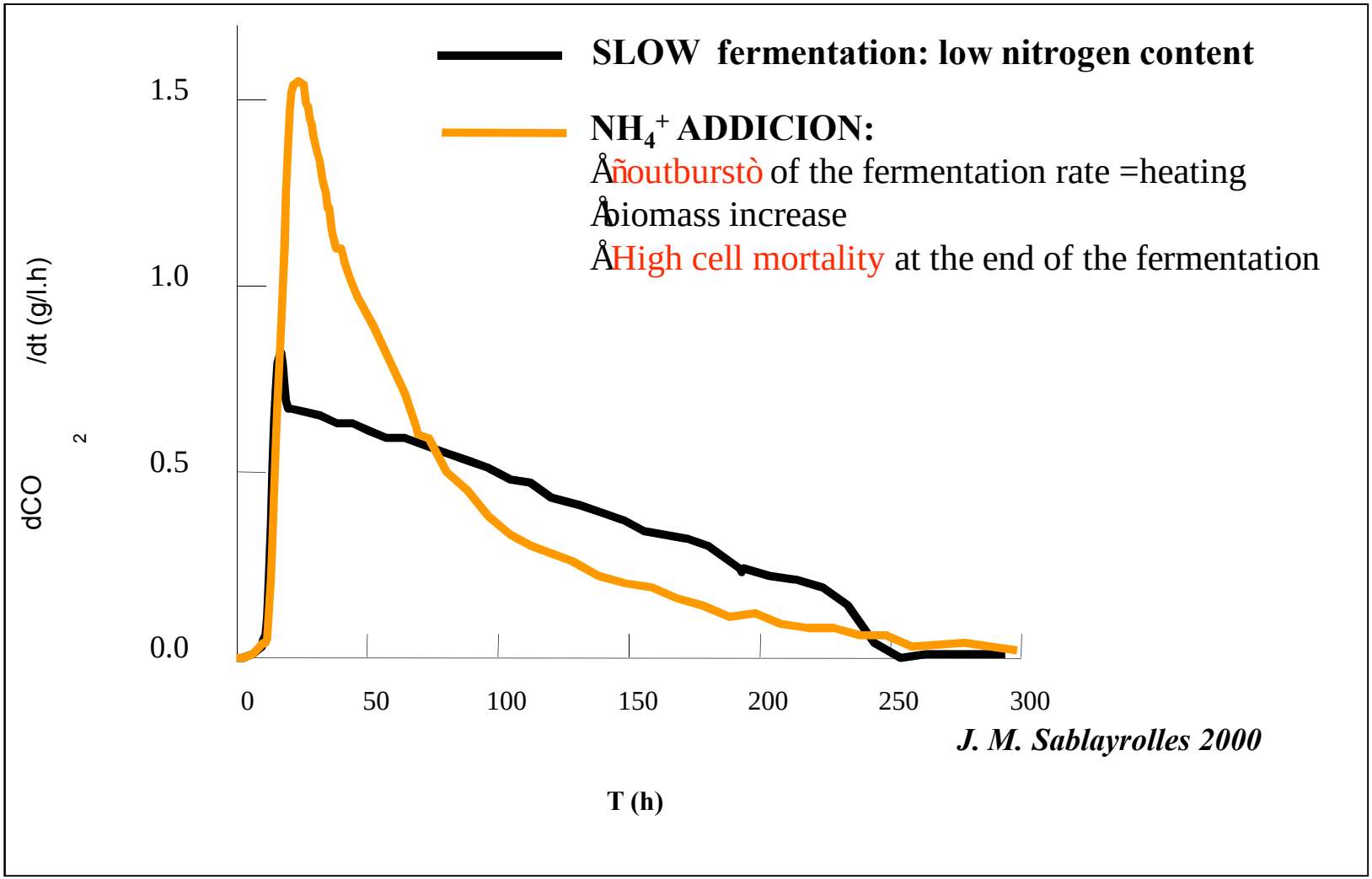
WHY NITROGEN IS ESSENTIAL?

- **Protein synthesis/ Sugar Transport**
  (Basturia and Lagunas,1986)

- **Cell growth : maximum CO2 production rate correlated with assim. nitrogen content of the must**
  (Bely *et al*., 1991)

•Fermentation rate –
a minimum level of assimilable nitrogen is required : 150mg/l
(Jiranek, 1993)

mediate" nitrogen assimilation problematic?



c vs. Inorganic Nitrogen

The research to date…

→ impact of nitrogen source on the yeast esters production (several yeast strains tested) :
L. Bisson, 2007 , Hernadez-Orte, 2005-2006, V. Ferreira 2007-2008

→ impact of nitrogen source on volatile thiols production :
M. Ugliano AWRI, 2008

Experimental matrix on Chardonnay grapes from Yalumba

(Beo:11.6, pH 3.34, TA 5.94, FSO2 14, TSO2 52,YAN 204).

	Inoculation	1/3 of AF	Total YAN
Control	-	-	-
DAP (L)	12.5 g/hl	12.5 g/hl	50 mg/l
DAP (H)	25 g/hl	25 g/hl	100 mg/l
Fermaid O	40 g/hl	20 g/hl	24 mg/l
DAP/ Fermaid O	15.2 g/hl	4.5g/hl 40g/hl	50 mg/l
GFP/ Fermaid O → *300mg/l*	20 g/hl	20 g/hl	24mg/l

The Australian Wine Research Institute

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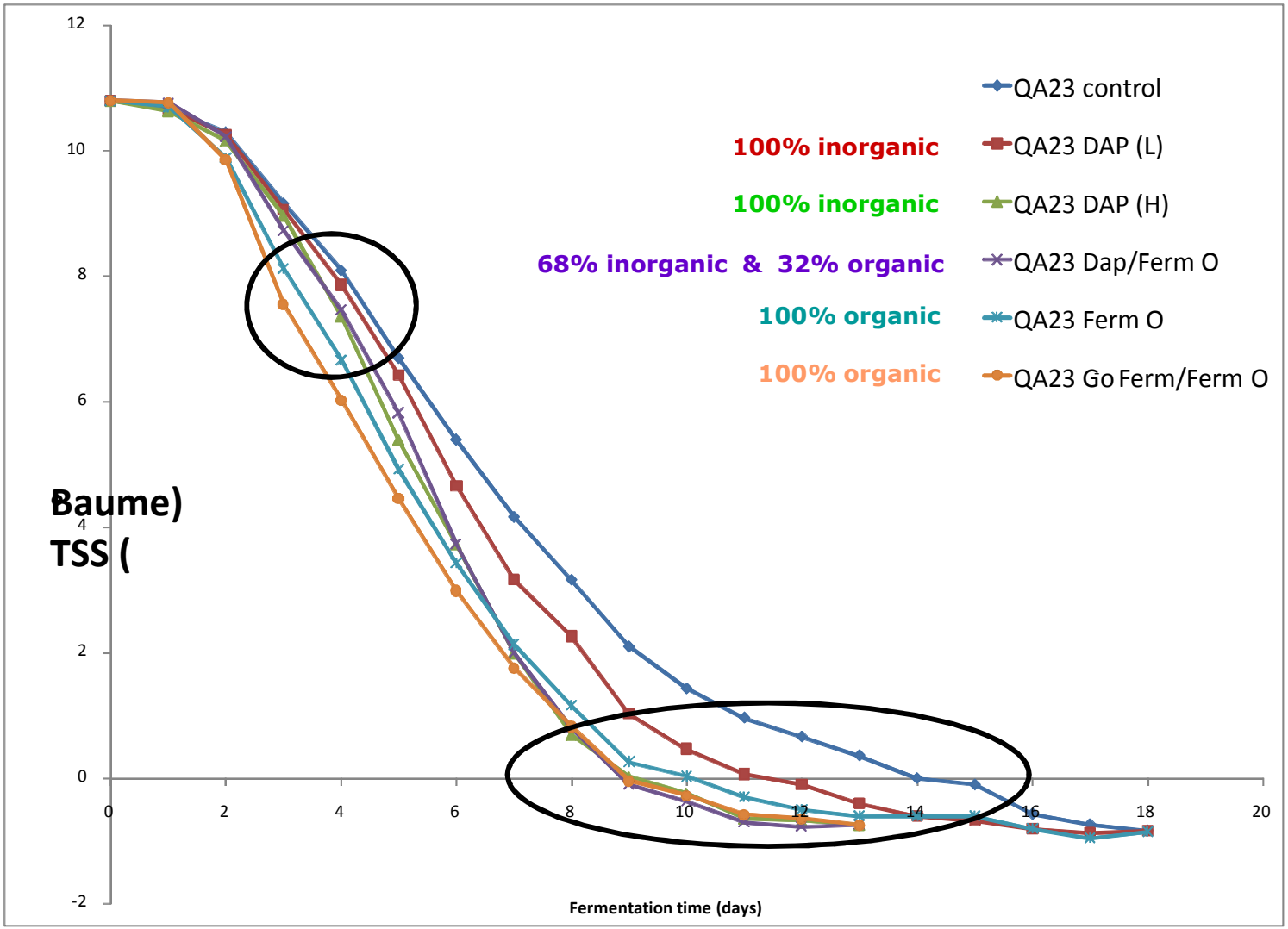

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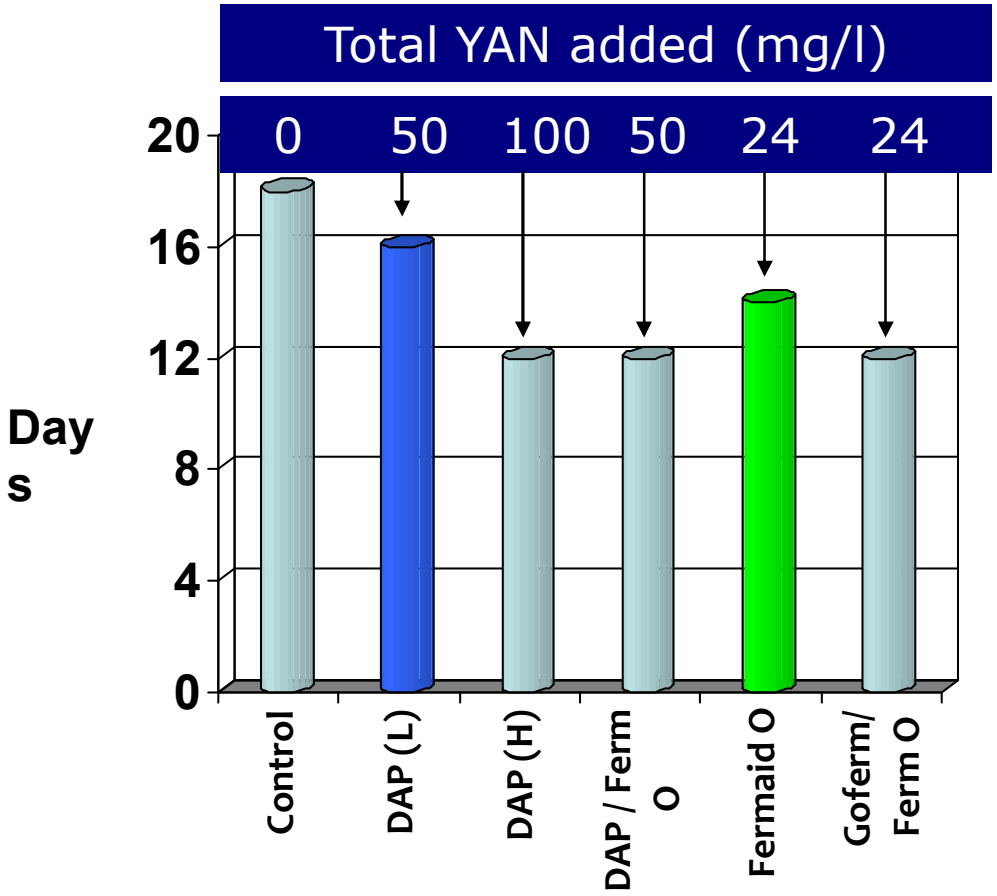
›act on yeast fermentative activity



- **24 mg/l of « 100% organic YAN » is significantly more efficient than 50 mg/l of « 100% inorganic YAN »**
- **Balanced nutrition better adapted to yeast nutrient requirements compared to 100% inorganic N2.**

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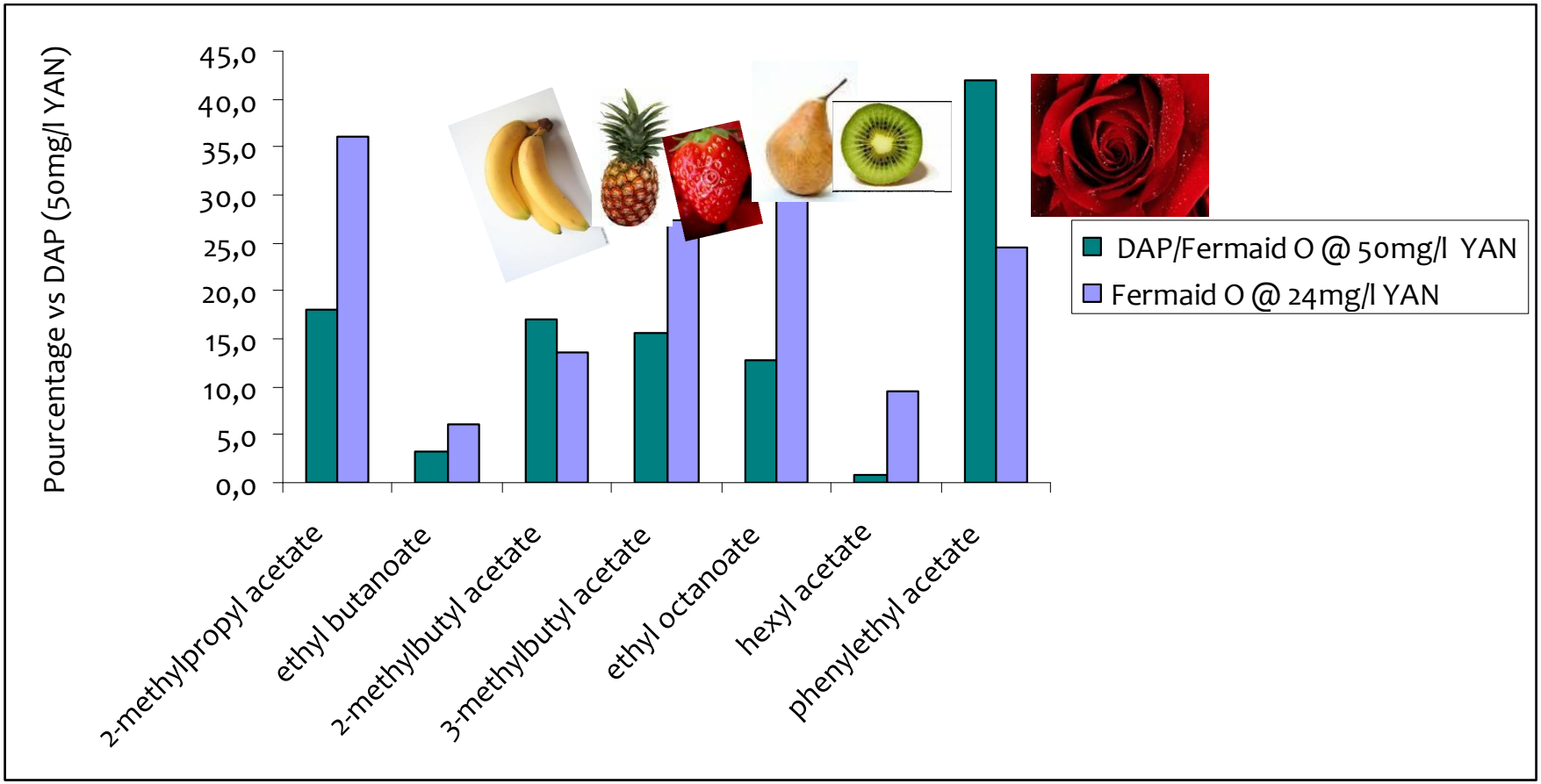
pact of N2 source on aromas



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Best approach to Nutrient adds.

- Determine YANC
- Only supplement if necessary
- 2 stage approach
  - Initial supplement with a complex nutrient
  - Make up remainder of requirement with DAP

Nitrogen levels

- 3 levels
  - **Low <150ppm (deficient)**
  - Medium (150 – 250 ppm)
  - High (>250 ppm)

- Is there a relationship between low N and other essential nutrients?

Survey of available Nitrogen

	White	Red	Rose	Botrytized
No. of Samples	**32**	**55**	**48**	**9**
Min. value	36	46	42	22
Max. value	270	354	294	157
Mean	181.9	157	119	82.8
Std. Deviation	32	55	48	9
Deficient (%)	**22**	**49**	**60**	**89**

Riberereau-Gayon

EGRATED NUTRITIONAL STRATEGY FOR WINE YEAST

JUICE YANC	rehydration	end of lag	1/3 AF
HIGH N > 225 mgN/l	Go-Ferm 2.5lb/kgal	-----	------
MEDIUM N > 125 mgN/l < 225 mgN/l	Go-Ferm 2.5lb/kgal	-----	FERMAID K 2lb./kgal
LOW N < 125 mgN/l	Go-Ferm 2.5lb/kgal	DAP 2.5lb/kgal or more	FERMAID K 2lb/kgal

Go-Ferm & FERMAID

FERMAID : IS IT USELESS NOW?

In high sugar - nitrogen deficient musts
a YAN addition (at 1/3 AF) is still needed

Go-Ferm	provides ab. 10 mgN/l at 30 g/hl (100% **a**-amino)
FERMAID	provides ab. 30 mgN/l at 30 g/hl (mix of **a**-amino and ammonia)
DAP	provides ab. 60 mgN/l at 30 g/hl (100% ammonia)

Sugar-Nitrogen Relationship

Brix	YAN
21	200
23	250
25	300
27	350

(Butzke)

Supplementation decisions

- Always go for complex first
  - More efficient
  - Better aromatics
  - Controlled growth
  - Controlled fermentation
- Back up if needed with DAP

o supplement and when- Summary

- Beginning of Fermentation
  - Macronutrients
  - Micronutrients
  - Oxygen
  - Vitamins

- Mid- Fermentation
  - Nitrogen
  - Sterols

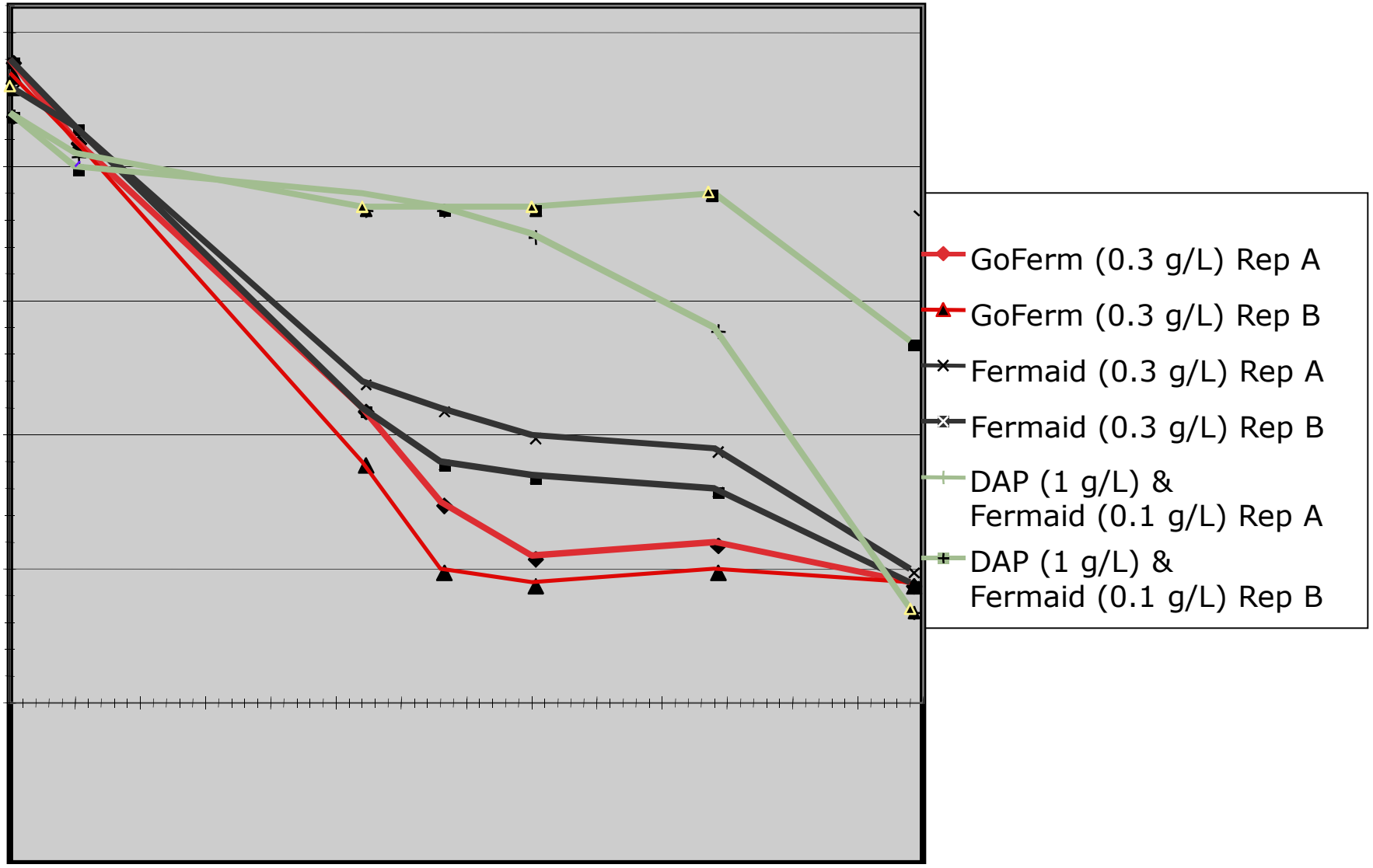
- Late Fermentation (<10 Brix)
  - Nothing, cells can not accumulate anything but sugar, due to the repressive effects of Ethanol

I can’t resist…

impact on MLF – 2006 Chardonnay (NY State)

CV D254 + ML bacteria strain: ALPHA

(Thomas Henick-Kling, Cornell University)



ak temperature **under the cap** maximums relative to the initial osmotic shock (in warm or hot climate regions)

- 20 Brix
- 21 Brix
- 22 Brix
- 23 Brix
- 24 Brix or more

- 35°C
- 32°C
- 30°C
- 26°C
- 24°C

It integrates warm or hot climate grape constraints for the yeast

“Fix it” phase

What happened and when?

with a Stuck Alcoholic Fermentation

- Refer to websites for protocols
- Blend
- Sterile Filter
- Long acclimatization, build-up with sugar
- Short acclimatization with high inoculation rate
- How many times should you try to restart a stuck ferment? When can you start tasting the yeast?
- Use of yeast hulls
- Addition of nutrients?

nhibitory saturated fatty acids come from?

From the yeast when stressed.

- High sugar content
- Low must turbidity

Stressed yeast Increase the production of short & medium saturated fatty acids (decanoic and octanoic)

TOXIC FOR THE YEASTS RESULTING IN STUCK FERMENTATIONS!

Stuck Alcohol Fermentation

- **Prepare the stuck wine**
  - **Nutrient VitEnd**
  - **Lallzyme LysoEasy**
- **Prepare the rescue yeast**
  - **Enoferm Rhône 2226 or Uvaferm 43**
  - **NATSTEP Protection**
- **Adapt the prepared rescue yeast to the stuck wine**
  - **Fermaid K**
- **Start the fermentation and add the stuck wine in batches**
  - **SIY Cell Hulls**

What to do in case of stuck fermentation

1. protect and prepare the "stuck" wine

2. prepare the yeast

3. Re-start the fermentation

ection-preparation of "stuck" wine

1. Analyse the wine: pH, alcohol, residual sugars, VA, free and total SO_2

2. Rack the wine avoiding air contact, to eliminate the lees

3. Add SO_2 according to the analysis results

4. Top off the containers carefully

5. Keep the wine temperature at around 20 °C

6. Filter (if possible) to avoid spoilage

7 Add inactive yeast residues (yeast hulls) to adsorb toxic substances for yeasts (C_8, C_{10} and C_{12} fatty acids)

Yeast hulls
25-30 g/hL

Keep in contact for 24-48 hours, stirring lightly once in a while

Let the yeast residues settle out

rack or filter

Add → FERMAID K 25 g/hL

In the most difficult cases → Cellulose 50 g/hL

ast preparation

Protocol Based on

100 hL of "stuck" wine or must

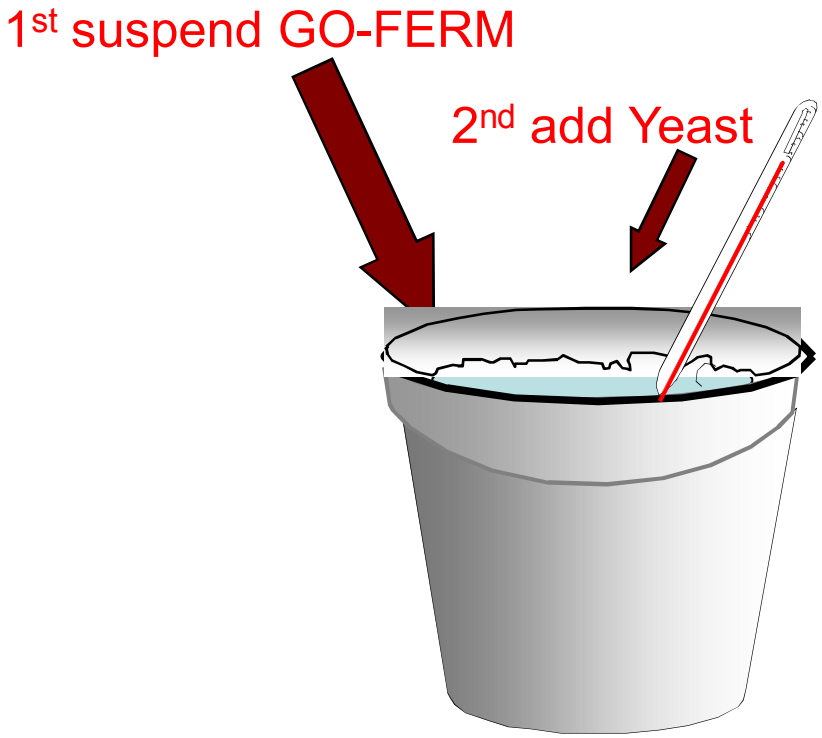
With:

12 % alcohol

15 g/L of residual sugars

PROPER YEAST REHYDRATION FOR STARTING 100hL STUCK WINE…

- ☺ 50 L Clean water 110°F
- ☺ Suspend 5 kg GO-FERM
- ☺ Wait until suspension temperature drops to 104°F before adding 5kg rescue yeast such as Uvaferm 43
- ☺ Light mixing to break up any clumps
- ☺ 15-30 minutes

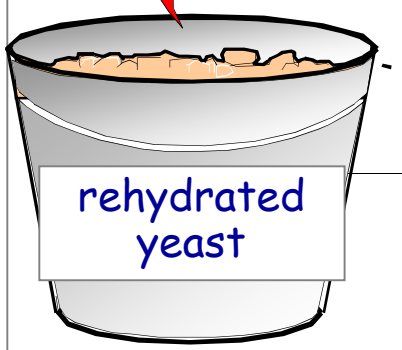


DO NOT WAIT LONGER! Go to the next step

djustment to the alcohol content

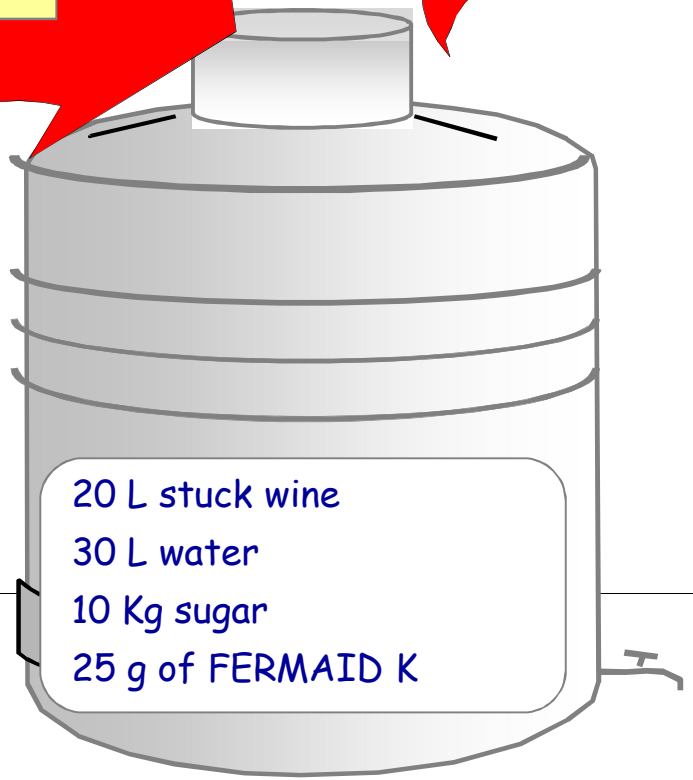
Add the 120 L to:

Add the 60 L to:



rehydrated yeast

0 % alcohol



20 L stuck wine
30 L water
10 Kg sugar
25 g of FERMAID K

keep at 25°C for about 6-8 hours
Mix once in a while

2,4 % alcohol
70-80 g/L sugars

DO NOT WAIT MORE THAN 8 hrs!

djustment to the alcohol content

preparation

add the 120 L to:

200 L stuck wine
100 L water
20 Kg sugar
250 g of FERMAID K

keep at 20-22°C for about 10-12 hrs

Check for the occurrence of fermentation

5 % ALCOHOL

60-70 g/L sugars

Add the 500 L to:

500 L of stuck wine

Keep at 20°C for about 12-24 hrs

Check for the occurrence of fermentation

Add the 10 hL to:

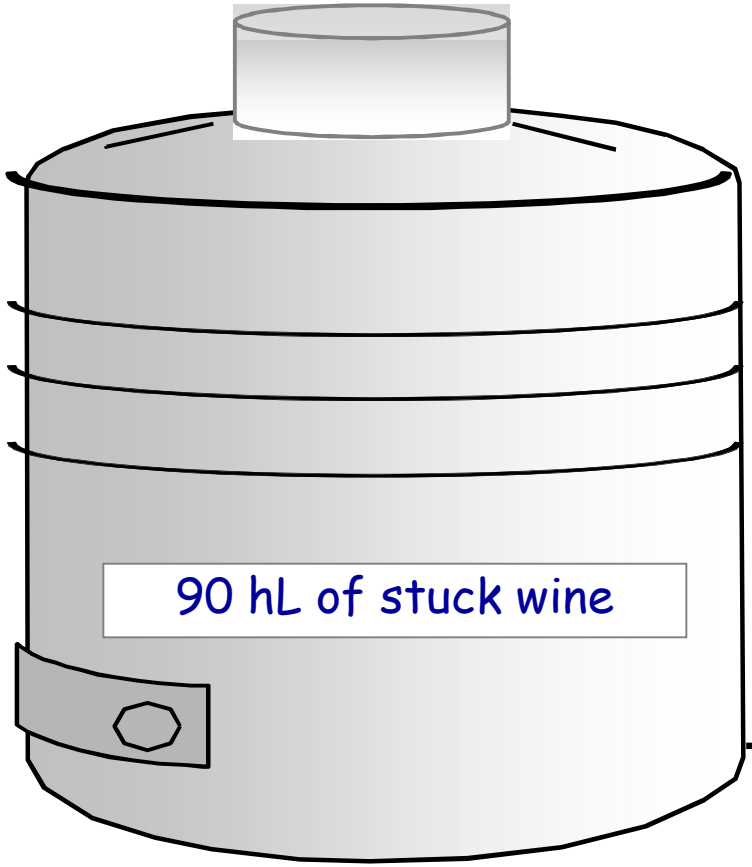
8,5 % ALCOHOL

15-25 g/L sugars

ATTENTION! Sometimes longer times are needed

nentation re-start
INOCULATION

Add the 10 hL to:



TEMPERATURE

- avoid temperature below 18 °C
- if necessary, warm up to 20-22 °C

TIME

- from 5 to 20 days
- sometimes longer than 20 days

Fermentation re-start until the residual sugars gone

Very important parameters to succeed in restarting a stuck fermentation…

Yeast quantity used for the inoculation (at least 10 million cells/mL - 50 g/hL of wine)

Physiological yeast conditions: adjustment to alcohol is critical

Analytical wine characteristics (evaluate the risks and the difficulties of re-starting)

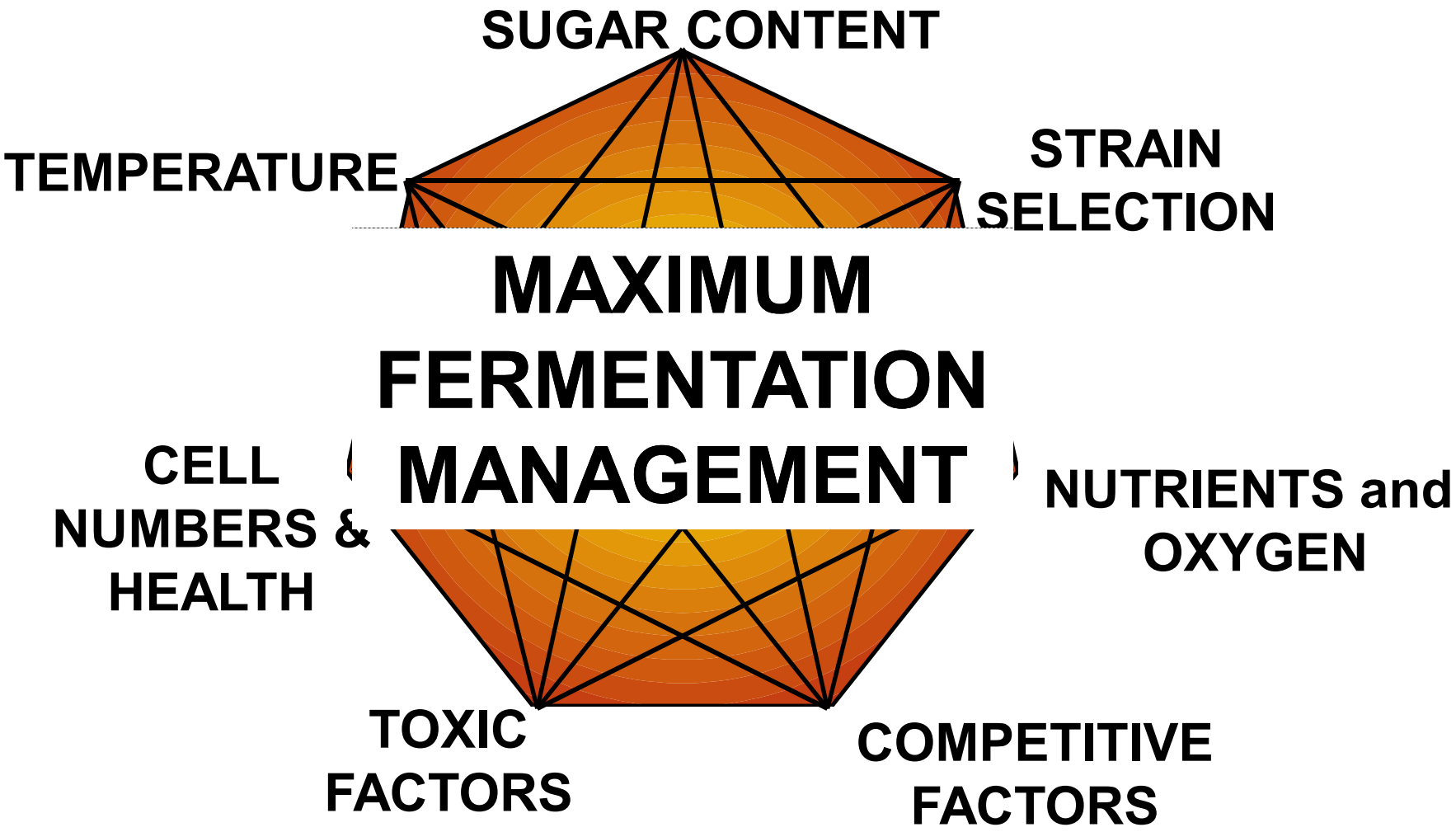
Yeast strain choice for the inoculation:
- It's better to avoid the same yeast strain used at the beginning
- It is very important the rapidity of fermentation re-start

Keep the cellar very clean, wines with residual sugars are more sensitive to microbial spoilage

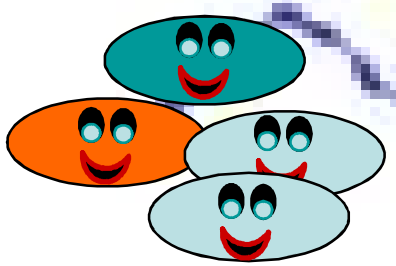
And you thought I would forget?

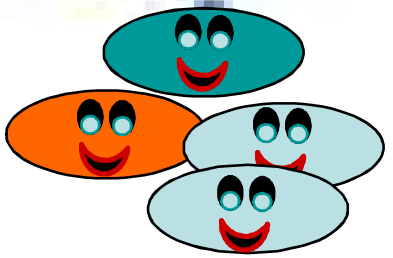
!!!!

rrelationships of Factors Affecting Fermentation



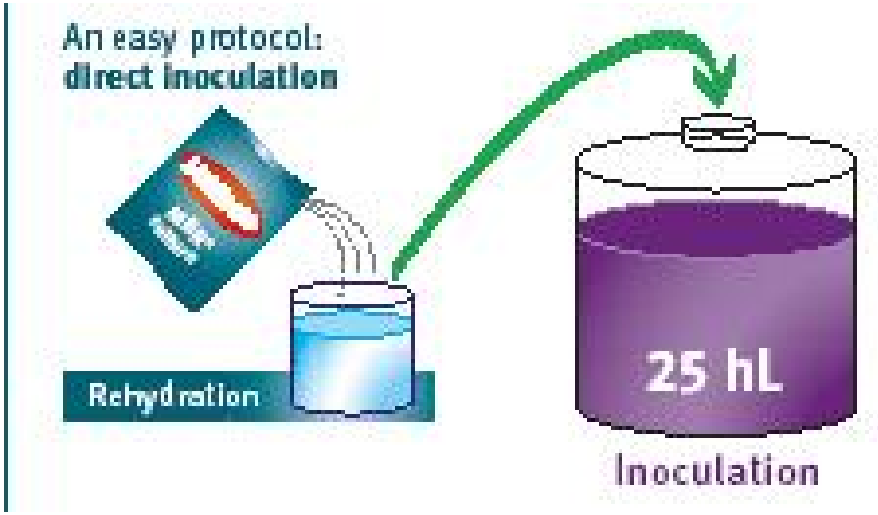
Management of MLF



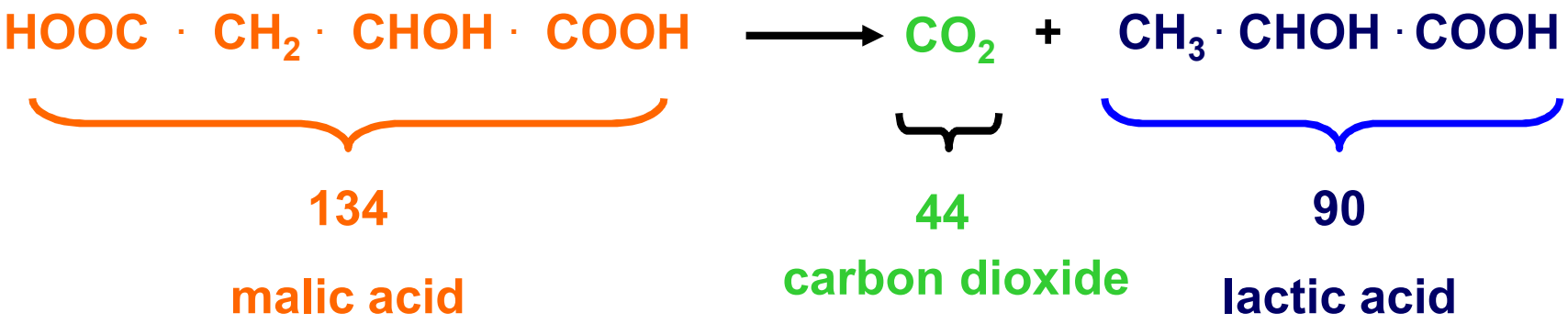


MBR Culture Rehydration

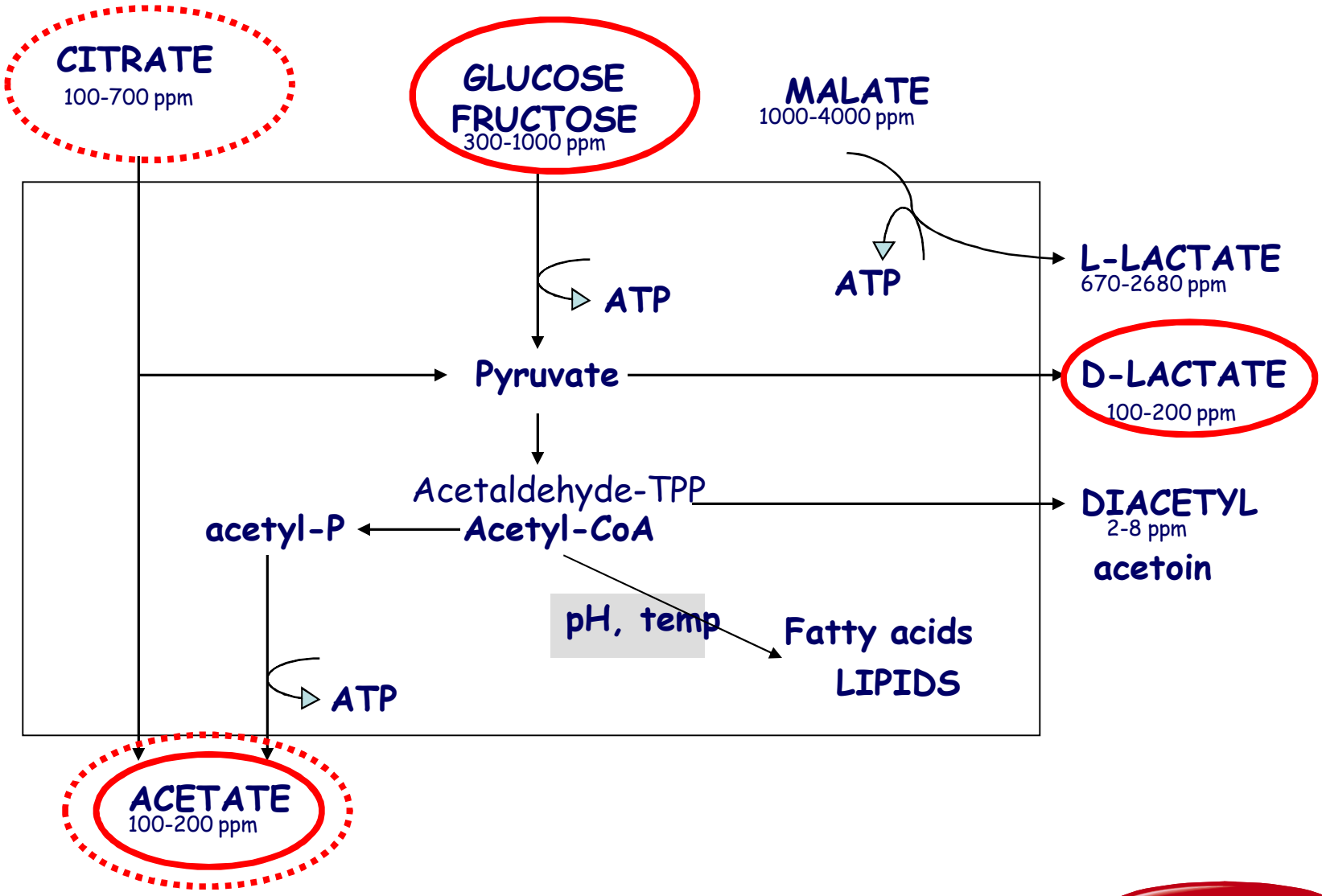
- When rehydrating MBR cultures, respect the 15 minute time limit otherwise loss of viability (>1 log at 1 hour)
- The safest optimum temperature for rehydration is 20°C



THE CHEMISTRY...



sm in heterofermentative Lactic Acid Bacteria



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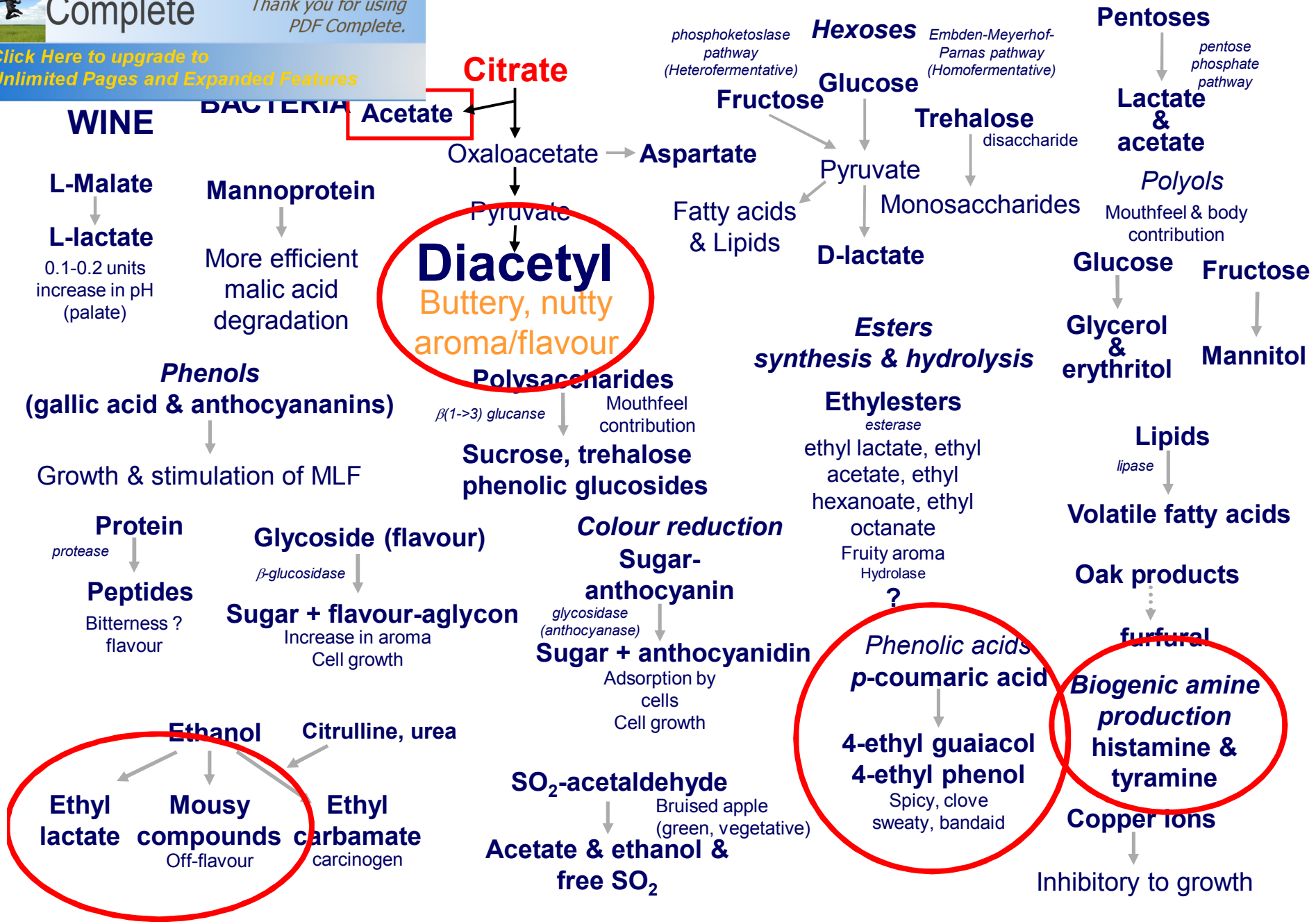
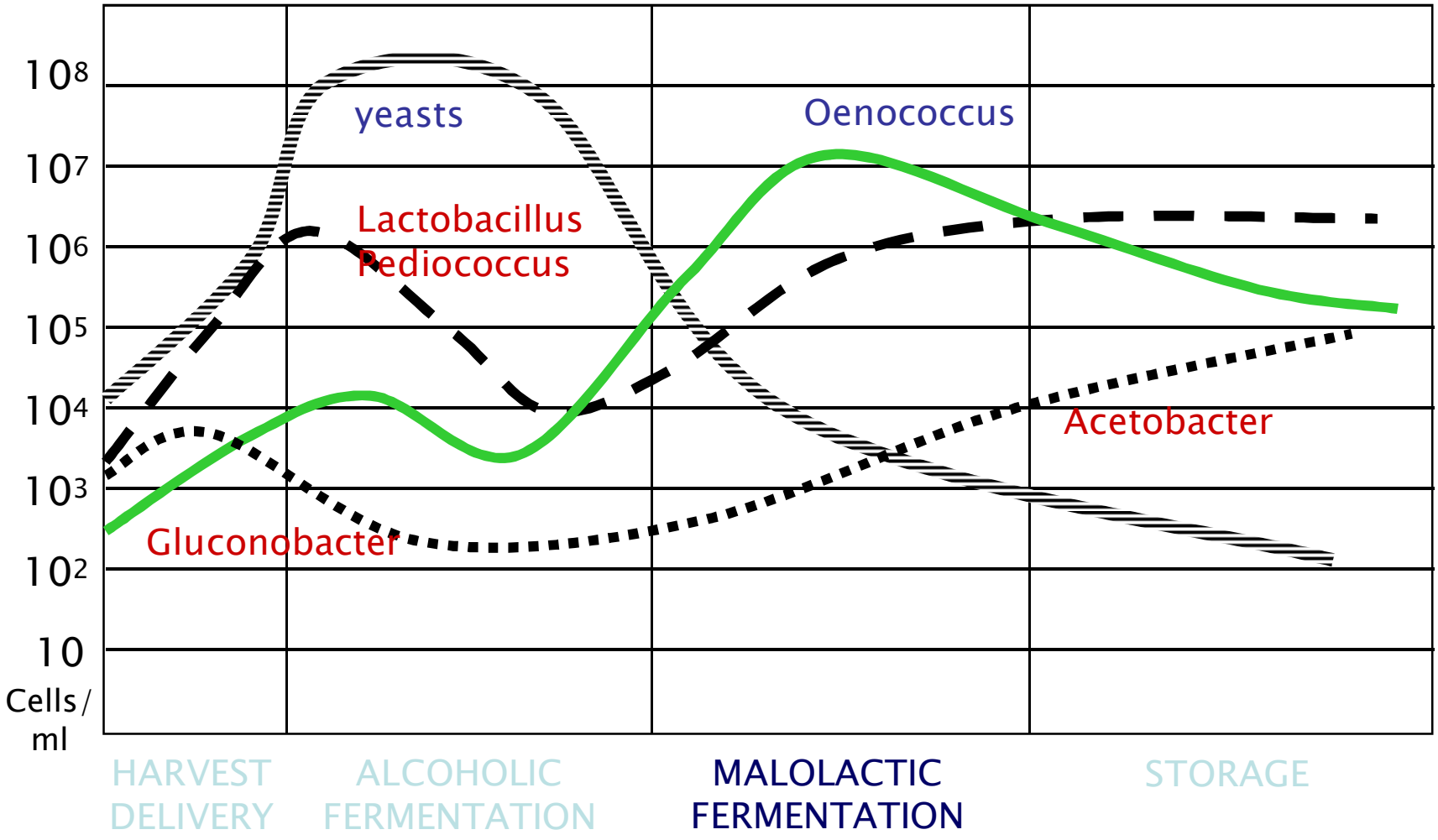
The more you know…

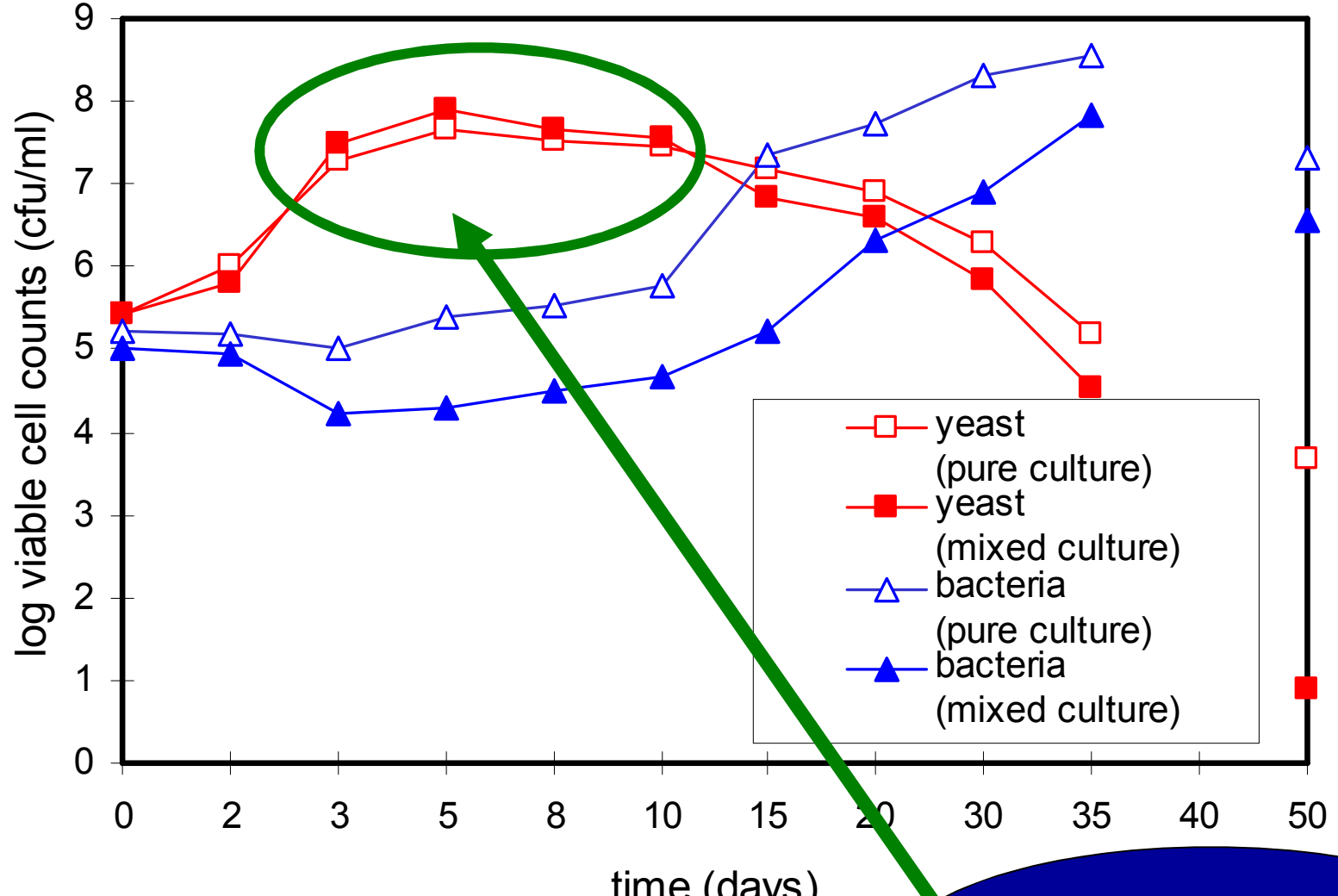
…the better!



Eveline Bartowski, AWRI, 2004

BACTERIA EVOLUTION FAVOURABLE CONDITIONS





(King and Beelmann 1986)

NO INFLUENCE OF *O. oeni* ON AF

are the risks of not inoculating?

- Depends on the pH
- High levels of biogenic amines
- High V.A.
- Undesirable aromas and flavors

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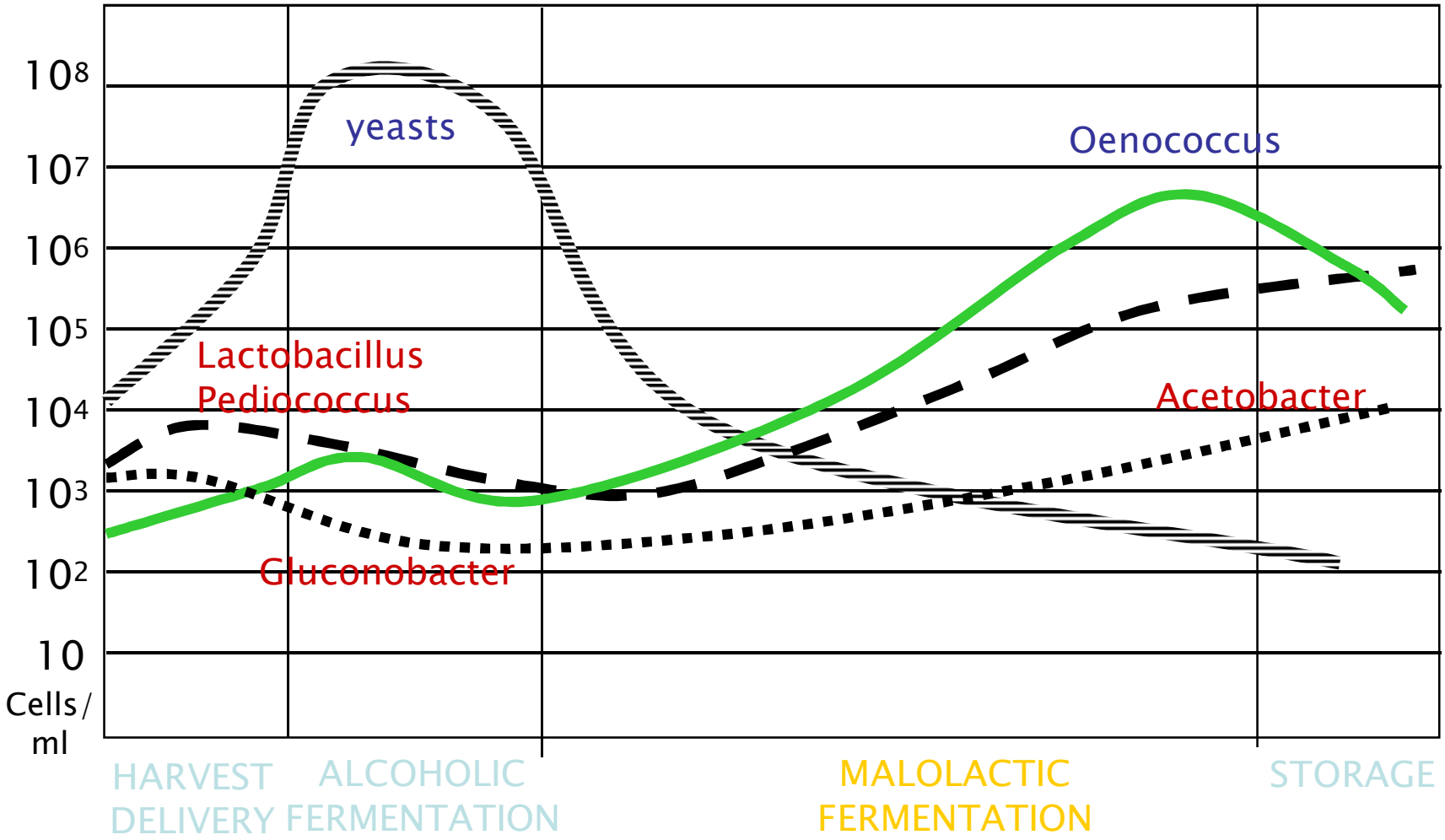
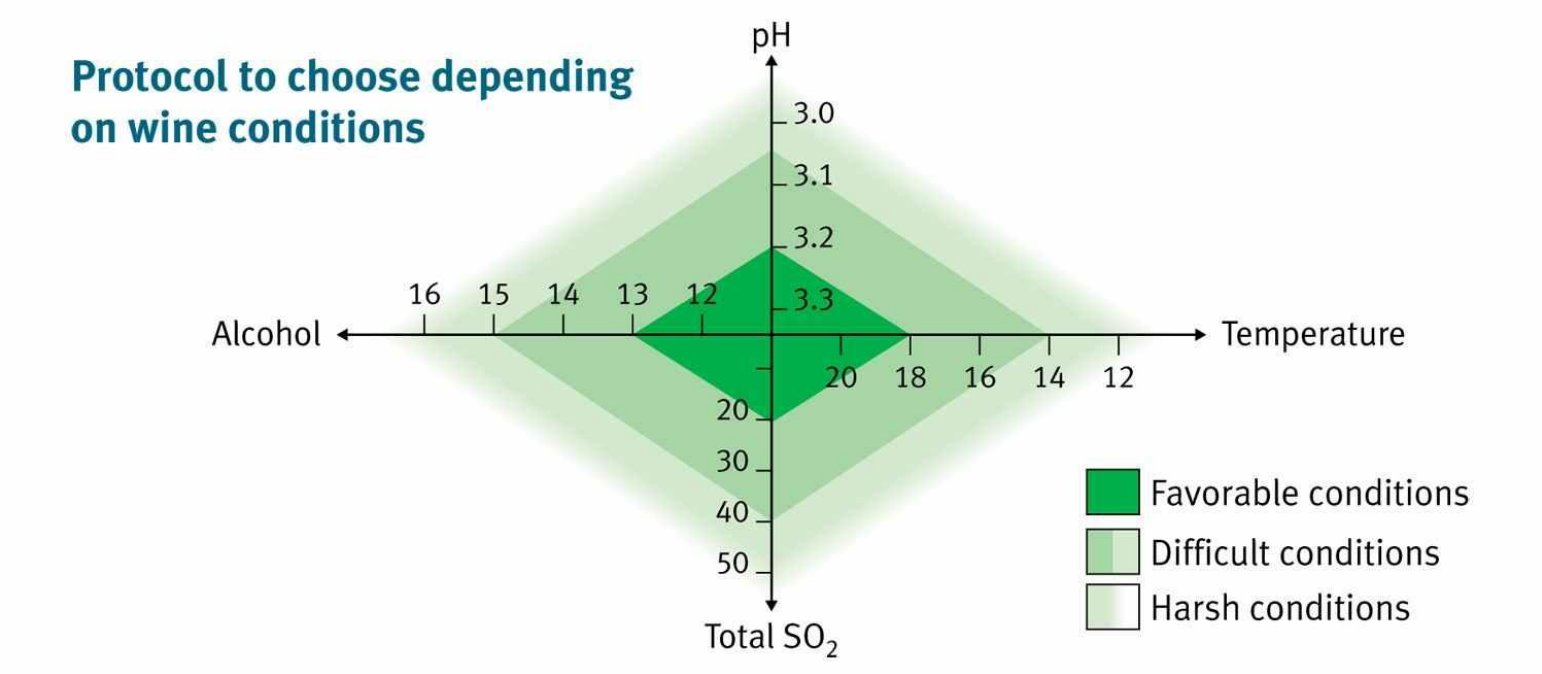
ACTERIA EVOLUTION
ER DIFFICULT CONDITIONS



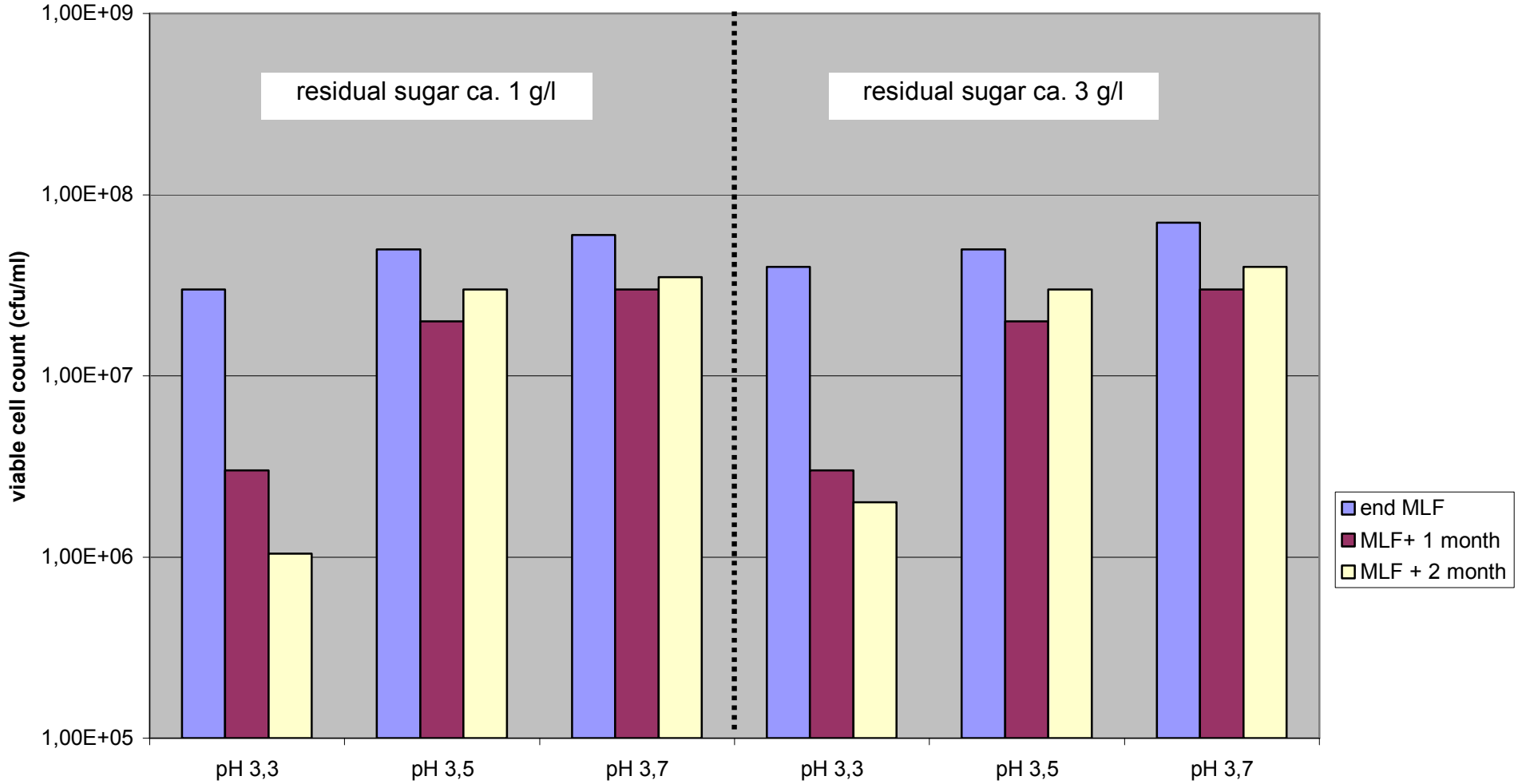
ITERACTION OF PARAMETERS



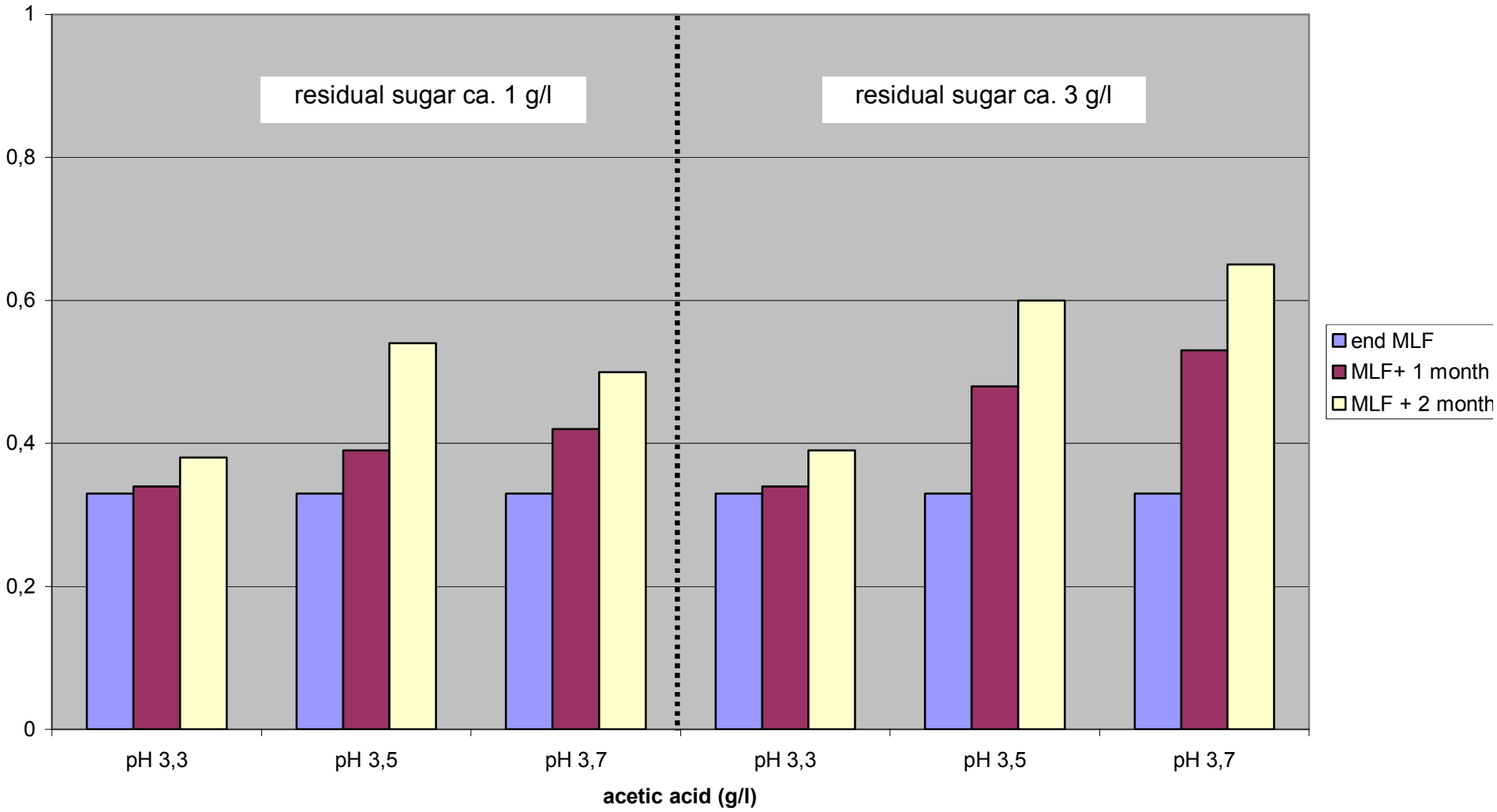
Conditions for a MLF

FAVOURABLE	DIFFICULT
pH 3,3-3,5	pH < 3,2
SO_2 total < 30 mg/l	SO_2 total > 50 mg/l
SO_2 free < 5 mg/l	SO_2 free > 10 mg/l
Temperature > 18°C	Temperature < 15°C
Alcohol < 12 %	Alcohol > 13,5 %

# of a complex Oenococcus oeni population after MLF at ferent pH and residual sugar levels

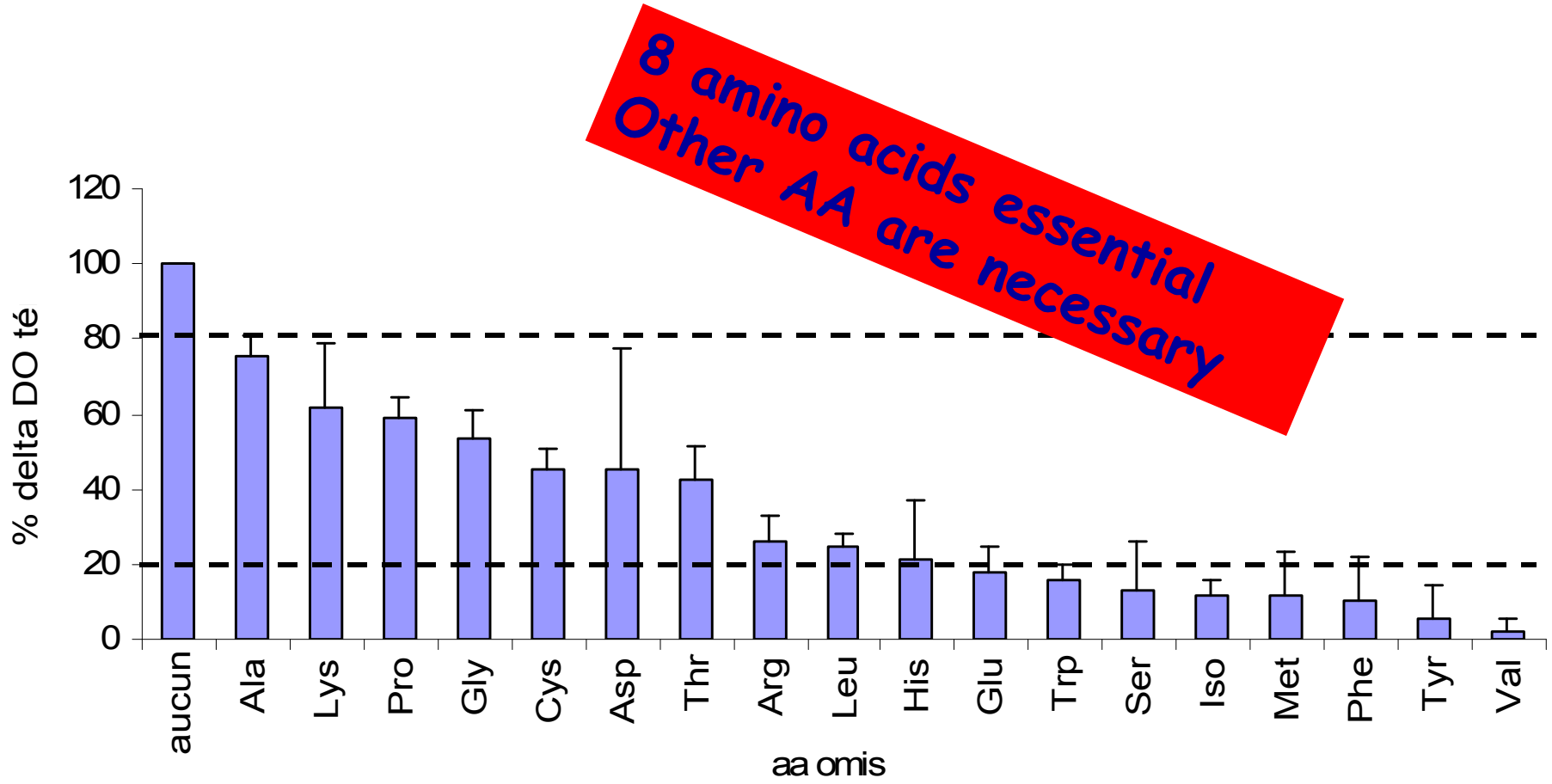


...ric acid in a Pinot Noir after MLF: ... of pH and residual sugar levels



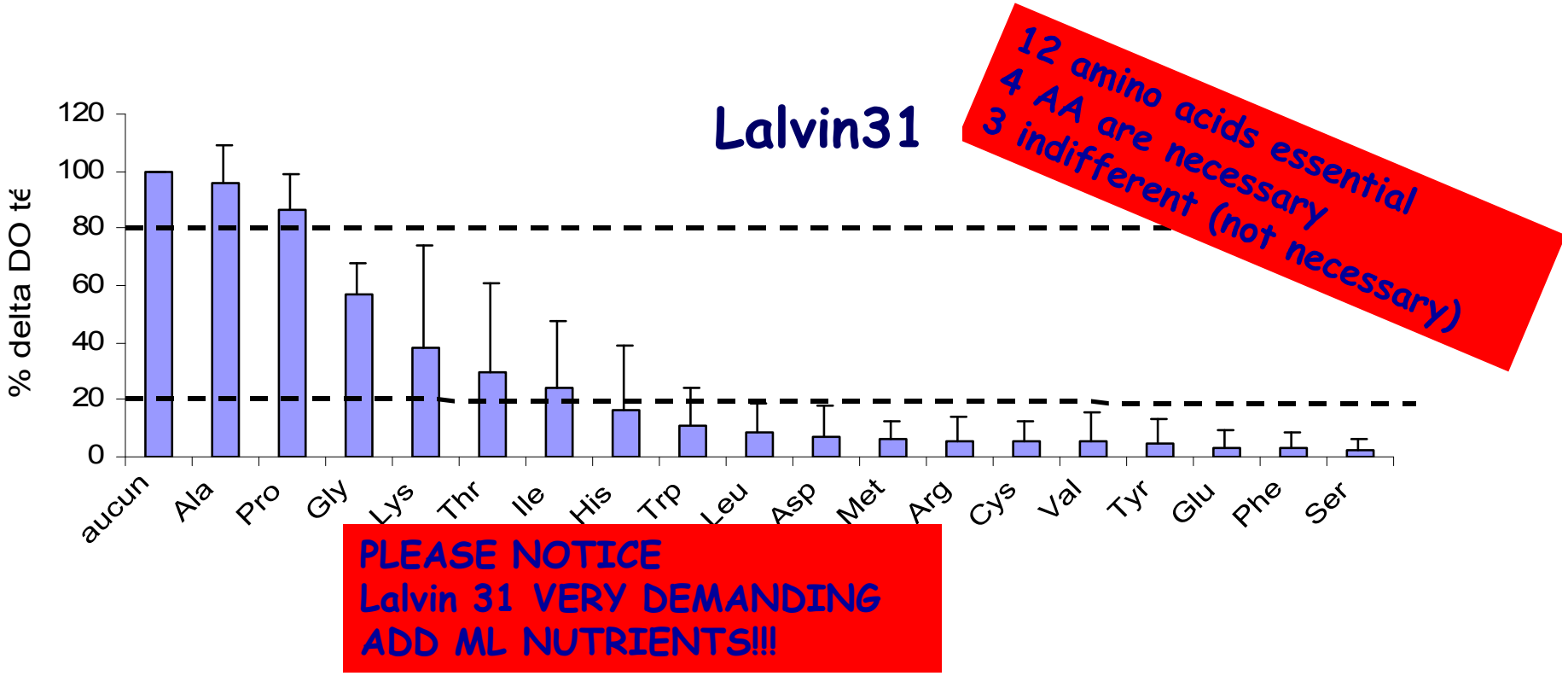
...in Oenococcus oeni VP41 in a synthetic minimal medium with a cocktail of amino acids added.

(values are expressed in percent growth of the OD 600 nm in presence of 18AAs)

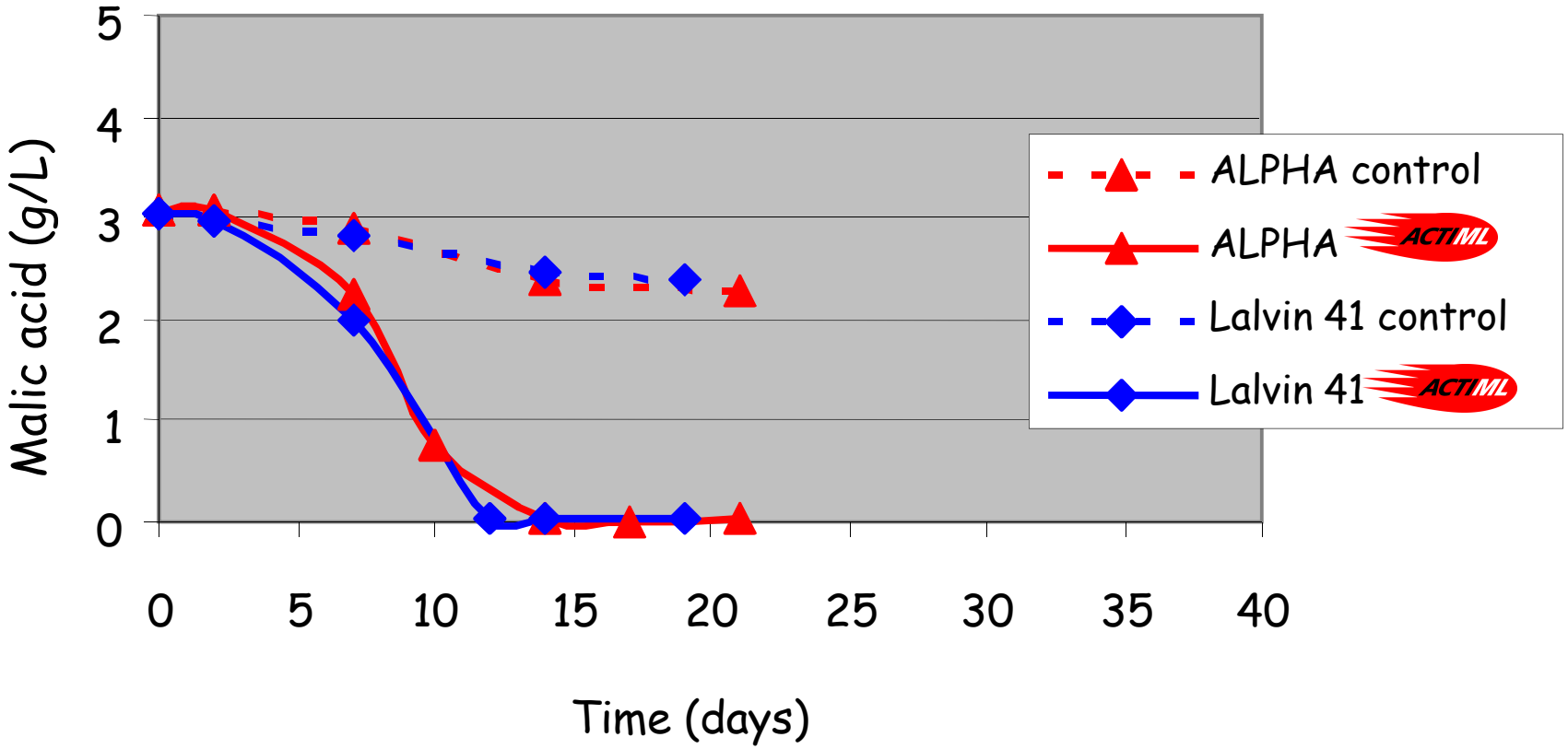


...ain Oenococcus oeni L31 in a synthetic minimal medium with a cocktail of amino acids added.

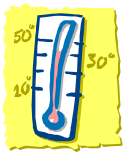
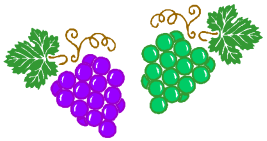
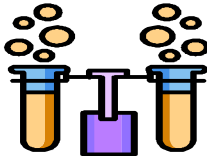
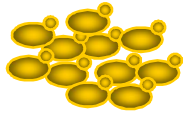
(values are expressed in percent growth of the OD 600 nm in presence of 18AAs)



Franc 2003 second inoculation %vol, T-SO2 43 ppm, pH 3,58) Malic acid degradation in presence



cetyl - management during winemaking

	Diacetyl conc^n		Diacetyl conc^n
 *O. oeni* strain	variable	 temperature	18°C - higher 25°C - lower
 wine type	white - lower red - higher	 SO_2	binds to diacetyl - sensorially inactive
 inoculation rate	10^4 - higher 10^6 - lower	 aeration	air - higher anaerobic - lower
 fermentation time	longer MLF - higher	 contact with yeast lees	long contact- lower
		 pH	lower pH may favour

From: Dr. Eveline Bartowski (AWRI) Trier (D) April 2008

THANK YOU!

For more information…

www.lallemandwine.us