Sacramento Home Winemaker Seminar

Sigrid Gertsen-Briand

Lallemand/ Scott Labs

July 21, 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. »

LALLEMAND

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

rivatives production – General steps

Yeast culture

Unique Oenological strain (ICV, etc.)
Specific strain (high sterol, mineral)

Enrichment procedures (glutathione, micronutrients…)

Yeast biomass

Inactivated yeast

- SIY33
- Noblesse
- GoFerm
- OptiWhite, Booster Blanc

Specific fractions

- OptiRed
- BoosterRouge
- Fermaid O

Autolysate

NATSTEP

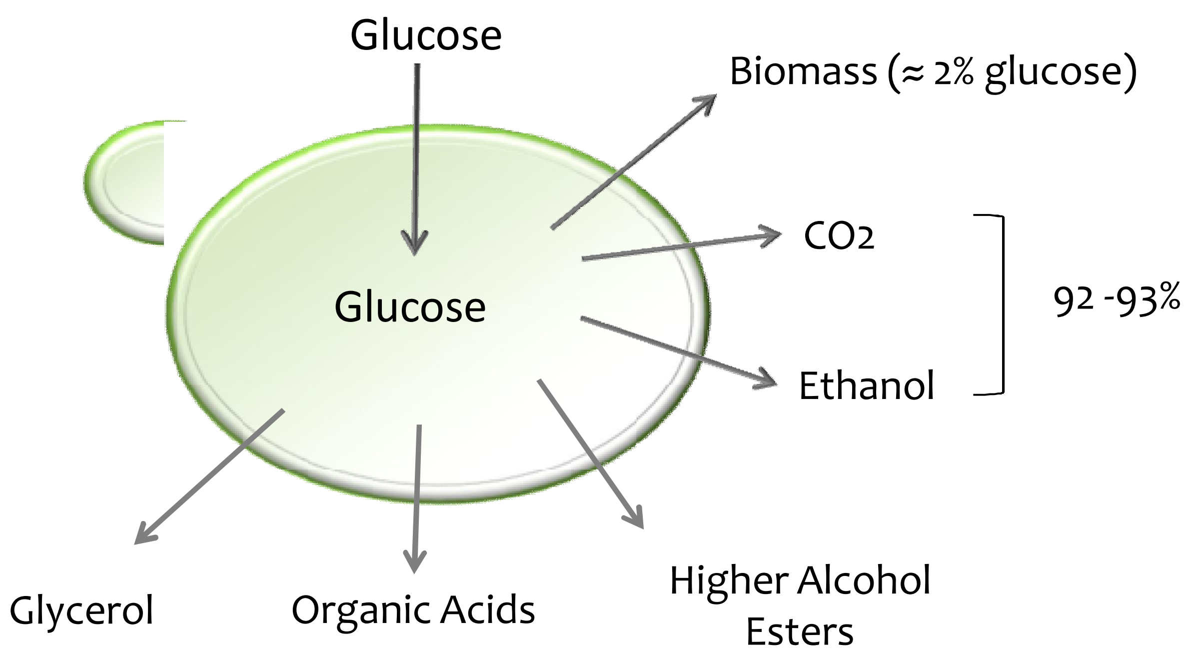
Centrifugation

Yeast extract

FNI

Yeast hulls

Alcoholic fermentation

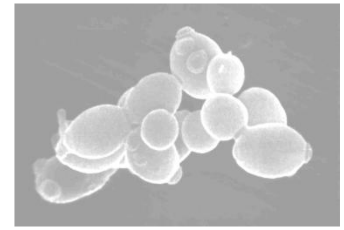


1° alcohol ←→ 16,8 g/l of glucose

even key points

- 1 Choice of yeast
- 2 Nutrition
- 3 Rehydration
- 4 Inoculation rate
- 5 Temperature
- 6 Oxygen
- 7 Micronutrient

YEAST STRAIN CHOICE

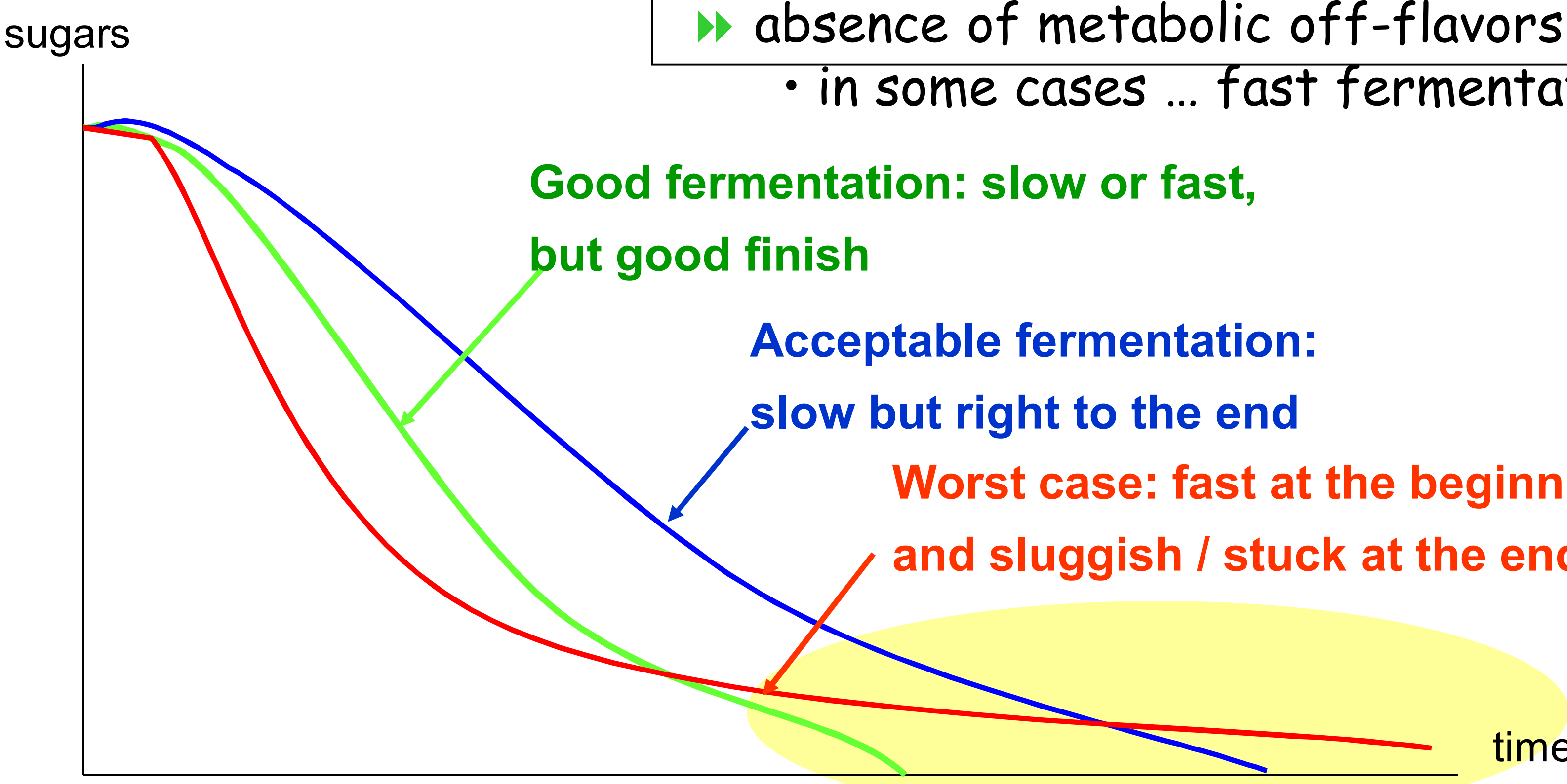


In function of:

- **potential alcohol and technical aspects (like microbial contamination, nutrition, T° control)**
- **Style & quality objectives**

SECURE FERMENTS

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



Key parameter: slope at the end

Defining Good Fermentation Practices

Good Fermentation Practices are considered options that will optimize:

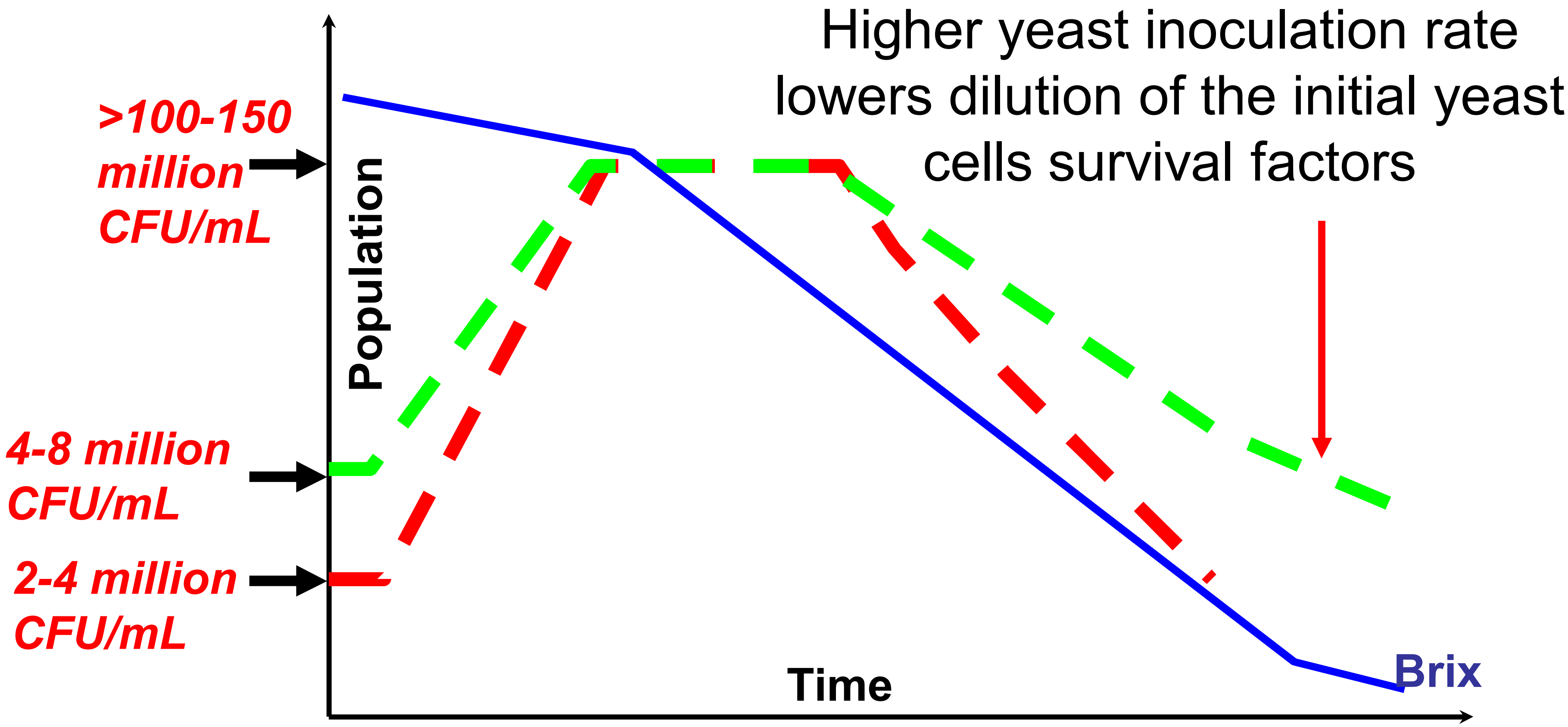
- **A complete and regular fermentation**
- **Achieving analytical and sensorial goals**

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

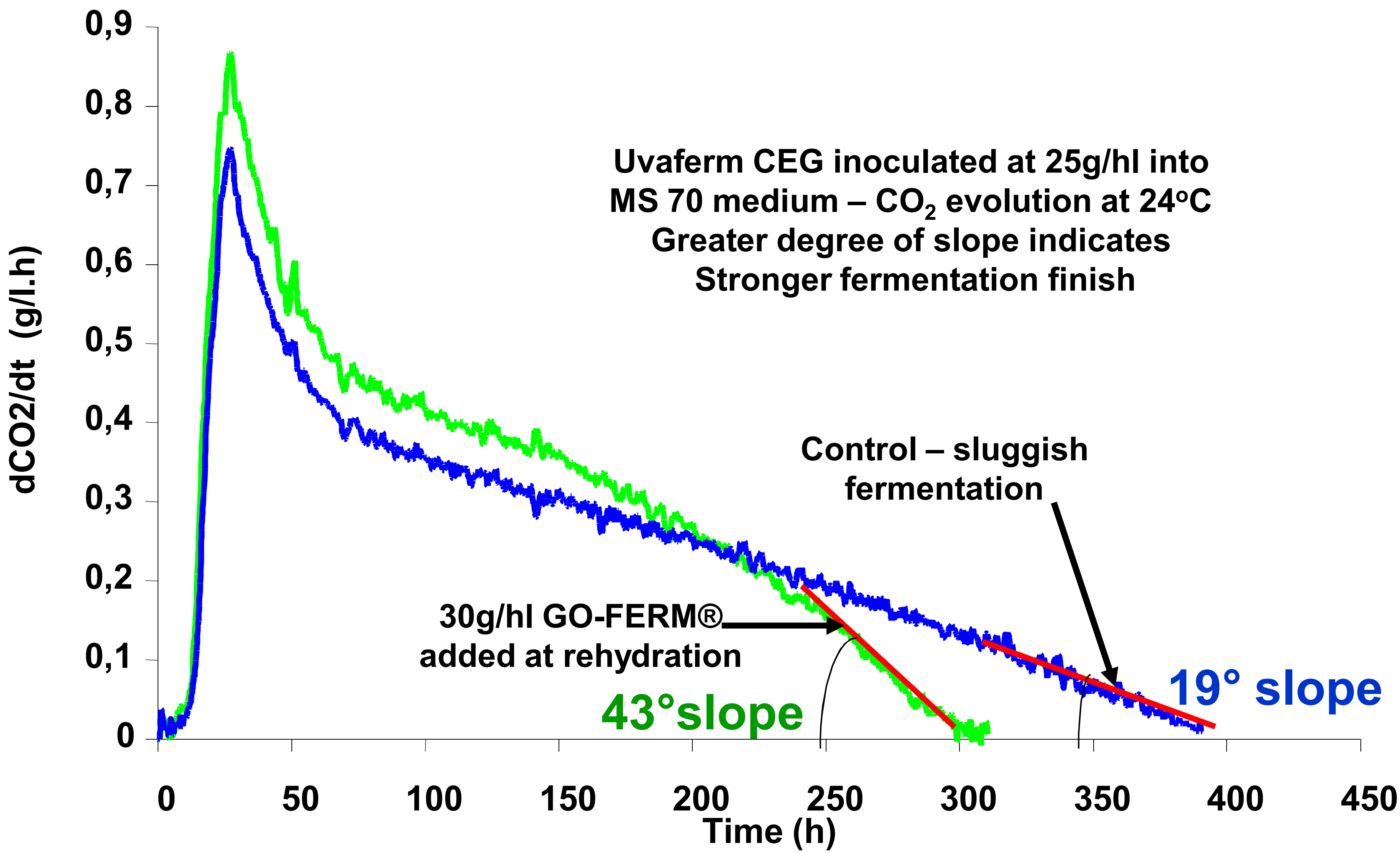
Yeast PROTECTION is essential

&

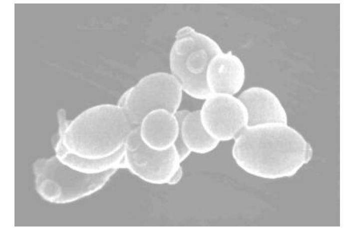
Yeast NUTRITION is vital.

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

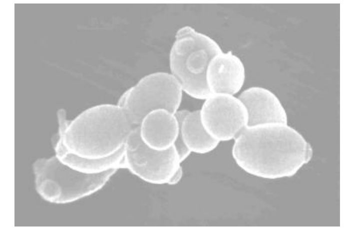
- No competition from other organisms (bacteria or other wild yeast)

- Biologically available
  - Either used initially
  - Stored in the cell until required

- Higher cell viability, More secure fermentation

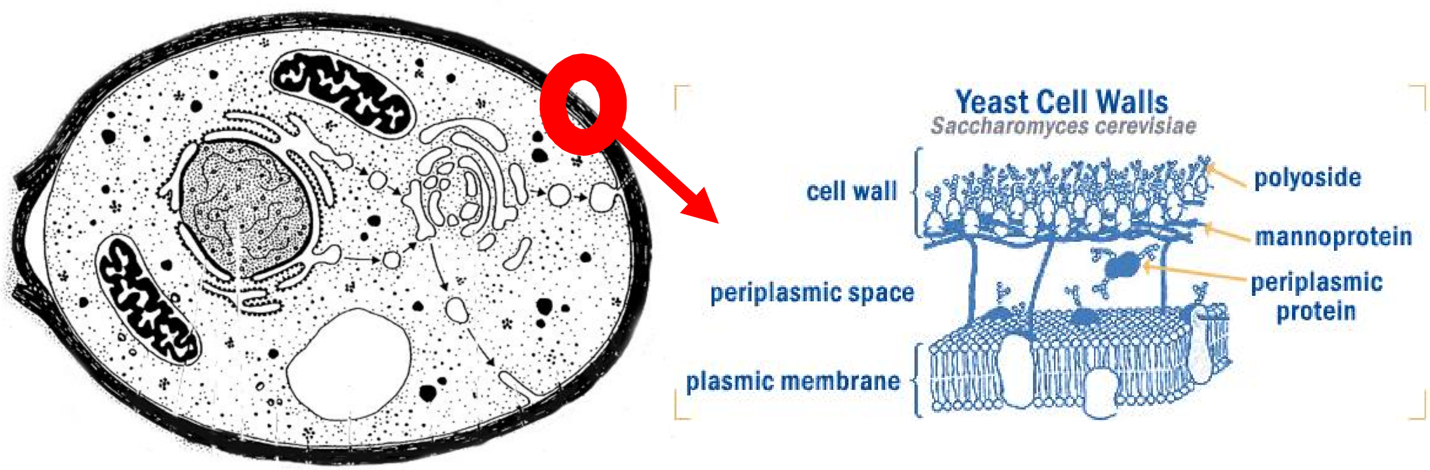
- Better acclimatized yeast

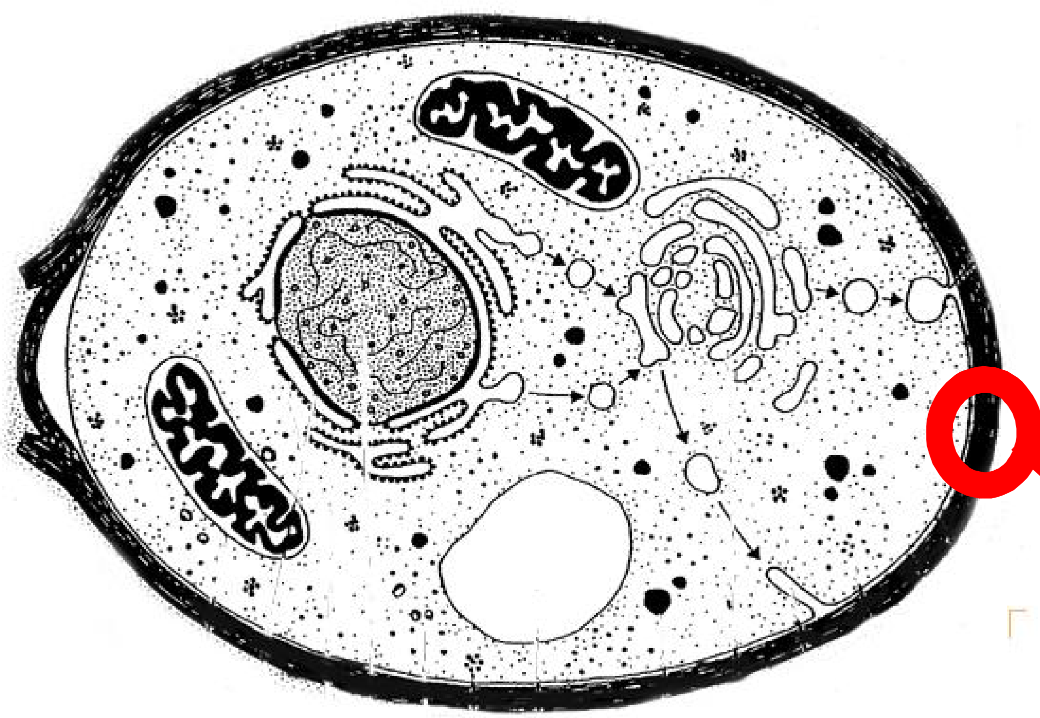
EHYDRATATION



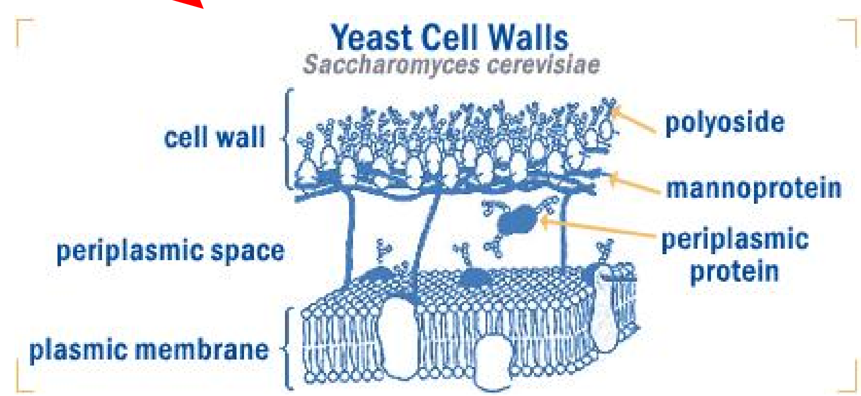
VERY IMPORTANT for YEAST LIFE

- 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

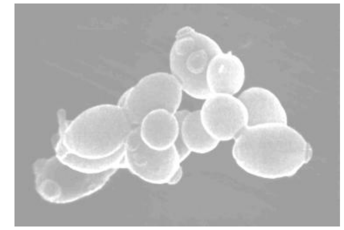


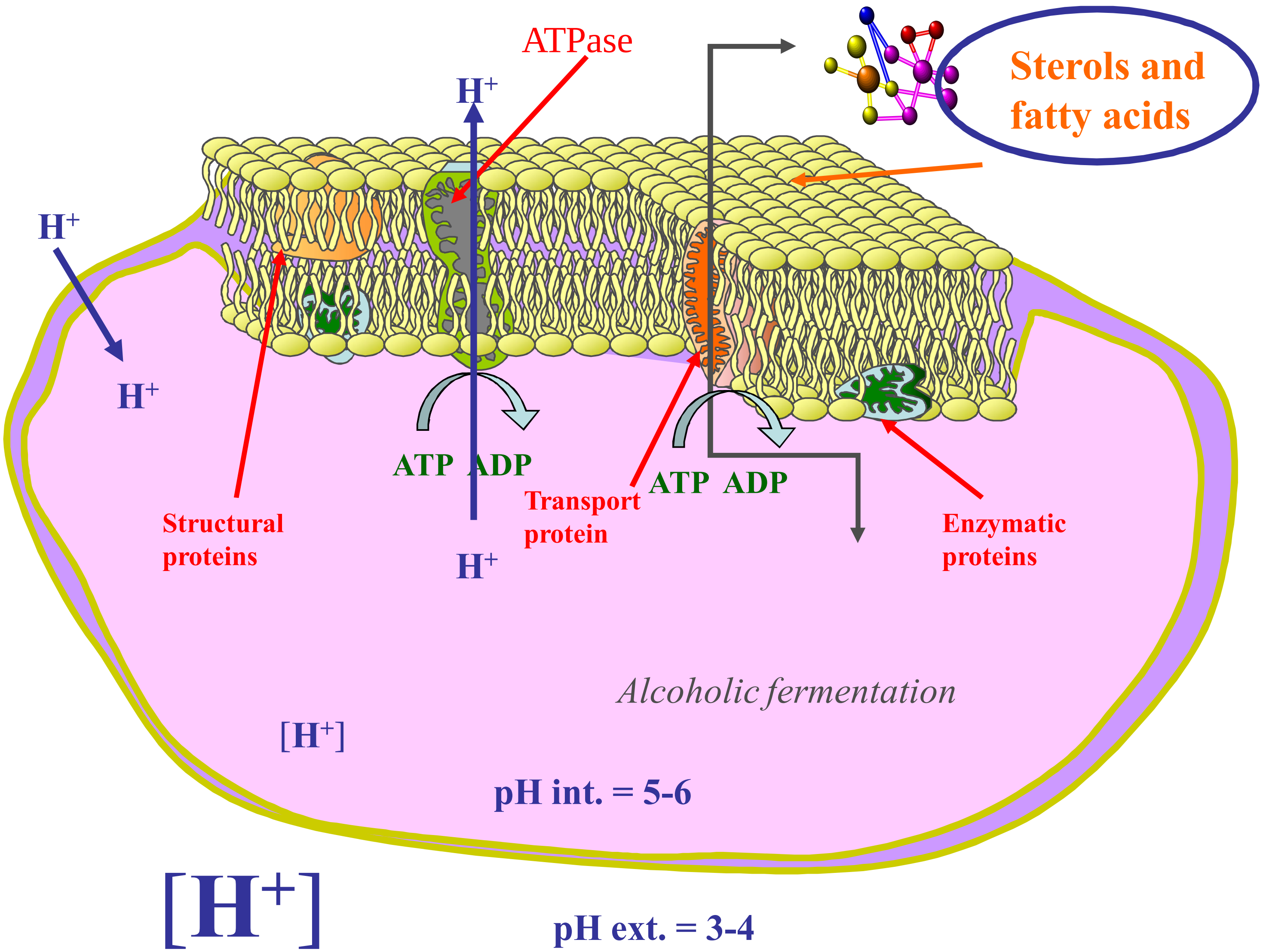


Yeast Cell Wall Cross section…

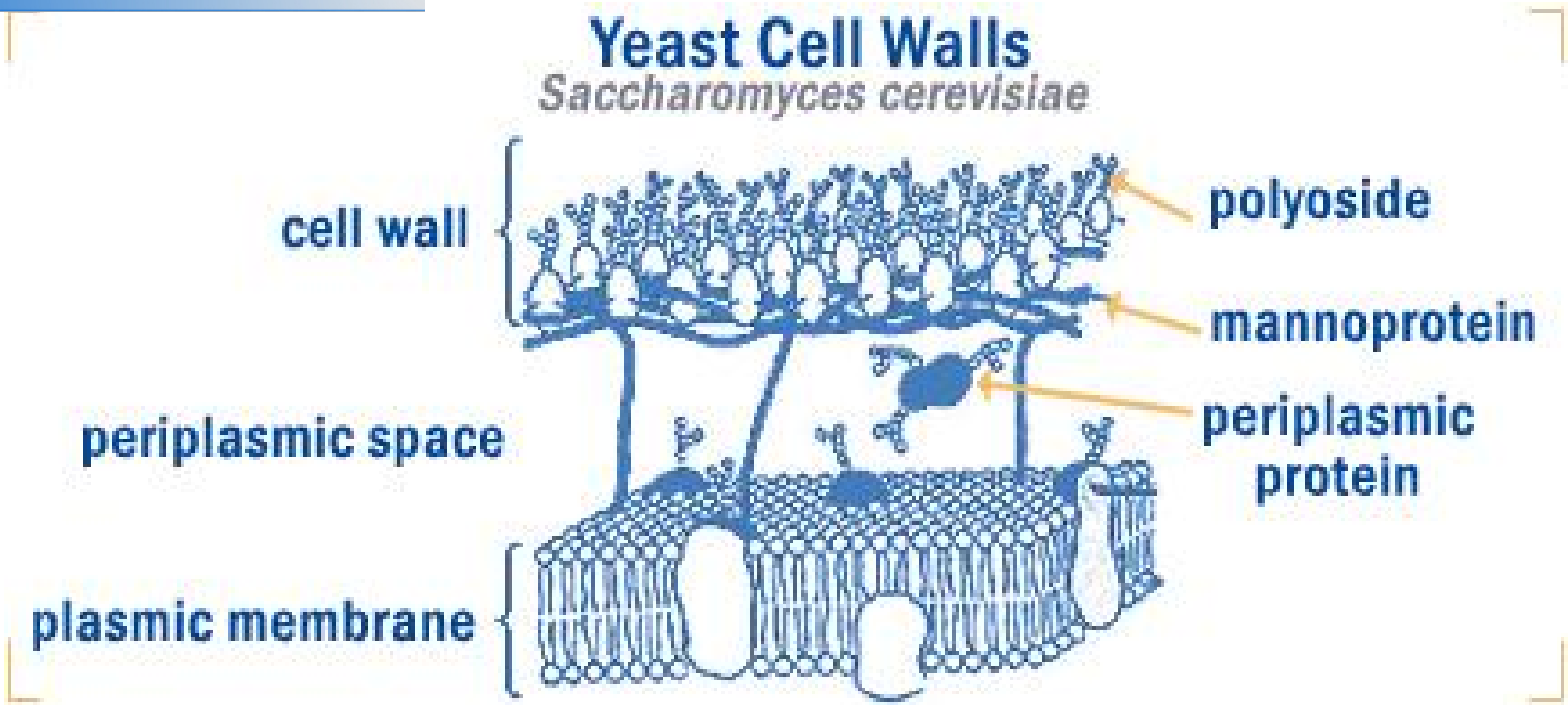


N- PLASMA MEMBRANE



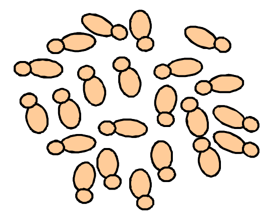


composition:



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

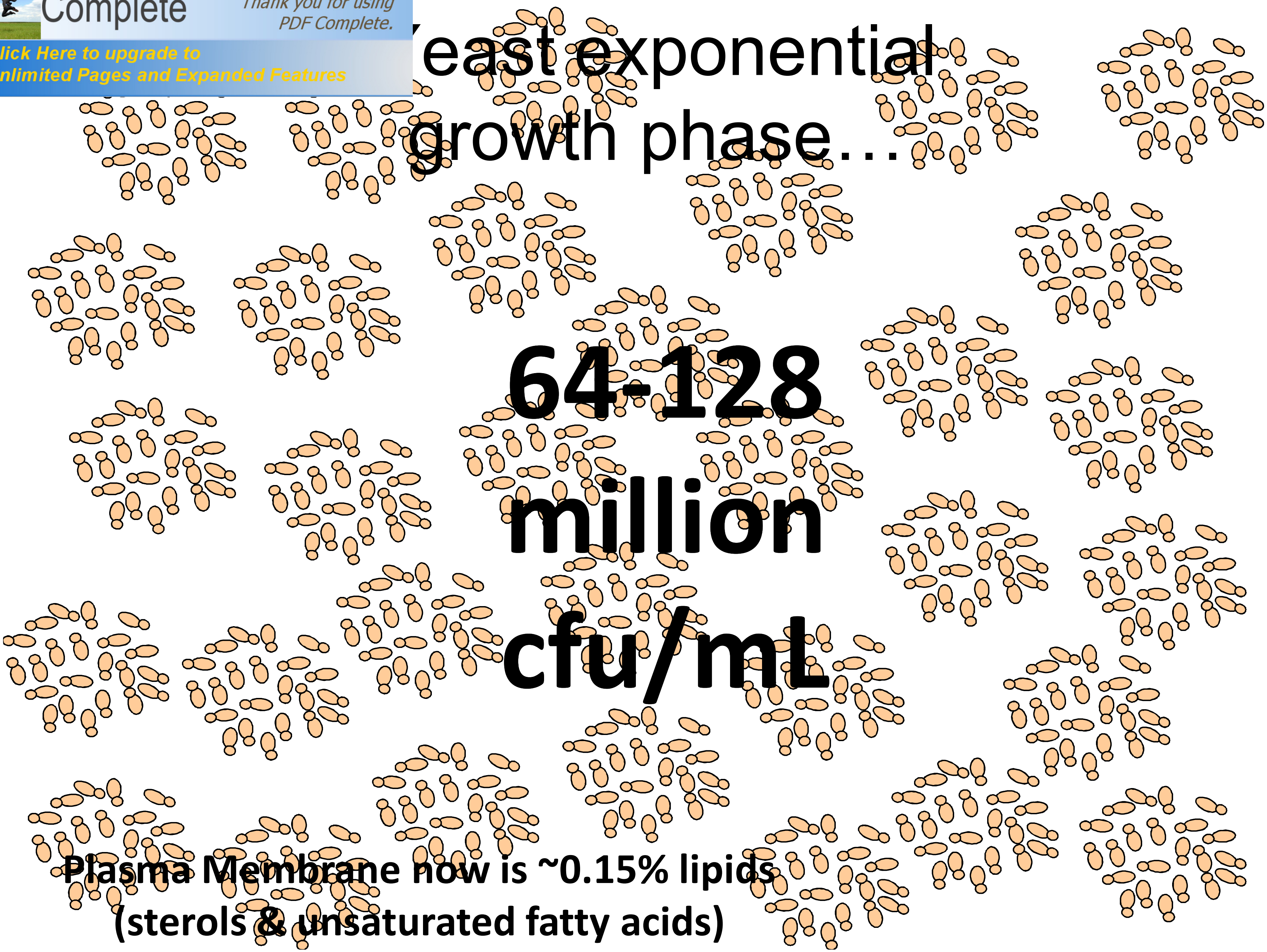
After yeast inoculation and lag phase begins yeast exponential growth phase…



2-4 million cfu/mL

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

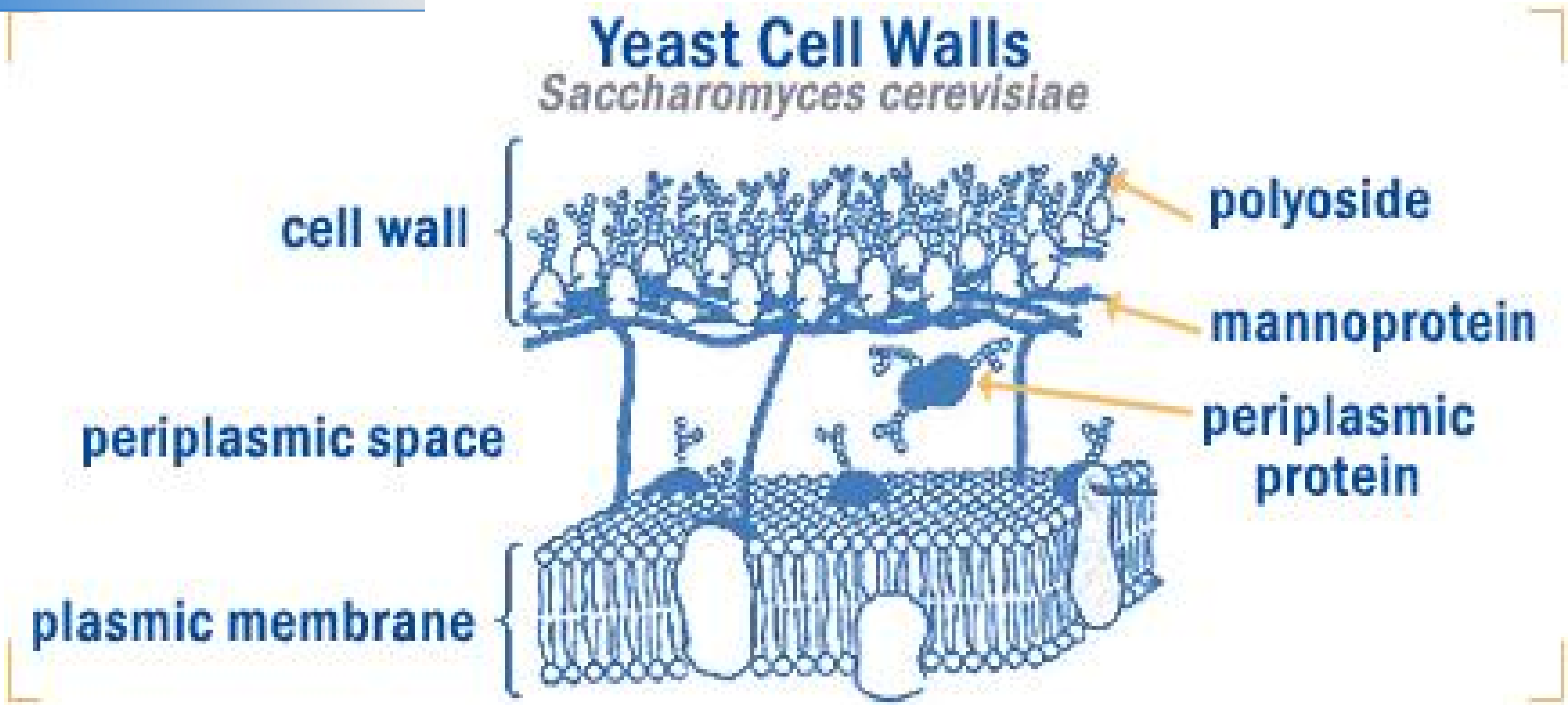
Yeast exponential growth phase…



64-128 million cfu/mL

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

composition:



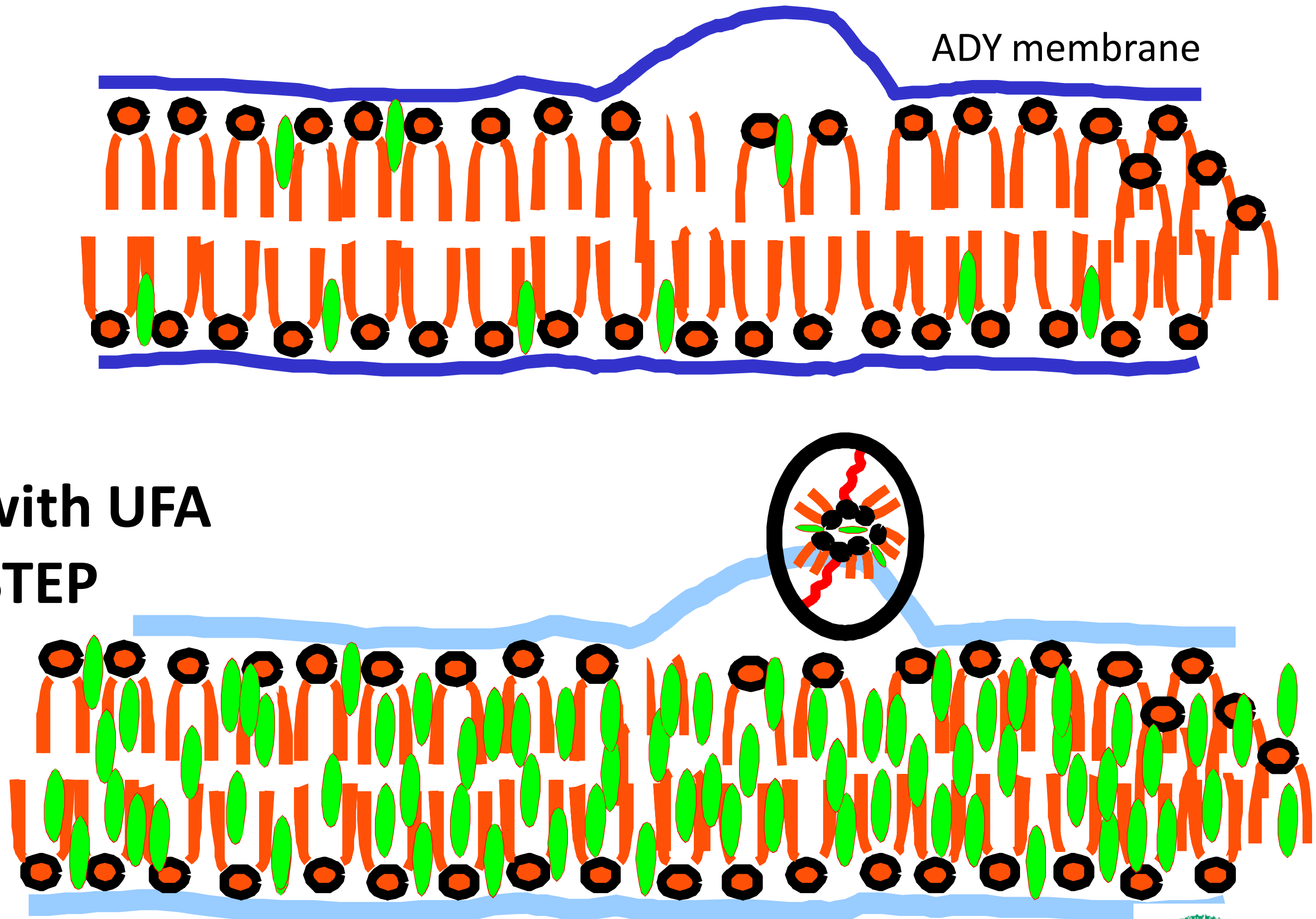
Plasma Membrane is now <0.2% lipids (sterols & unsaturated fatty acids)

A critically low level!

d lipid depletion, add them t rehydration

Rehydration Without Protection

Rehydration with UFA & Sterol NATSTEP Protection



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),

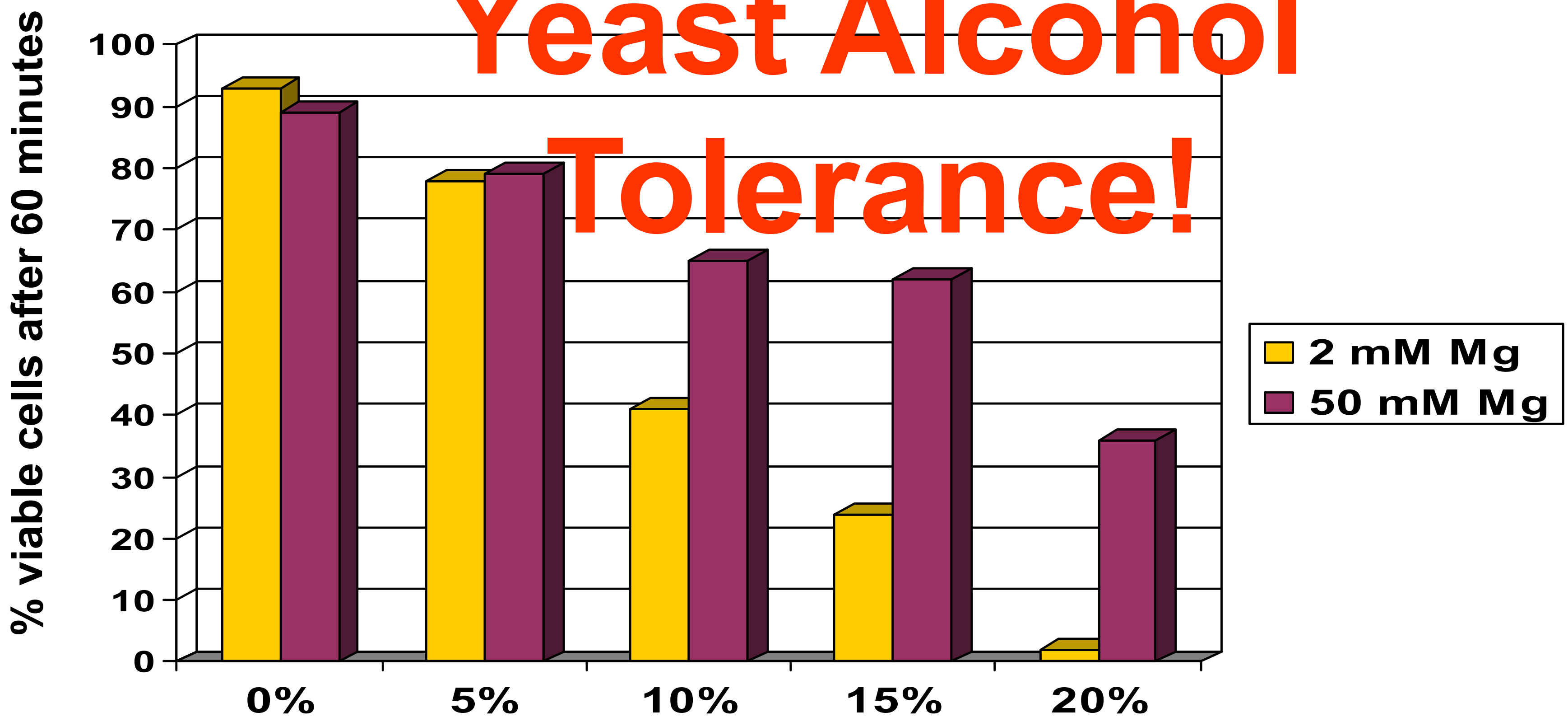
Manganese synergistic effect with Zn, shorter generation time

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

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

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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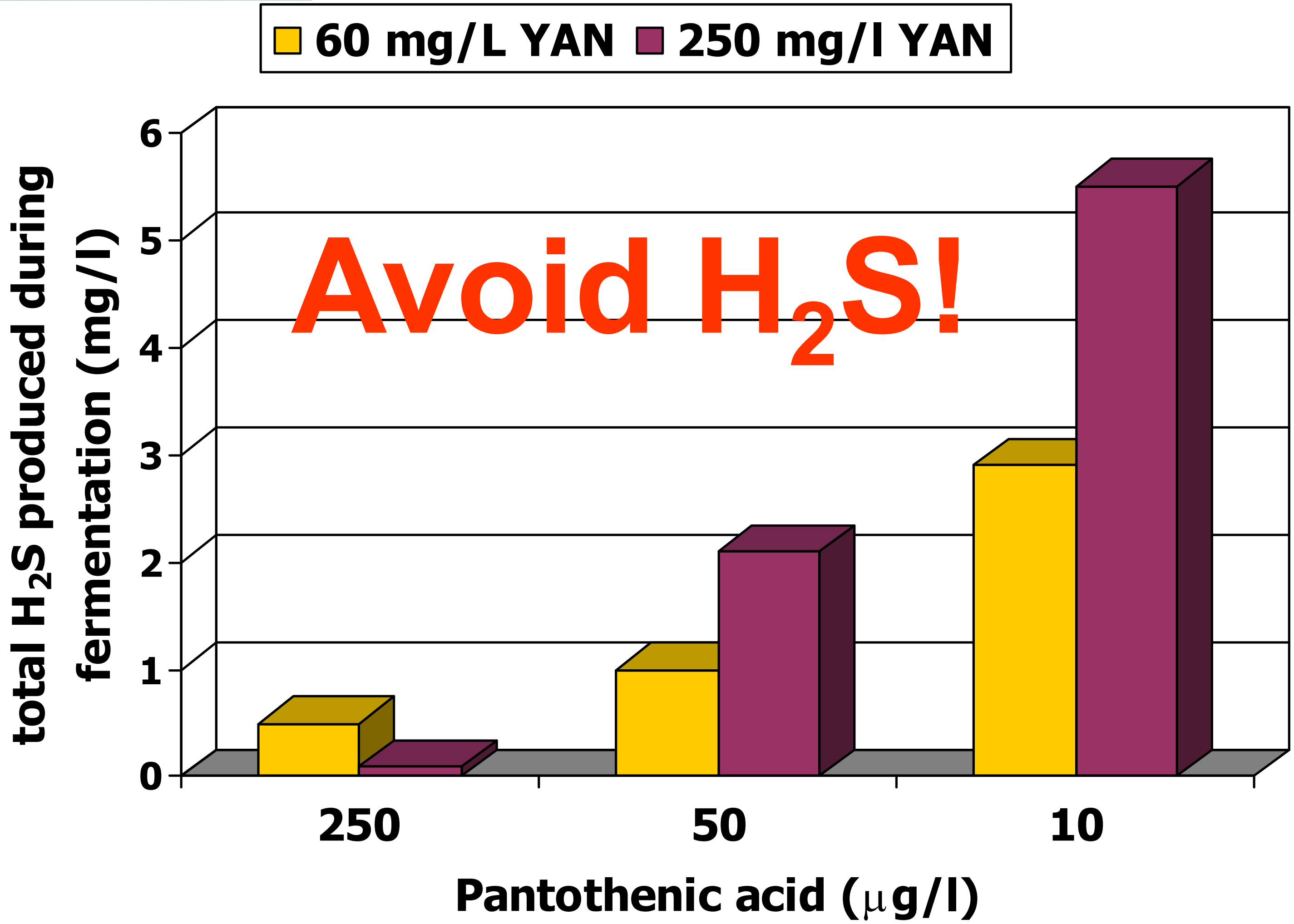
Pantothenic Acid Important?



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

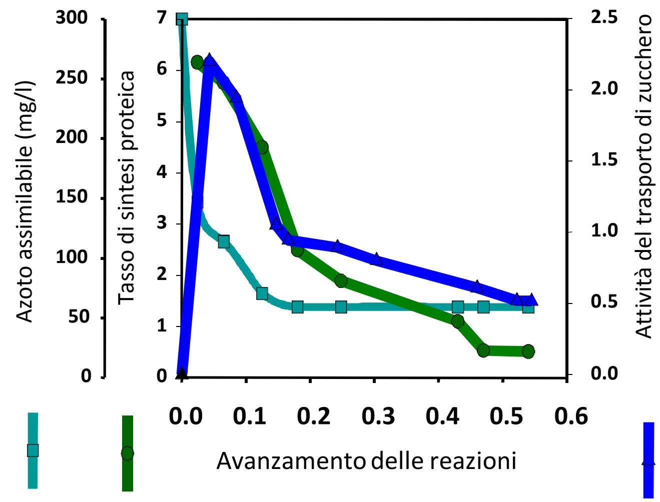
Formol titration	NOPA
Simple titration Hazardous waste NH4 and FAN (including Proline) Good estimation	Measures FAN (excluding proline) Measure Ammonia separately (ISE Probe) No waste Spectrometry

ctors influencing accumulation

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

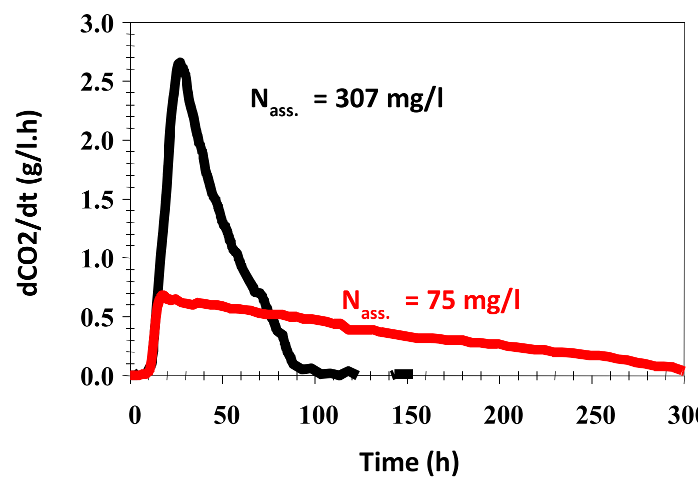
WHY NITROGEN IS ESSENTIAL?

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

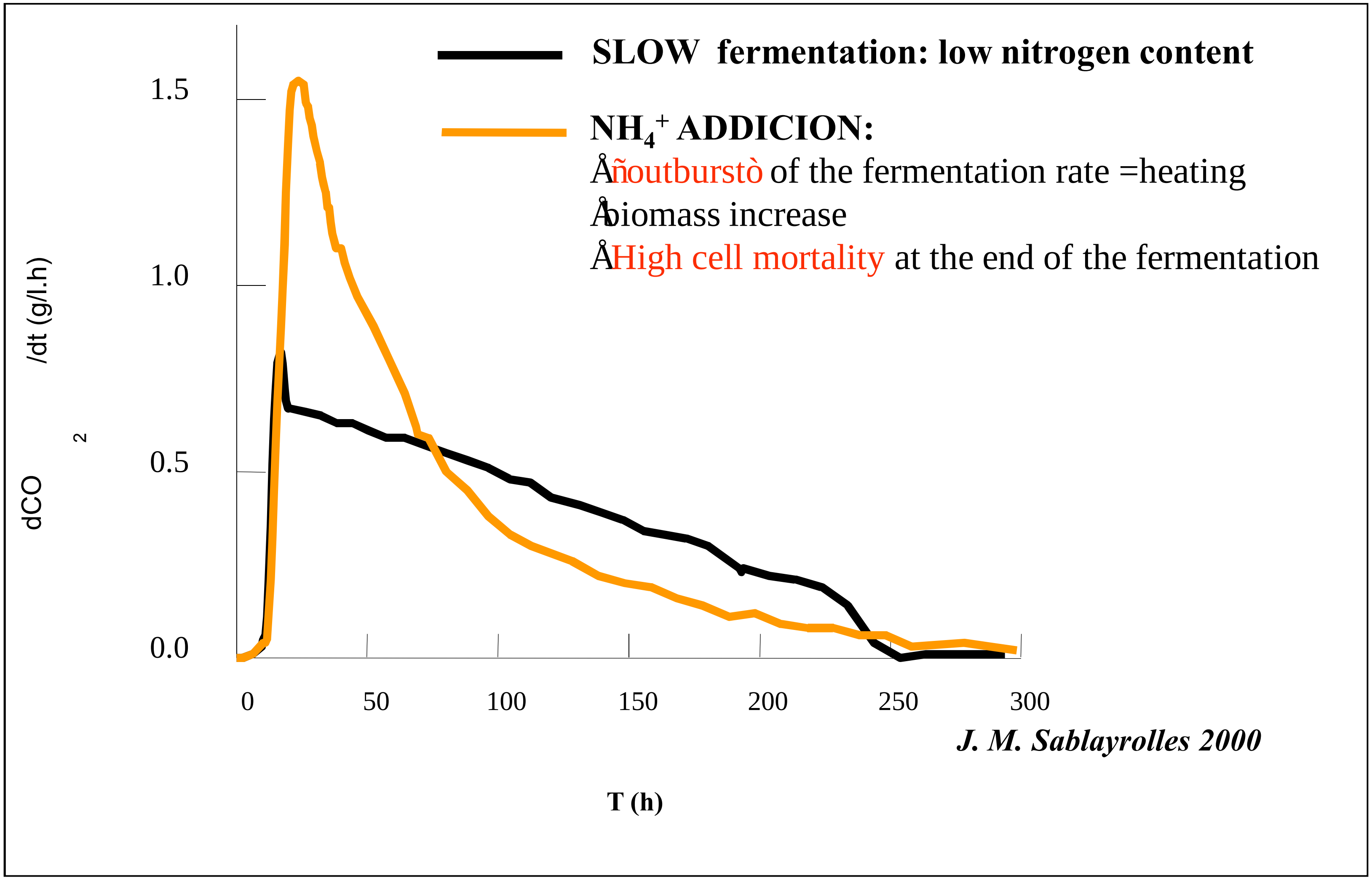


- **Cell growth : maximum CO2 production rate correlated with assim. nitrogen content of the must**
  (Bely *et al*., 1991)

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



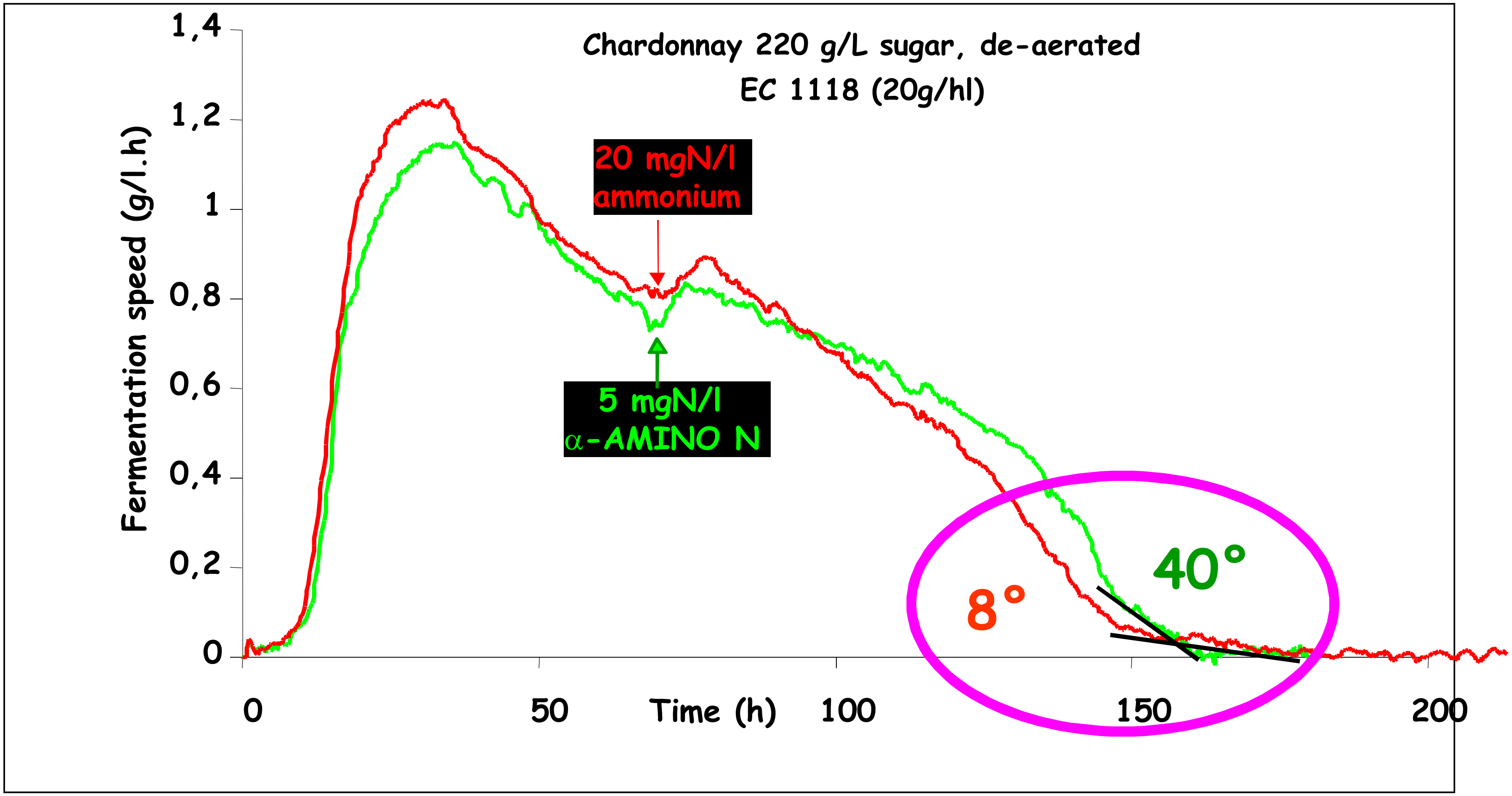
…mediate” nitrogen assimilation problematic?



. Inorganic nitrogen:

Security at the end of the fermentation:

BETTER RESULTS, EVEN WITH LOW ADDITION



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

g Chardonnay aroma and flavour with yeast-strain specific nutrient regimes"

Objectives

- To improve understanding of the yeast-nutrients-flavour matrix
- To quantify improvements in yeast performance in response to fermentation nutrients
- To demonstrate sensorial differences in wines made using different yeasts with and without fermentation nutrients
- To characterise the nature of chemical differences in wines exhibiting distinct sensory profiles

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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

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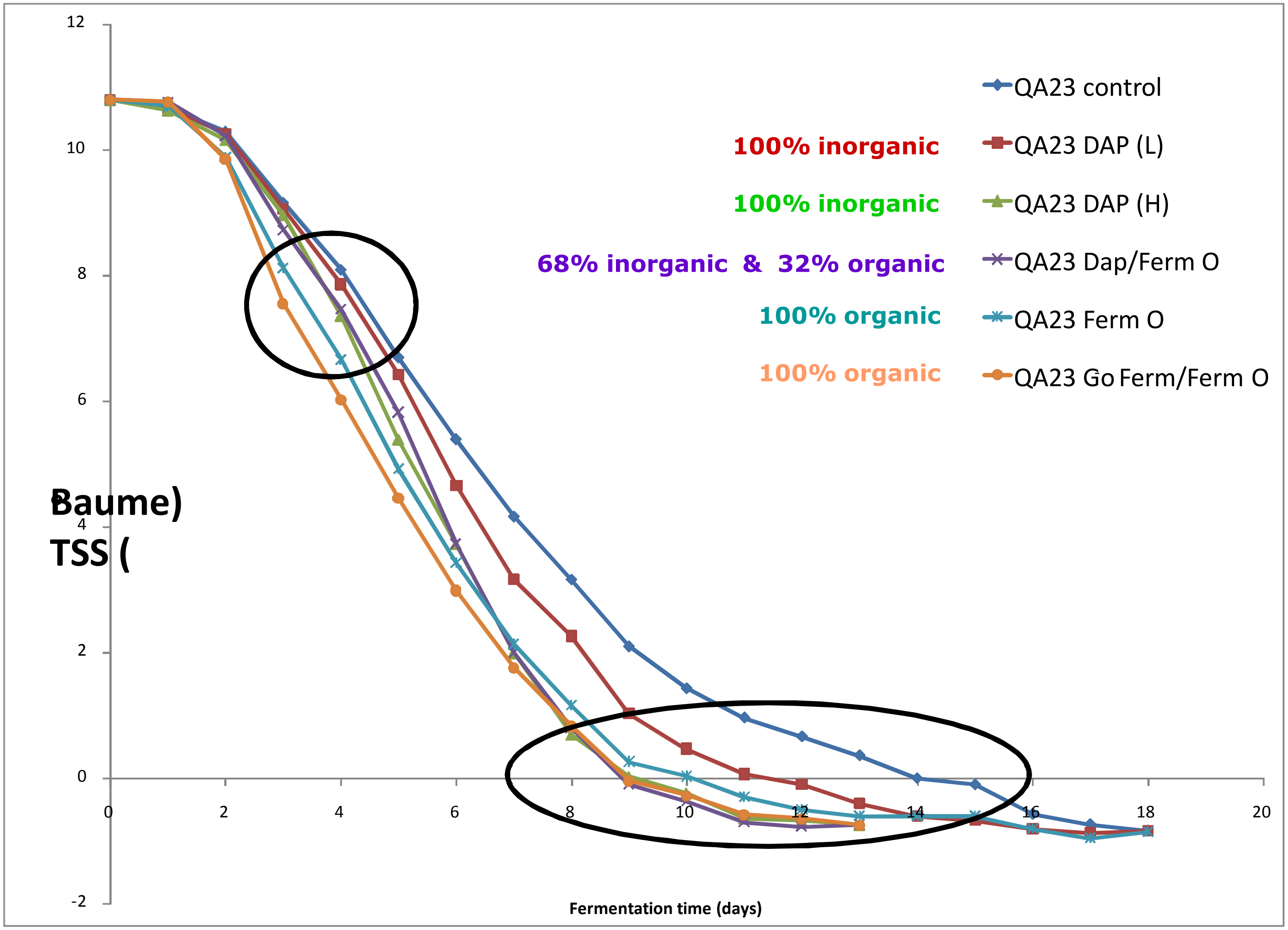

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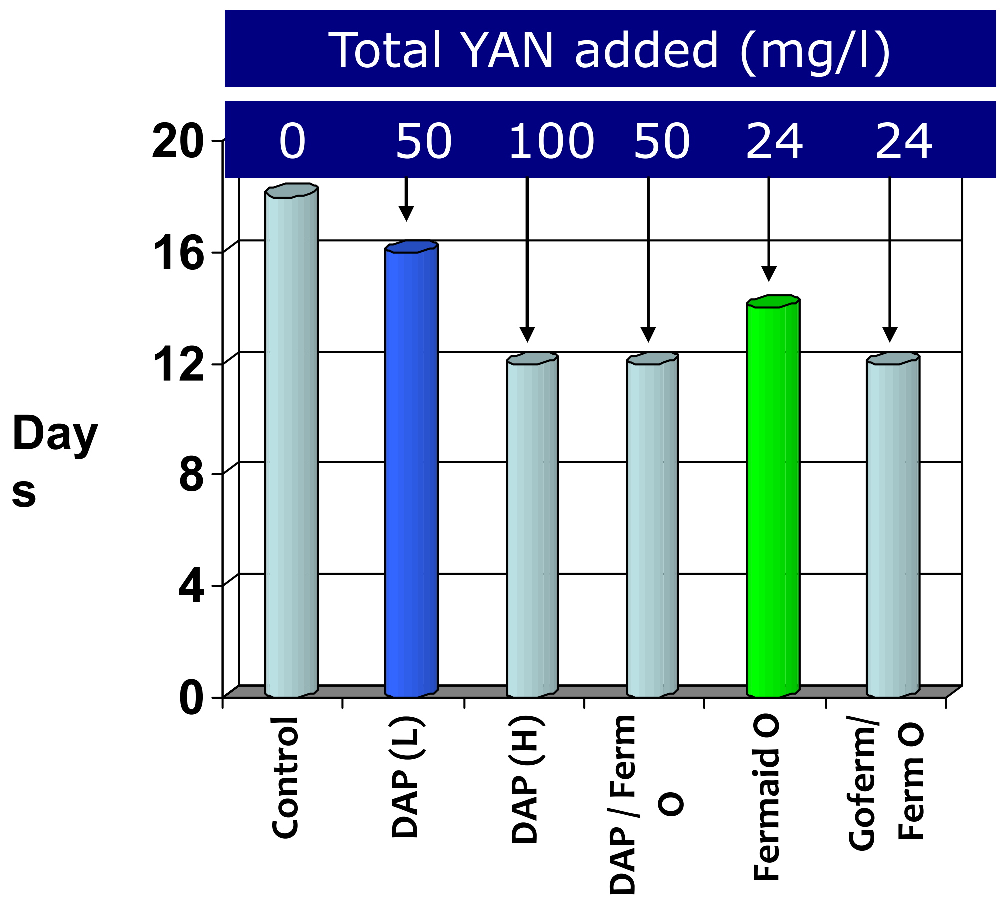
act on yeast fermentative activity



- 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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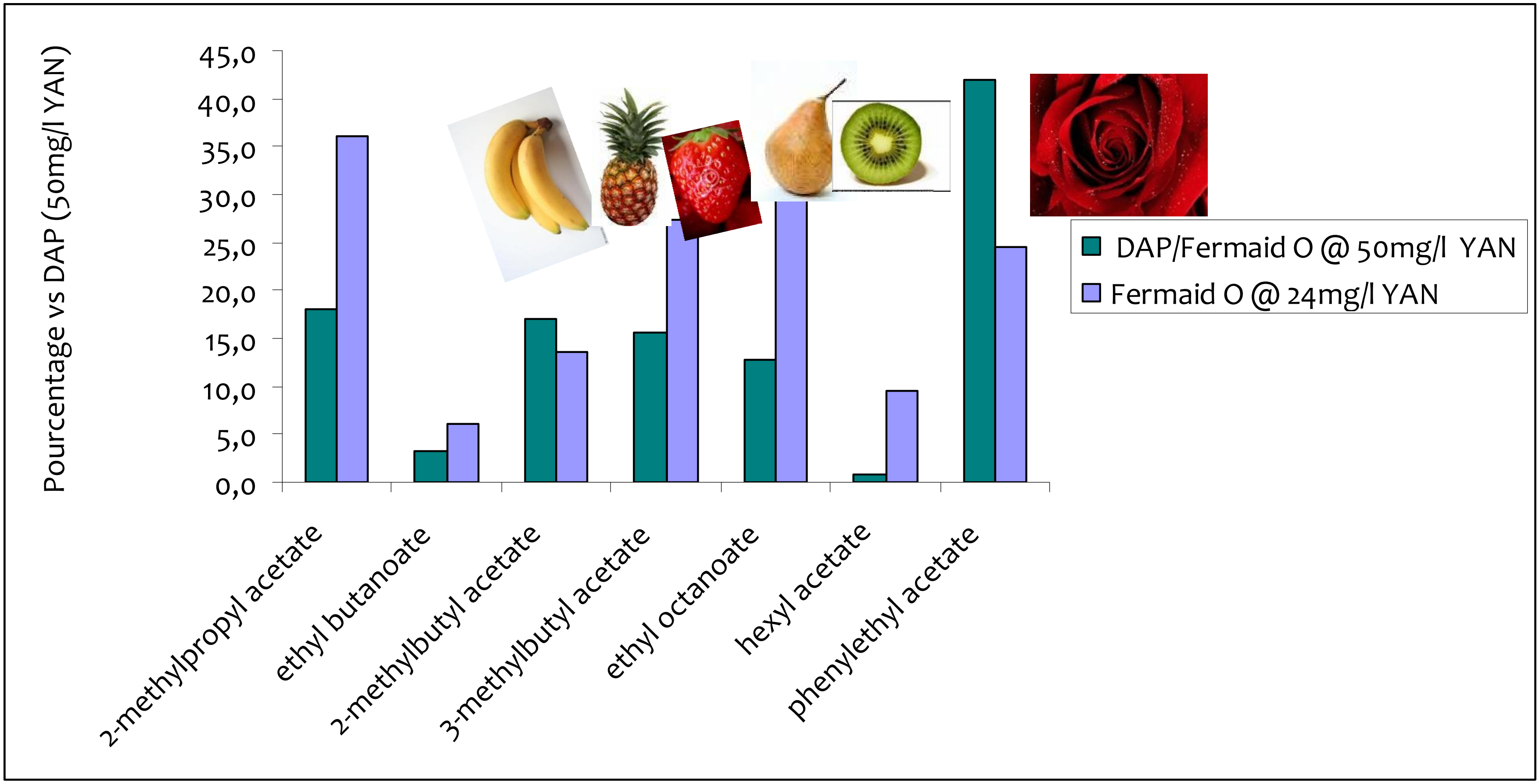
pact of N2 source on aromas



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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

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

ture Control in Red Must

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

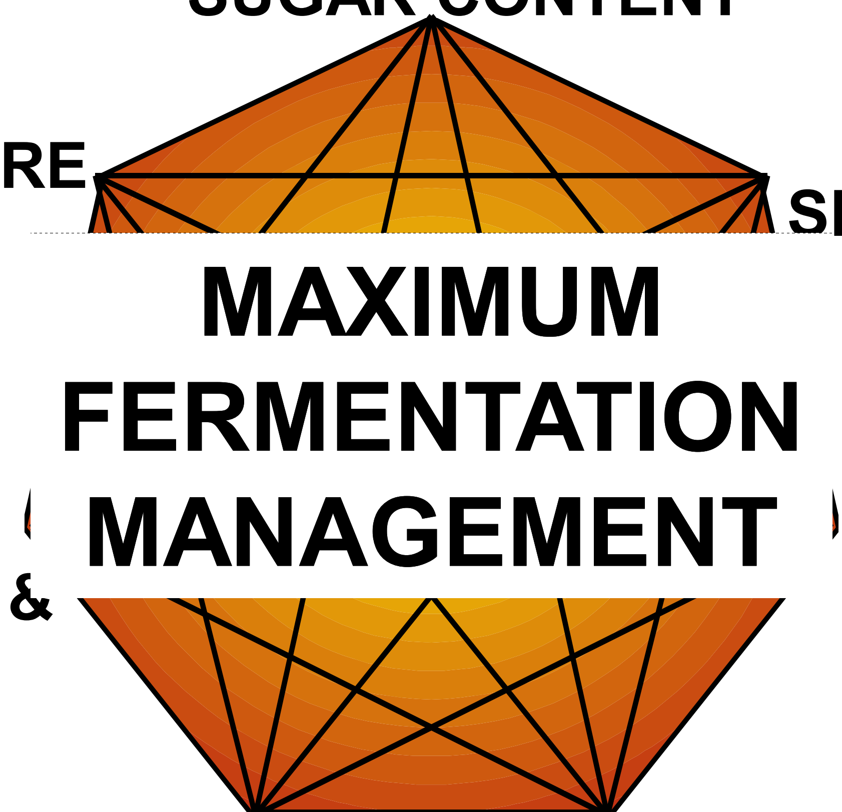
Max. Temperature

• 20 Brix	• 95°F
• 21 Brix	• 90°F
• 22 Brix	• 85°F
• 23 Brix	• 80°F
• >24 Brix	• 76°F

And you thought I would forget?

!!!!

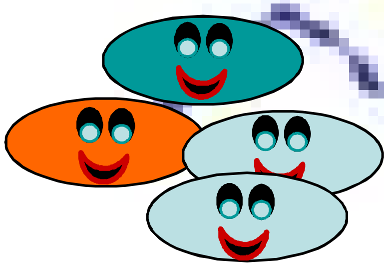
rrelationships of Factors Affecting Fermentation

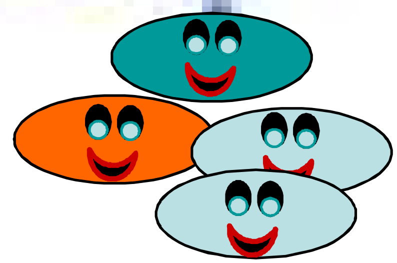


Management

of

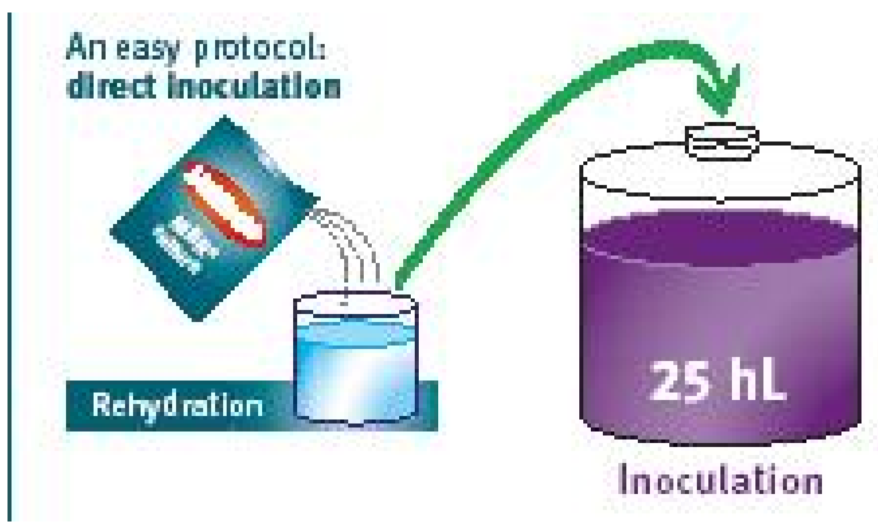
MLF





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^{o}C

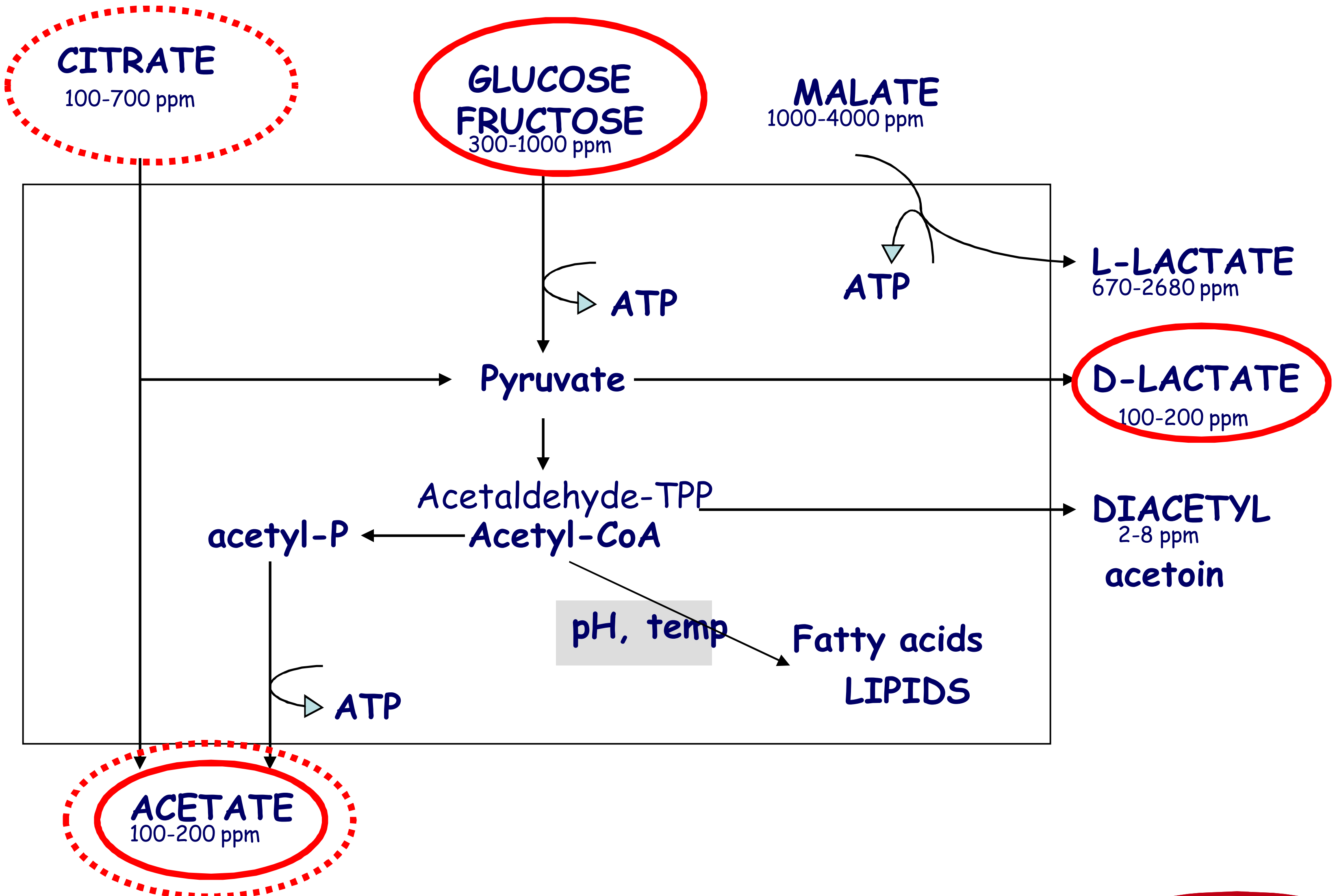


THE CHEMISTRY...

$$HOOC \cdot CH_2 \cdot CHOH \cdot COOH \longrightarrow CO_2 + CH_3 \cdot CHOH \cdot COOH$$

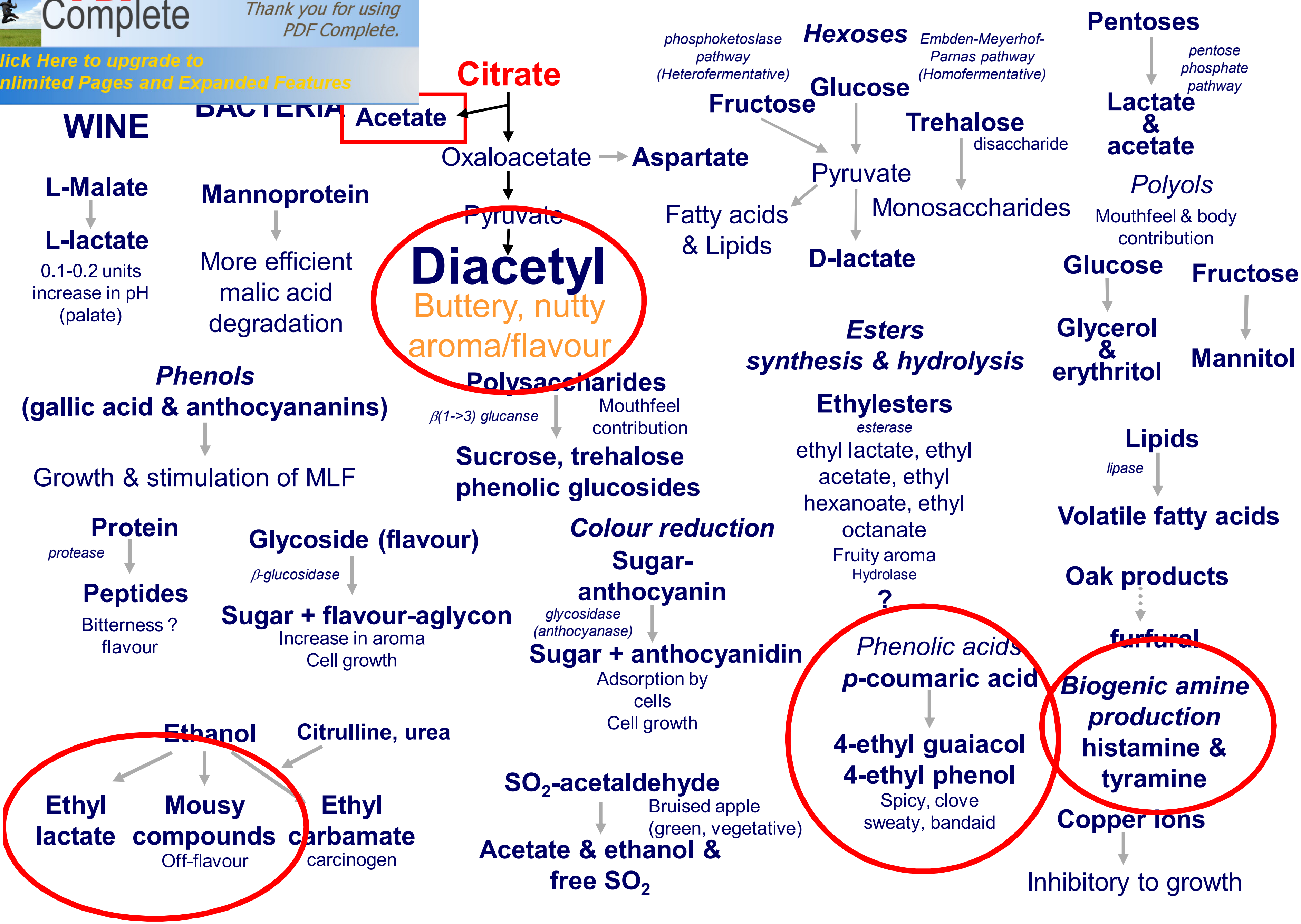
malic acid	carbon dioxide	lactic acid
134	44	90

sm in heterofermentative Lactic Acid Bacteria



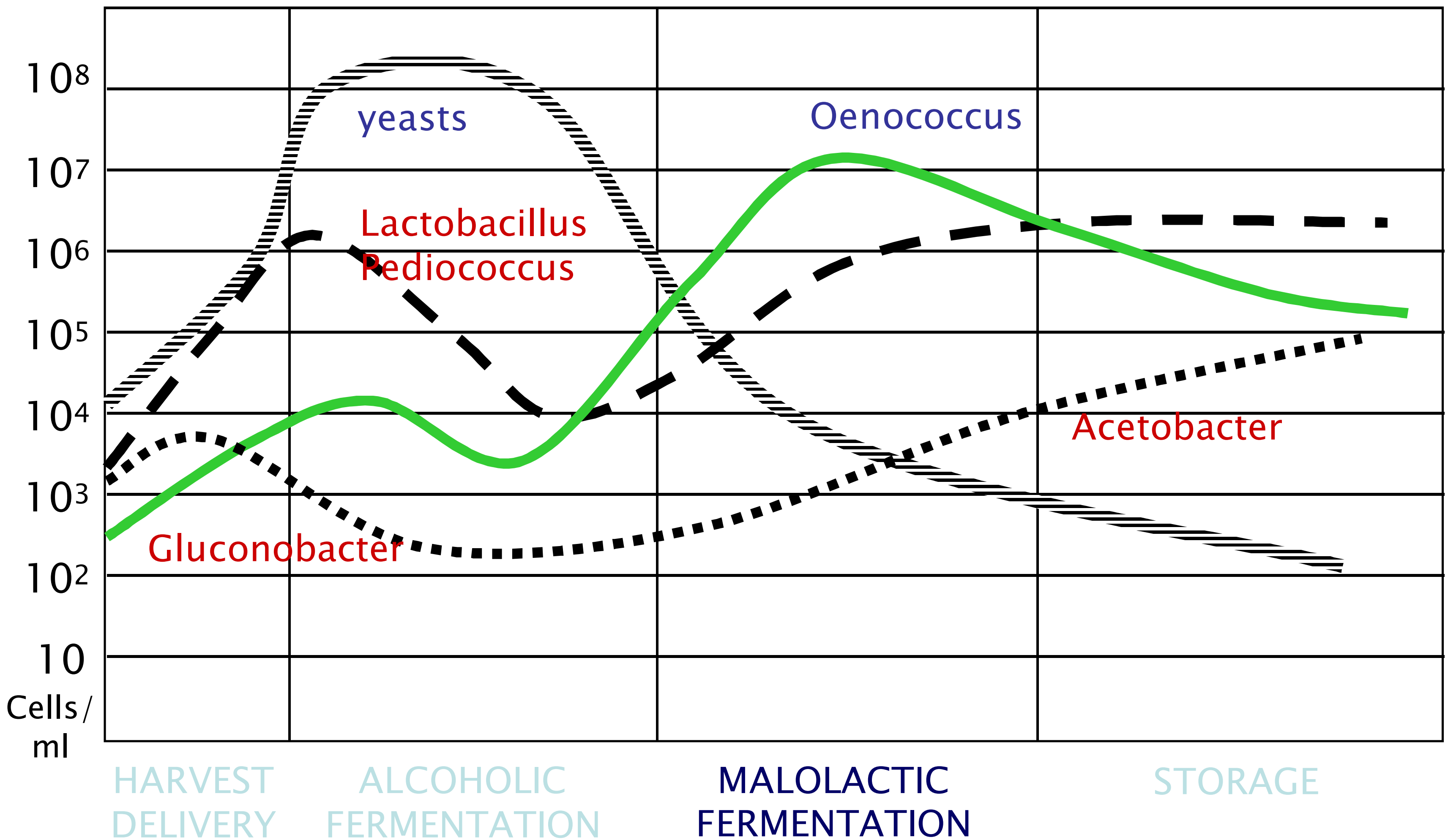
The more you know…

…the better!



Eveline Bartowski, AWRI, 2004

ACTERIA EVOLUTION
FAVOURABLE CONDITIONS



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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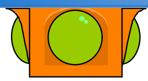
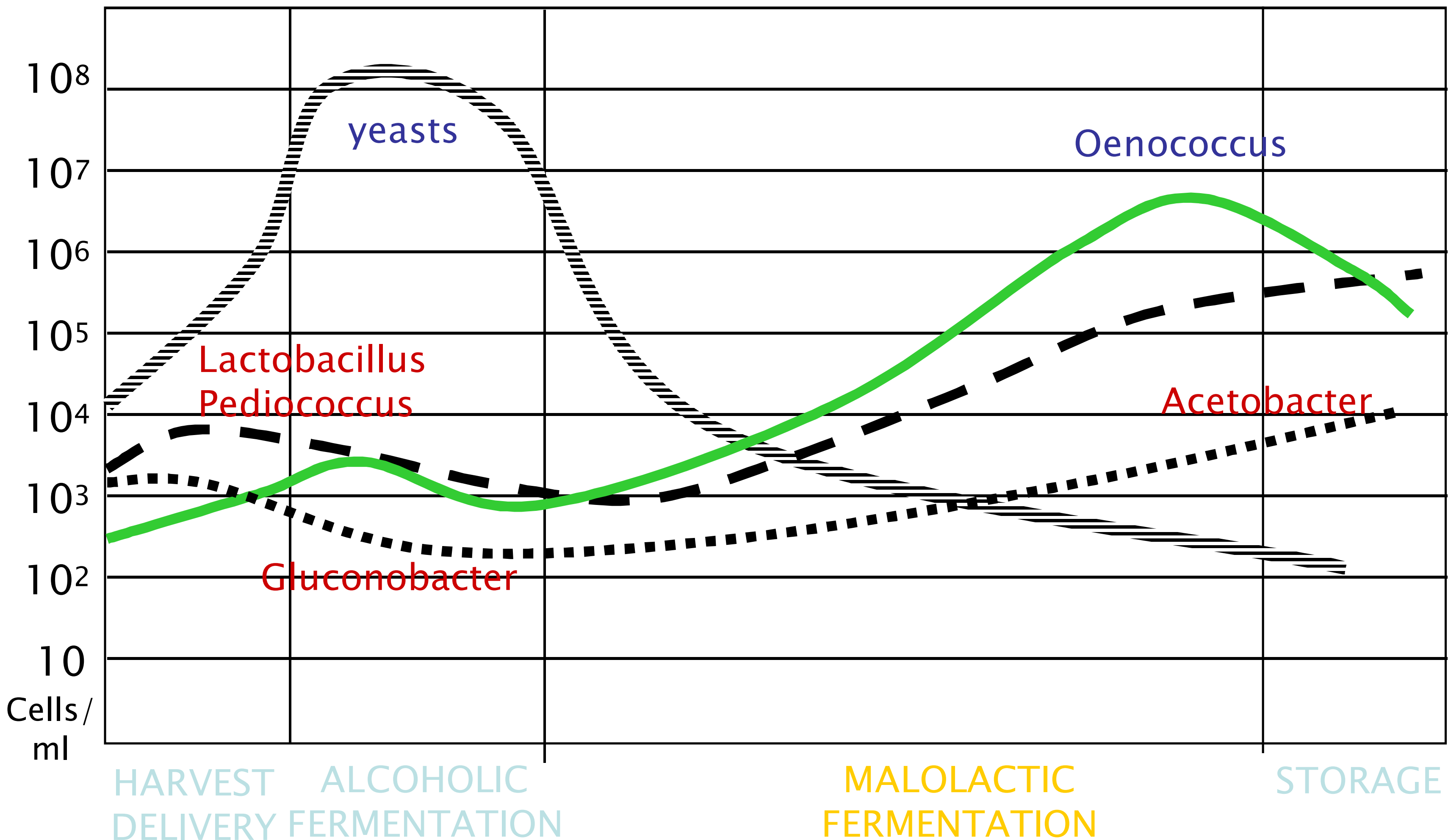
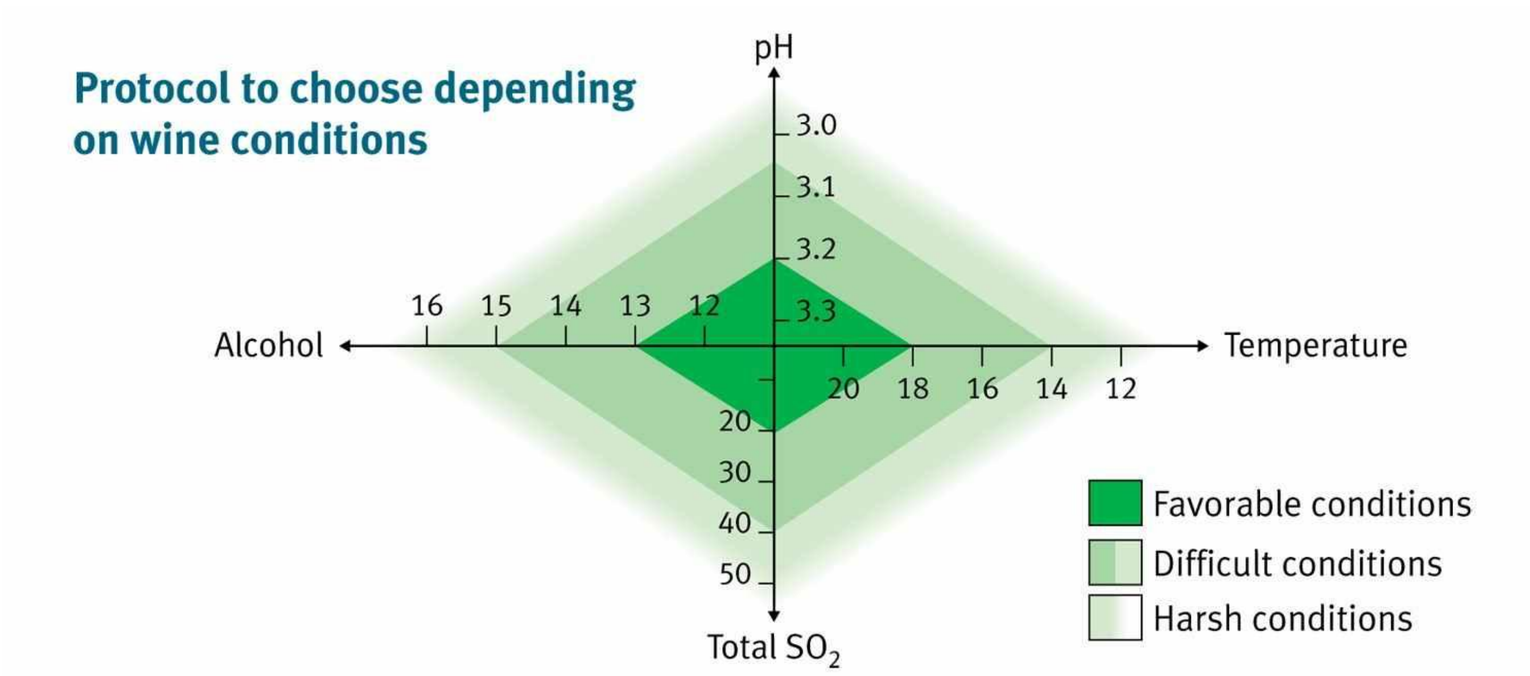
ACTERIA EVOLUTION
ER DIFFICULT CONDITIONS





ITERACTION OF PARAMETERS

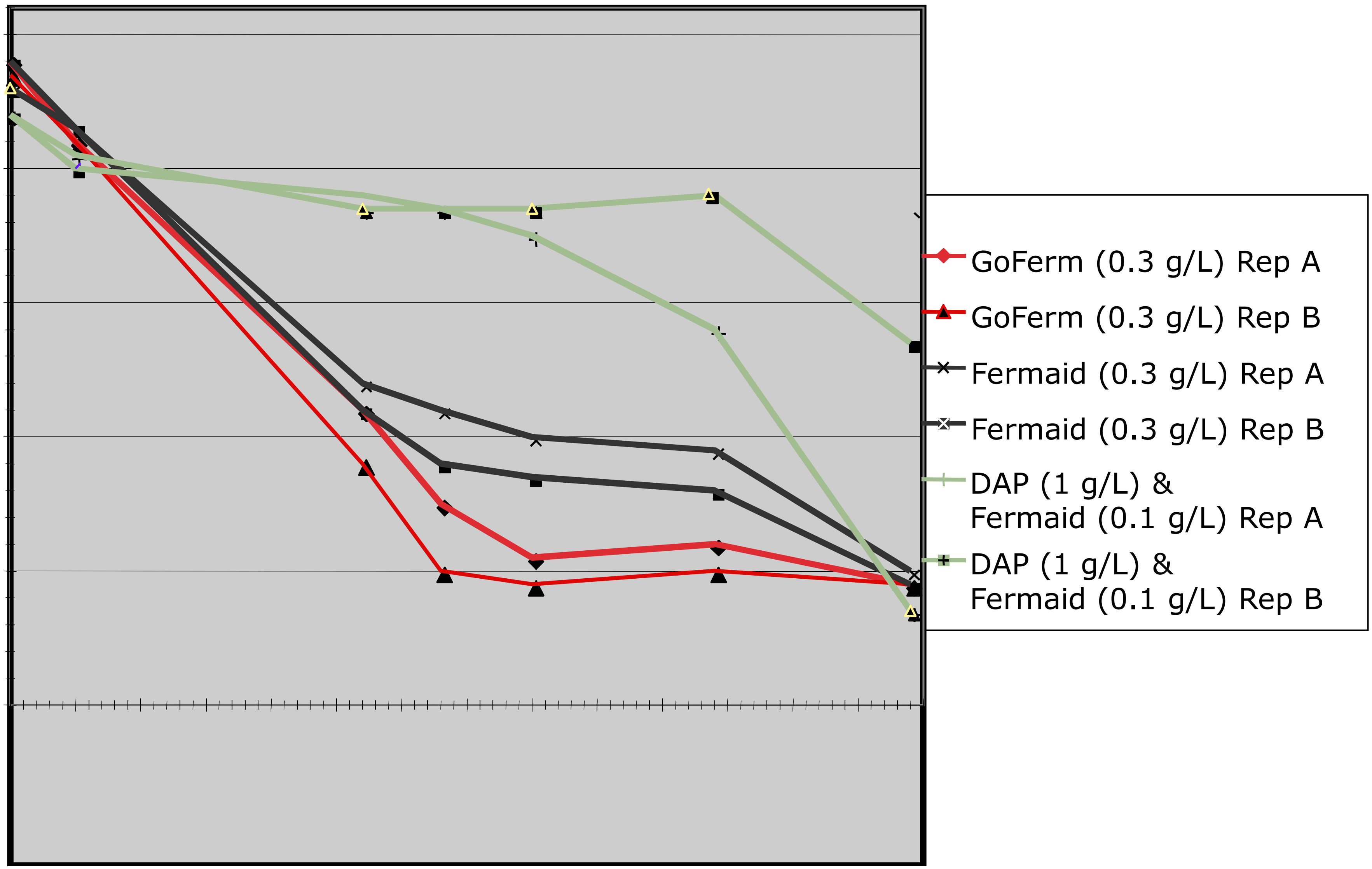


Conditions for a MLF

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 %

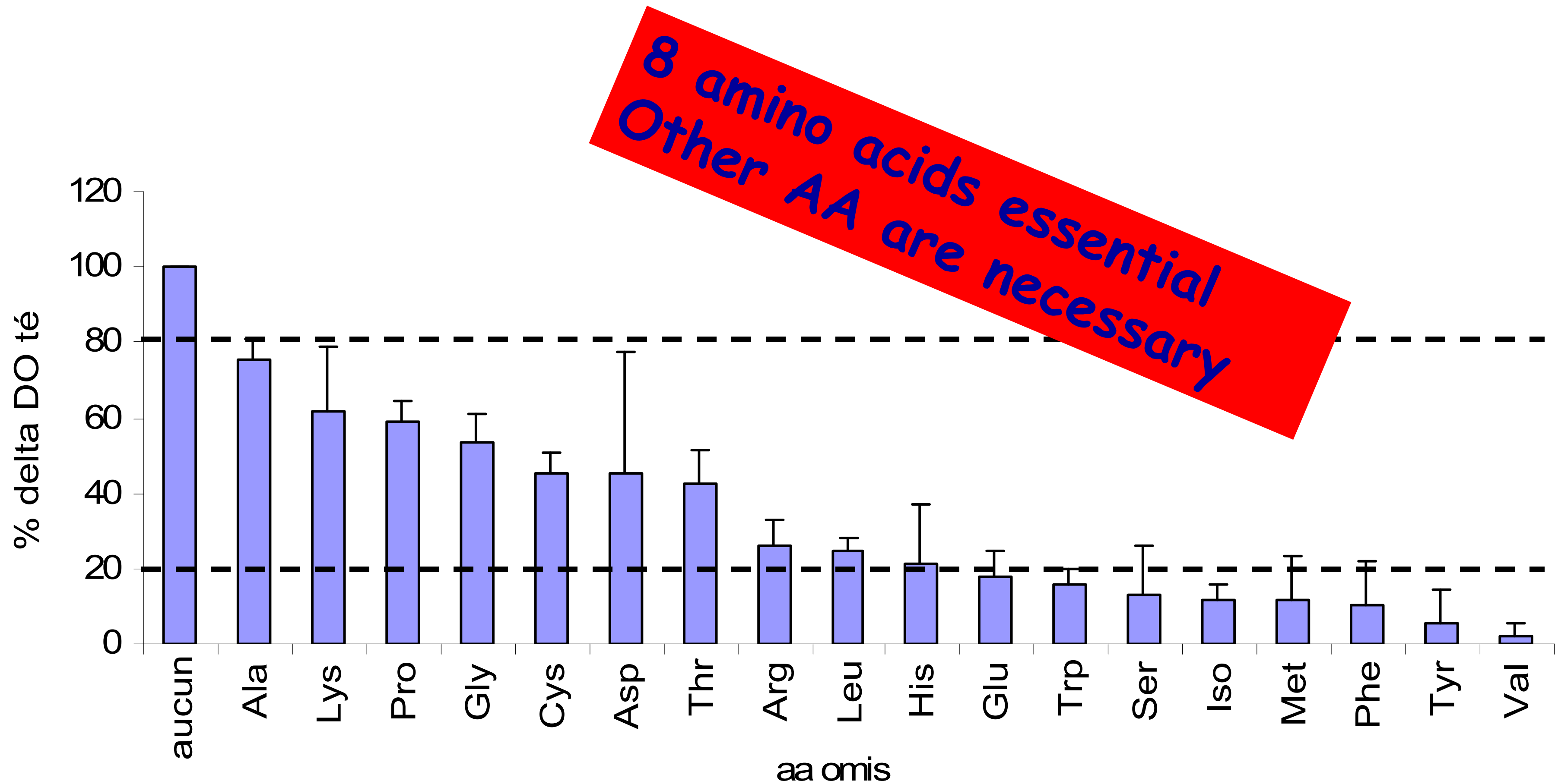
impact on MLF – 2006 Chardonnay (NY State)
CV D254 + ML bacteria strain: ALPHA

(Thomas Henick-Kling, Cornell University)



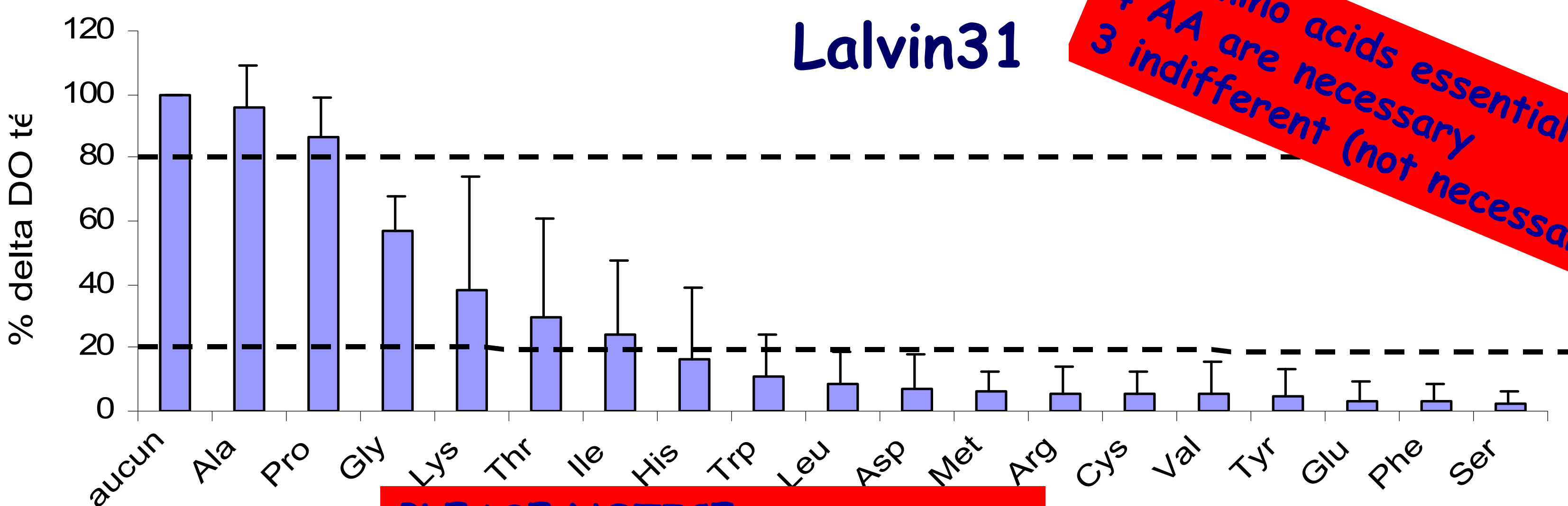
in *Oenococcus oeni* VP41 in a synthetic minimal medium with a cocktail of amino acids added.

(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.

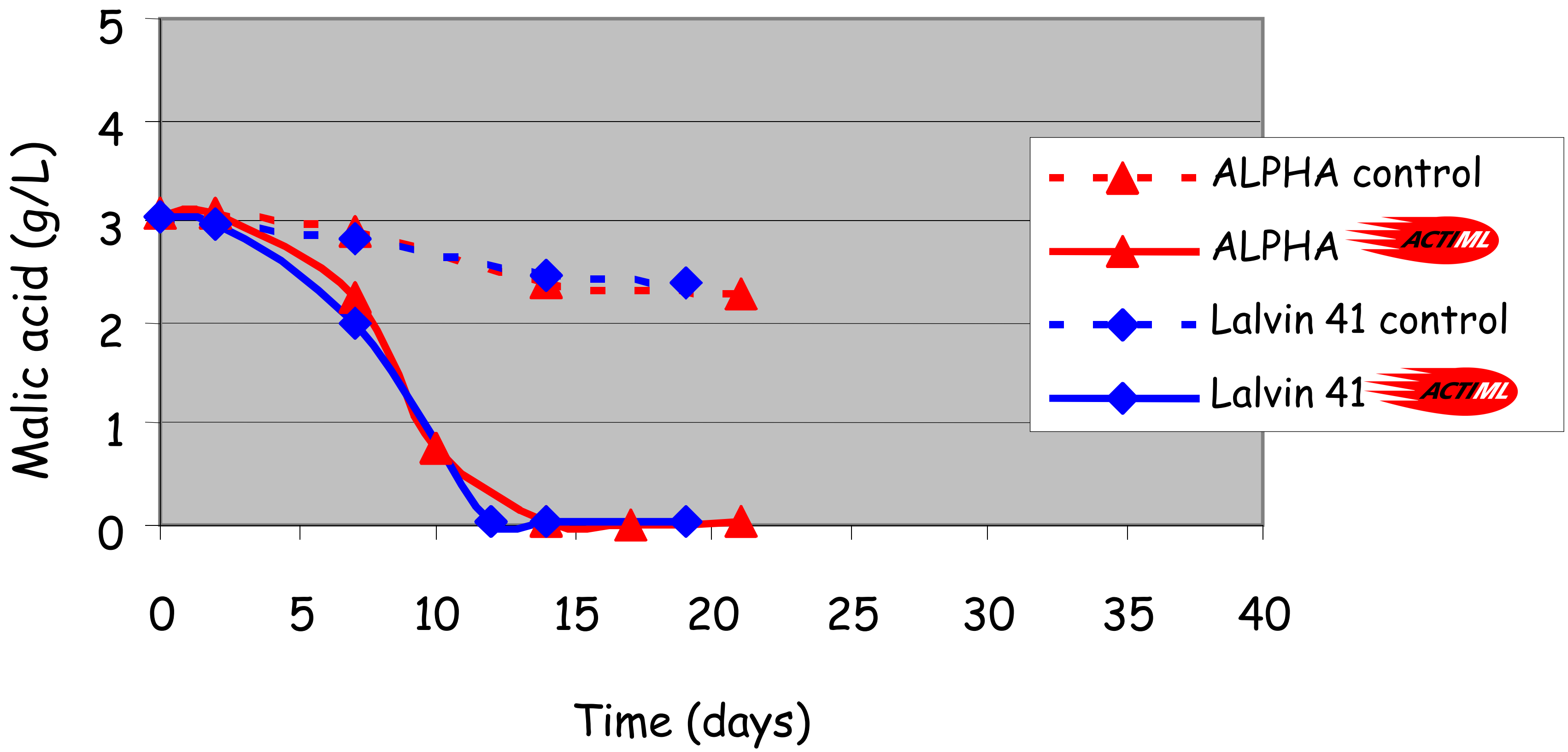
(values are expressed in percent growth of the OD 600 nm in presence of 18AAs)



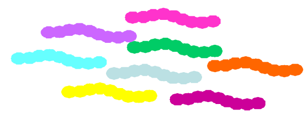
12 amino acids essential
4 AA are necessary
3 indifferent (not necessary)

PLEASE NOTICE
Lalvin 31 VERY DEMANDING
ADD ML NUTRIENTS!!!

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

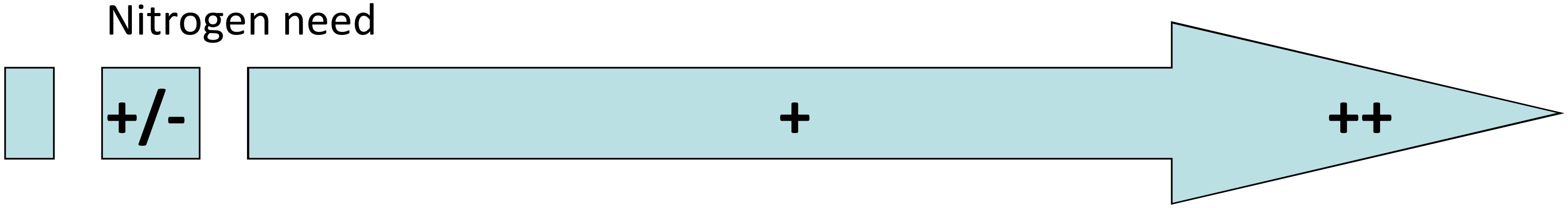


Nutrient Need



O. oeni strain

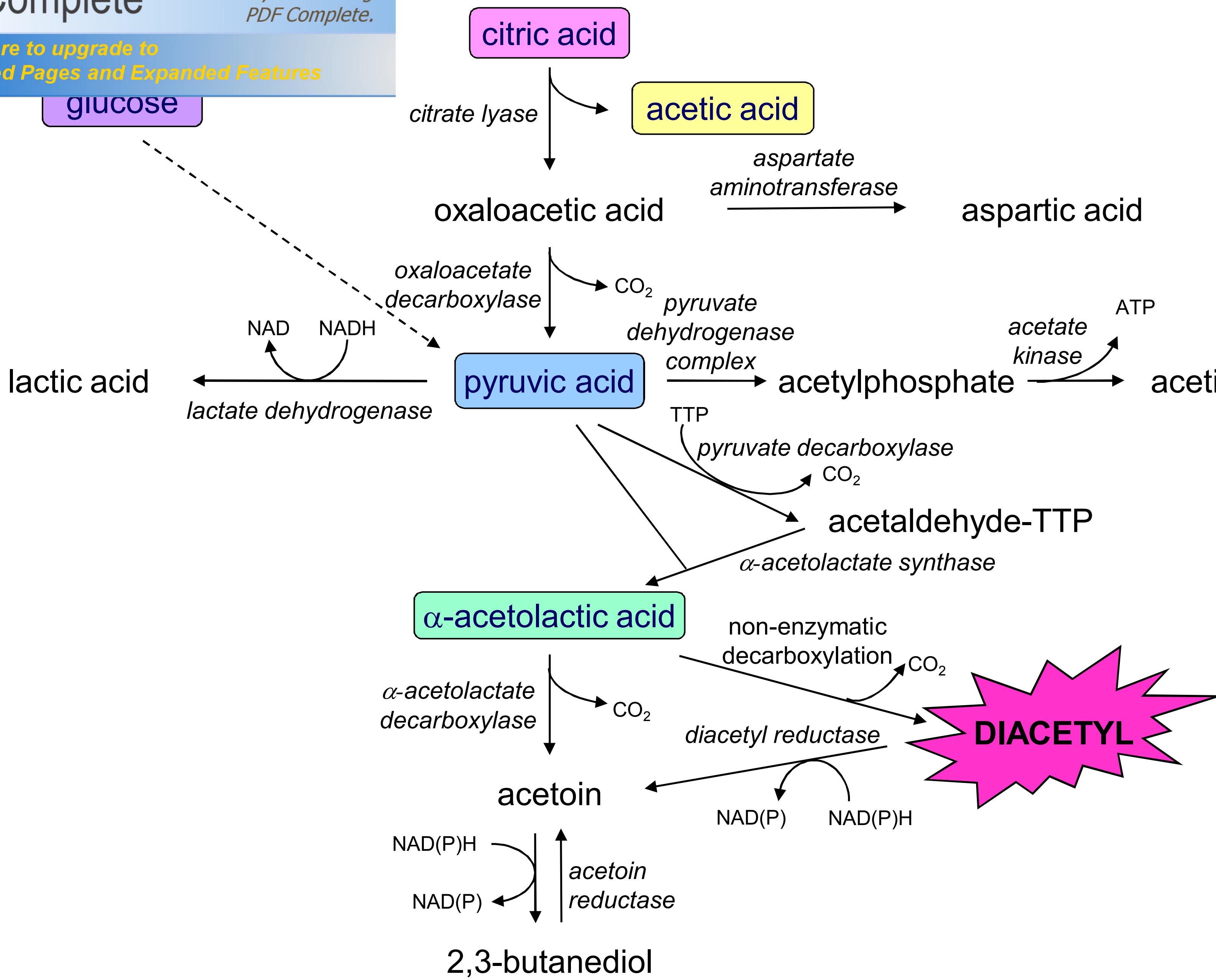
Nitrogen need



VP41	ALPHA	PN4	Elios 1	MT01	Lalvin31	Beta
Will benefit. Especially in difficult conditions, high alcohol, low pH or high SO_2	Will benefit. Especially in difficult conditions, high alcohol, low pH	Will benefit. Especially in difficult conditions, high alcohol, low pH	Will benefit. Especially in difficult conditions	Obligatory in the pied-de-cuve step, but also beneficial in the base wine	ALWAYS Especially in low pH conditions	ALWAYS Especially in sequential inoculation

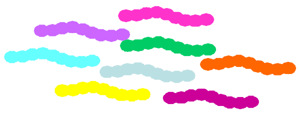
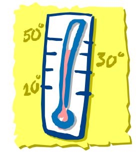
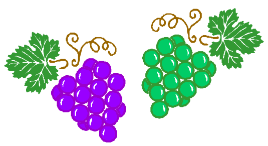
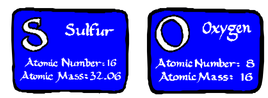
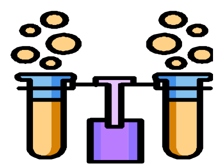
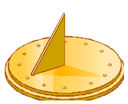
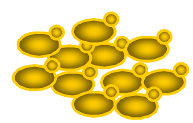
Table of compatibility with MLF

Level of compatibility	5	4	3	2	1	0
	++	+	+ -	-	--	No information
Yeast strains	QA23	2056	EC1118	CEG	M1	C1108
	ICV D254	DV10	PM	SIMI White	K1	C or R7
	71B	R2	ICV D21	2323	WAM	FC9
	AMH	M2	BDX	2226	Opale	QD145
	W15	W	BRL97	RHST		T73
		W27	SLO	T306		ALB
		6U	Cross evolution	2056		Syrah
		CY3079	BGY	RA17		M69
		W46	CSM	BM45		CGC62
		RC212	VQ15	BM4x4		CK
		ICV D80	228	BA11		GHM
		VN	ICVD47			MCS
		VRB				SVG
		ICV GRE				
		RHST				
		Rhone 4600				
		CM				
		CS2				
		299				
		NEM				
		PMA				
		BC				
		CEG				
		43				



Eveline Bartowski, AWRI, 2004

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

even key points

- 1 Choice of bacteria
- 2 Nutrition
- 3 Rehydration
- 4 Inoculation rate
- 5 Temperature
- 6 Oxygen
- 7 Micronutrient

THANK YOU!

For more information…

www.lallemandwine.us