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. »
Oenology Product Range

• Active Dry Wine Yeast Strains
  – ~150 *Saccharomyces* (>1000 in collection)
  – Brands include Lalvin, Enoferm, Uvaferm, VI-A-DRY
• Encapsulated 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
Yeast derivatives production – General steps

Yeast culture

- Yeast biomass
  - Inactivated yeast
  - Specific fractions
  - Autolysate (Centrifugation)
    - Yeast extract
    - Yeast hulls
Prevention
Alcoholic fermentation

Glucose → Biomass (≈ 2% glucose)
Glucose → CO₂
Glucose → Ethanol
Glucose → Glycerol
Glucose → Organic Acids
Glucose → Higher Alcohol Esters

92-93%

1° alcohol ↔ 16.8 g/l of glucose
 SECURE FERMENTS

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

- Good fermentation: slow or fast, but good finish
- Acceptable fermentation: slow but right to the end
- Worst case: fast at the beginning and sluggish / stuck at the end

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

Higher yeast inoculation rate lowers dilution of the initial yeast cells survival factors.

- Normal Fermentation Curve
- Population vs. Time
- Brix

- >100-150 million CFU/mL
- 4-8 million CFU/mL
- 2-4 million CFU/mL
Yeast **PROTECTION** is essential & Yeast **NUTRITION** is vital.
Effect on Fermentation Kinetics of GO-FERM® Micronutrient Addition During Yeast Rehydration

A. Julien, J. Sablayrolles - INRA Montpellier 2001

Uvaferm CEG inoculated at 25g/hl into MS 70 medium – CO₂ evolution at 24°C
Greater degree of slope indicates Stronger fermentation finish

Control – sluggish fermentation
30g/hl GO-FERM® added at rehydration
43° slope
19° slope
ADDITIONS IN REHYDRATION

- 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
REHYDRATATION

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...
ATP  ADP

$[\text{H}^+]$

$pH$ ext. = 3-4

$pH$ int. = 5-6

Alcoholic fermentation

Sterols and fatty acids

Enzymatic proteins

Transport protein

ATPase

H$^+$

Structural proteins

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Yeast cell wall 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)
64–128 million cfu/ml

Yeast exponential growth phase...

Plasma Membrane now is ~0.15% lipids (sterols & unsaturated fatty acids)
Plasma Membrane is now <0.2% lipids (sterols & unsaturated fatty acids) A critically low level!

Yeast cell wall composition:

- Plasma Membrane is now <0.2% lipids (sterols & unsaturated fatty acids)
  A critically low level!
To help avoid lipid depletion, add them during yeast rehydration.

Rehydration Without Protection

Rehydration with UFA & Sterol NATSTEP Protection
<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon</td>
<td>Structural element, energy source</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>Proteins and enzymes</td>
</tr>
<tr>
<td><strong>Oxygen</strong></td>
<td>Fatty acid and sterol production</td>
</tr>
<tr>
<td>Hydrogen</td>
<td>Transmembrane proton motive force</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Energy transduction, membrane structure and nucleic acids</td>
</tr>
<tr>
<td>Potassium</td>
<td>Ionic balance, enzyme activity</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Cell structure, enzyme activity</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Sulphydryl amino acids, vitamins</td>
</tr>
</tbody>
</table>
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)
**Micronutrients: Vitamins**

- **Pantothenate**: avoids $\text{H}_2\text{S}$ 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
Production of hydrogen sulphide by *S. cerevisiae* in a synthetic juice at different concentrations of Yeast Assimilable Nitrogen and Panthotenate (WSU, C. Edwards 2001)

**Pantothenic Acid Important?**

Avoid H$_2$S!

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

Why is Pantothenic Acid Important?

Avoid H$_2$S!
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

<table>
<thead>
<tr>
<th>Formol titration</th>
<th>NOPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple titration</td>
<td>Measures FAN (excluding proline)</td>
</tr>
<tr>
<td>Hazardous waste</td>
<td>Measure Ammonia separately (ISE Probe)</td>
</tr>
<tr>
<td>NH4 and FAN (including Proline)</td>
<td>No waste</td>
</tr>
<tr>
<td>Good estimation</td>
<td>Spectrometry</td>
</tr>
</tbody>
</table>
Factors 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)
Why is a fast and immediate" nitrogen assimilation problematic?

- **SLOW fermentation**: low nitrogen content
- **NH₄⁺ ADDICION**:
  - Outburst of the fermentation rate = heating
  - Biomass increase
  - High cell mortality at the end of the fermentation

![Graph showing CO₂ production vs. time](image)
The research to date...

→ impact of nitrogen source on the yeast esters production (several yeast strains tested):

→ 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).

<table>
<thead>
<tr>
<th></th>
<th>Inoculation</th>
<th>1/3 of AF</th>
<th>Total YAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAP (L)</td>
<td>12.5 g/hl</td>
<td>12.5 g/hl</td>
<td>50 mg/l</td>
</tr>
<tr>
<td>DAP (H)</td>
<td>25 g/hl</td>
<td>25 g/hl</td>
<td>100 mg/l</td>
</tr>
<tr>
<td>Fermaid O</td>
<td>40 g/hl</td>
<td>20 g/hl</td>
<td>24 mg/l</td>
</tr>
<tr>
<td>DAP/Fermaid O</td>
<td>15.2 g/hl</td>
<td>4.5 g/hl</td>
<td>50 mg/l</td>
</tr>
<tr>
<td>GFP/Fermaid O</td>
<td>300 mg/l</td>
<td>20 g/hl</td>
<td>24 mg/l</td>
</tr>
</tbody>
</table>
TSS (°Baume) vs. Fermentation time (days)

- QA23 control
- 100% inorganic
- 100% inorganic
- 68% inorganic & 32% organic
- 100% organic
- 100% organic
- 100% organic
- QA23 DAP (L)
- QA23 DAP (H)
- QA23 Dap/Ferm O
- QA23 Ferm O
- QA23 Go Ferm/Ferm O

The Australian Wine Research Institute

LALLEMAND
Impact 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.
Impact of N2 source on aromas

Pourcentage vs DAP (50mg/l YAN)

- DAP/Fermaid O @ 50mg/l YAN
- Fermaid O @ 24mg/l YAN

Ingredients:
- 2-methylpropyl acetate
- Ethyl butanoate
- 2-methylbutyl acetate
- 3-methylbutyl acetate
- Ethyl octanoate
- Hexyl acetate
- Phenylethyl acetate
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 $<150$ ppm (deficient)
  - Medium (150 – 250 ppm)
  - High ($>250$ ppm)

- Is there a relationship between low N and other essential nutrients?
Survey of available Nitrogen

<table>
<thead>
<tr>
<th></th>
<th>White</th>
<th>Red</th>
<th>Rose</th>
<th>Botrytized</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Samples</td>
<td>32</td>
<td>55</td>
<td>48</td>
<td>9</td>
</tr>
<tr>
<td>Min. value</td>
<td>36</td>
<td>46</td>
<td>42</td>
<td>22</td>
</tr>
<tr>
<td>Max. value</td>
<td>270</td>
<td>354</td>
<td>294</td>
<td>157</td>
</tr>
<tr>
<td>Mean</td>
<td>181.9</td>
<td>157</td>
<td>119</td>
<td>82.8</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>32</td>
<td>55</td>
<td>48</td>
<td>9</td>
</tr>
<tr>
<td>Deficient (%)</td>
<td>22</td>
<td>49</td>
<td>60</td>
<td>89</td>
</tr>
</tbody>
</table>

Riberereau-Gayon
INTEGRATED NUTRITIONAL STRATEGY FOR WINE YEAST

<table>
<thead>
<tr>
<th>JUICE YANC</th>
<th>rehydration</th>
<th>end of lag</th>
<th>1/3 AF</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIGH N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 225 mgN/l</td>
<td>Go-Ferm</td>
<td>-----</td>
<td>------</td>
</tr>
<tr>
<td></td>
<td>2.5lb/kgal</td>
<td></td>
<td>------</td>
</tr>
<tr>
<td>MEDIUM N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 125 mgN/l</td>
<td>Go-Ferm</td>
<td>-----</td>
<td>FERMAID K</td>
</tr>
<tr>
<td>&lt; 225 mgN/l</td>
<td>2.5lb/kgal</td>
<td></td>
<td>2lb./kgal</td>
</tr>
<tr>
<td>LOW N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 125 mgN/l</td>
<td>Go-Ferm</td>
<td>DAP</td>
<td>FERMAID K</td>
</tr>
<tr>
<td></td>
<td>2.5lb/kgal</td>
<td>or more</td>
<td>2lb/kgal</td>
</tr>
</tbody>
</table>
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% α-amino)

FERMAID provides ab. 30 mgN/l at 30 g/hl
(mix of α-amino and ammonia)

DAP provides ab. 60 mgN/l at 30 g/hl
(100% ammonia)
# Sugar-Nitrogen Relationship

<table>
<thead>
<tr>
<th>Brix</th>
<th>YAN</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>200</td>
</tr>
<tr>
<td>23</td>
<td>250</td>
</tr>
<tr>
<td>25</td>
<td>300</td>
</tr>
<tr>
<td>27</td>
<td>350</td>
</tr>
</tbody>
</table>

(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
I can’t resist...
Yeast nutrition impact on MLF – 2006 Chardonnay (NY State) 
CV D254 + ML bacteria strain: ALPHA
(Thomas Henick-Kling, Cornell University)
peak temperature **under the cap**
maximums relative to the initial osmotic shock (in warm or hot climate regions)

- 20 Brix • 35°C
- 21 Brix • 32°C
- 22 Brix • 30°C
- 23 Brix • 26°C
- 24 Brix or more • 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?
Where do inhibitory 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
Prevention-preparation of “stuck” wine

Avoid

Oxidation

Development of spoilage
Micro-organisms
(acetic acid and lactic acid bacteria)
1. Analyse the wine: pH, alcohol, residual sugars, VA, free and total SO₂

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

- May contain substances responsible for spoilage
- Carriers of undesired micro-organisms
- May contain substances which are toxic for the yeasts
3. Add $\text{SO}_2$ 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
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

Add Cellulose 50 g/hL
Yeast preparation

Protocol Based on

100 hL of “stuck” wine or must

With:

- 12 % alcohol
- 15 g/L of residual sugars
PROPER YEAST REHYDRATION FOR RESTARTING 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
Adjustment to the alcohol content

Add the 120 L to:
- 20 L stuck wine
- 30 L water
- 10 Kg sugar
- 25 g of FERMAID K

Add the 60 L to:
- 0 % alcohol
- 70-80 g/L sugars

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

DO NOT WAIT MORE THAN 8 hrs!
Yeast preparation

Add the 500 L to:
- Keep at 20-22°C for about 10-12 hrs
- Check for the occurrence of fermentation

Add the 120 L to:
- 200 L stuck wine
- 100 L water
- 20 Kg sugar
- 250 g of FERMAID K

Add the 10 hL to:
- 500 L of stuck wine

Keep at 20°C for about 12-24 hrs
- Check for the occurrence of fermentation

5% ALCOHOL
- 60-70 g/L sugars

8.5% ALCOHOL
- 15-25 g/L sugars

ATTENTION! Sometimes longer times are needed
Add the 10 hL to:

90 hL of stuck wine

Fermentation re-start

INOCULATION

TEMPEPERATURE
- 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?

!!!!
Key Interrelationships of Factors Affecting Fermentation

- Sugar Content
- Temperature
- Cell Numbers & Health
- Toxic Factors
- Competitive Factors
- Strain Selection
- Nutrients and Oxygen
- Maximum Fermentation Management
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...

\[
\begin{align*}
\text{HOOC} \cdot \text{CH}_2 \cdot \text{CHOH} \cdot \text{COOH} & \rightarrow \text{CO}_2 + \text{CH}_3 \cdot \text{CHOH} \cdot \text{COOH} \\
134 & \quad 44 \quad 90 \\
\text{malic acid} & \quad \text{carbon dioxide} \quad \text{lactic acid}
\end{align*}
\]
The more you know…

…the better!
wine

bacteria

phenols (gallic acid & anthocyanins)
growth & stimulation of mlf

phenols

growth & stimulation of mlf

mannoprotein

more efficient malic acid degradation

mannose

pentoses

polyols

volaile fatty acids

lipids

protein protease

peptides

bitterness ? flavour

glycoside (flavour)

beta-glucosidase

sugar + flavour-aglycon

increase in aroma

cell growth

colour reduction

sugar-anthocyanin

glycosidase (anthocyaninase)

sugar + anthocyanidin

adsorption by cells

cell growth

phenolic acids

p-coumaric acid

4-ethyl guaiacol

4-ethyl phenol

spicy, clove

sweaty, bandaid

copper ions

inhibitory to growth

so2-acetaldehyde

bruised apple

(green, vegetative)

acetate & ethanol & free so2

citrulline, urea

ethanol

ethyl lactate

mossey compounds

off-flavour

ethy carbamate

carcinogen

copper ions

pentoses

polyols

mouthfeel & body contribution

hexoses

embden-meyerhof-

parnas pathway (homofermentative)

pentose phosphate pathway

fructose

trehalose disaccharide

esters

synthesis & hydrolysis

ethylesters esterase

ethyl lactate, ethyl acetate, ethyl hexanoate, ethyl octanate

fruity aroma

lipids

lipase

volatile fatty acids

oak products

furfural

biogenic amine production

histamine & tyramine

copper ions

inhibitory to growth

eveline bartowski, awri, 2004
BACTERIA EVOLUTION
FAVOURABLE CONDITIONS

Cells/ml

yeasts
Lactobacillus

Gluconobacter

Oenococcus

Pediococcus

Acetobacter

HARVEST DELIVERY
ALCOHOLIC FERMENTATION
MALOLACTIC FERMENTATION
STORAGE
NO INFLUENCE OF O. oeni ON AF

(King and Beelmann 1986)
What are the risks of not inoculating?

• Depends on the pH
• High levels of biogenic amines
• High V.A.
• Undesirable aromas and flavors
BACTERIA EVOLUTION UNDER DIFFICULT CONDITIONS

- Yeasts
- Oenococcus
- Lactobacillus
- Pediococcus
- Gluconobacter
- Acetobacter
INTERACTION OF PARAMETERS

Protocol to choose depending on wine conditions

Favorable conditions
Difficult conditions
Harsh conditions
## Conditions for a MLF

<table>
<thead>
<tr>
<th>FAVOURABLE</th>
<th>DIFFICULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 3,3-3,5</td>
<td>pH &lt; 3,2</td>
</tr>
<tr>
<td>$\text{SO}_2$ total &lt; 30 mg/l</td>
<td>$\text{SO}_2$ total &gt; 50 mg/l</td>
</tr>
<tr>
<td>$\text{SO}_2$ free &lt; 5 mg/l</td>
<td>$\text{SO}_2$ free &gt; 10 mg/l</td>
</tr>
<tr>
<td>Temperature &gt; 18°C</td>
<td>Temperature &lt; 15°C</td>
</tr>
<tr>
<td>Alcohol &lt; 12%</td>
<td>Alcohol &gt; 13,5%</td>
</tr>
</tbody>
</table>
Survival and growth of a complex *Oenococcus oeni* population after MLF at different pH and residual sugar levels.
Evolution of acetic acid in a Pinot Noir in dependence of pH and residual sugar levels

Evolution of acetic acid in a Pinot Noir after MLF:
- influence of pH and residual sugar levels
Growth of strain 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)
Growth of strain 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!!!
Cabernet Franc 2003 second inoculation (14% vol, T-SO2 43 ppm, pH 3.58)
Malic acid degradation in presence
Diacetyl - management during winemaking

- **Diacetyl conc**:
  - **O. oeni strain**: variable
  - **wine type**: white - lower, red - higher
  - **inoculation rate**: $10^4$ - higher, $10^6$ - lower
  - **fermentation time**: longer MLF - higher
  - **temperature**: 18°C - higher, 25°C - lower
  - **SO₂**: binds to diacetyl - sensorially inactive
  - **aeration**: air - higher, anaerobic - lower
  - **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