Malolactic fermentation seminar
Webinar
with the Northern Grapes project

January 8, 2012

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Lallemand & Scott Labs
Overview

• Intro to MLF & MLF without borders
• Biogenic amines
• pH and SO$_2$
• Timing of MLF (co-inoculation)
• Nutrition
• Sensory contribution

• (up my sleeve: Brett management, phenolic compound in grapes and ML)
Role of microbes in winemaking

• Yeast
  ◦ Conventional
    • *Saccharomyces* spp.
      • Primary function
      • Secondary function
  ◦ Non-Conventional
    • Native (un-inoculated)
    • *Torulaspora delbrueckii*, *Kluyveromyces marxianus*

• Bacteria
  ○ *O. oeni* only if you are planning on a MLF
  ○ *Lactobacillus plantarum*?

• Mold
  ○ Botrytis, only if making a Botrytis style wine
Microflora in must and wine

- Brettanomyces
- Lactobacillus
- Pediococcus
- Acetobacter and Oenococcus
- Saccharomyces
2 small comments to set the stage

• ML is not a separate entity

• ML is influenced by winery AND vineyard practices!
Yeast nutrition impact on MLF – 2006 Chardonnay (NY State)
Yeast: Lalvin ICV D254 - MBR® starter culture
(Thomas Henick-Kling, Cornell University)
Pesticide Residue Inhibition of Malolactic Bacteria

Plate Diffusion Test

Acid MRS-Agar (yellow: 10ppm, green: 100ppm, rosé: 1000ppm).

Fig. 1 cymoxanil + dithianon
Fig. 2 Cyprodinil
Fig. 3 fludioxonil
Pesticide Residue Inhibition of Malolactic Bacteria..

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Active Ingredient</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKTUAN</td>
<td>cymoxanil + dithianon</td>
<td>High</td>
</tr>
<tr>
<td>SCALA</td>
<td>pyrimethanil</td>
<td>Low</td>
</tr>
<tr>
<td>SWITCH</td>
<td>Cyprodinil or fludioxonil</td>
<td>Moderate</td>
</tr>
<tr>
<td>FRUCPICA</td>
<td>mepanipyrim</td>
<td>None</td>
</tr>
</tbody>
</table>
Factors affecting Fermentation Management - Key Interrelationships

- JUICE or MUST
- TEMPERATURE
- BACTERIA STRAIN
- CELL NUMBERS & HEALTH
- NUTRITIONAL FACTORS
- TOXIC FACTORS
- COMPETITIVE FACTORS

MAXIMUM FERMENTATION MANAGEMENT
The joy of malolactic fermentation!
THE CHEMISTRY

• L (-) malic acid converted into L(+) lactic acid (commercial additions of D(+) will remain untouched).
• Not really a ‘fermentation’ as no energy is produced
• Reduction of acidity by 1-3 g/L
• Production of diacetyl: good or bad?
Metabolism in heterofermentative Lactic Acid Bacteria

- **CITRATE**: 100-700 ppm
- **GLUCOSE**
- **FRUCTOSE**: 300-1000 ppm
- **MALATE**: 1000-4000 ppm
- **Acetate-P**: 100-200 ppm
- **Pyruvate**: ATP
- **Acetaldehyde-TPP**: Acetyl-CoA
- **L-Lactate**: 670-2680 ppm
- **D-Lactate**: 100-200 ppm
- **Diacetyl**: 2-8 ppm
- **Acetoin**: 2-8 ppm
- **pH, temp**: Fatty acids, LIPIDS
The more you know...

...the better!
Microbial metabolism
flavor-active compounds

From Swiegers, Bartowsky, Henschke & Pretorius, 2005
Eveline Bartowski, AWRI, 2009
Metabolism of sugars and organic acids during MLF

CELL GROWTH
- sugar catabolism
- no malate catabolism
- no citrate catabolism
- slight acetate production
- slight lactate production

STATIONARY PHASE I
- no sugar catabolism
- malate catabolism
- no citrate catabolism
- no acetate production
- lactate production

STATIONARY PHASE II
- no sugar catabolism
- no malate catabolism
- citrate catabolism
- acetate production
- no lactate production
A sugar metabolism could only be detected after consumption of the organic acids (malic and citric)!
Potential risk of spontaneous MLF

- pH influence

Very difficult
Possibly no MLF

Spontaneous MLF can be difficult

Increasing risk of MLF induced by *Lactobacillus* & *Pediococcus* sp.
Organoleptic deviations possible

Potential risk of sugar degradation by *O. oeni* at high pH
Degradation of Biogenic Amines

Either by Monoamine Oxidases (Monoamines) or Diamine Oxidases (Diamines)

Inhibited by Alcohol

Have Vitamin B6 as Cofactor
Selected strains do not produce biogenic amines

Spontaneous bacteria???
= It all depends on which strain took over to do the malolactic conversion??
Survival and growth of a complex O. oeni population after MLF at different pH and residual glucose levels.

- **pH 3.3**
- **pH 3.5**
- **pH 3.7**

**Viable cell count (cfu/ml)**
- **End MLF**
- **MLF + 1 month**
- **MLF + 2 month**

**Residual sugar**:
- **ca. 1 g/l**
- **ca. 3 g/l**
Evolution of acetic acid in a Pinot Noir after MLF:
- influence of pH and residual sugar levels
### Scorecard for determining the ease of malolactic fermentation

<table>
<thead>
<tr>
<th></th>
<th>My wine</th>
<th>Evaluation</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol (% vol.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Free SO₂ (mg/L)</td>
<td>&lt; 8</td>
<td>8 - 12</td>
<td></td>
</tr>
<tr>
<td>Total SO₂ (mg/L)</td>
<td>&lt; 30</td>
<td>30 - 40</td>
<td></td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>18 - 22</td>
<td>14 - 18 or 22 - 24</td>
<td></td>
</tr>
<tr>
<td>Yeast’s nutritional needs</td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>Ease of Alcoholic Fermentation</td>
<td>No problems</td>
<td>Transient yeast stress</td>
<td>Sluggish / stuck AF</td>
</tr>
<tr>
<td>Initial level of malic acid (g/L)</td>
<td>2 - 4</td>
<td>4 - 5</td>
<td>5 - 7 or 1 - 2</td>
</tr>
<tr>
<td>Maximum AF rate (maximum loss of brix/day)</td>
<td>&lt; 2</td>
<td>2 - 4</td>
<td>4 - 6</td>
</tr>
</tbody>
</table>

**NOTE:** Other, currently less well-known factors that are not considered in this scorecard may include the level of dissolved oxygen, polyphenolic content, free SO₂ compaction, pesticide residues, etc.

**Total score for the ease of malolactic fermentation:**

- **< 13 points**: Favorable
- **13 - 22 points**: Not so favorable
- **23 - 40 points**: Difficult
- **> 40 points**: Extreme
Other, currently less understood factors that may impact Malolactic Fermentation and are not considered in this scorecard may include the level of dissolved oxygen, polyphenolic content, lees compacting, pesticide residues, etc.
Effective SO₂ and pH

Molecular SO₂ (%) = \( \frac{100}{10^{\text{pH} - 1,81} + 1} \)

<table>
<thead>
<tr>
<th>pH</th>
<th>Free SO₂ mg/l</th>
<th>% molecular SO₂</th>
<th>Total molecular SO₂ mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>25</td>
<td>6.06 %</td>
<td>1.54</td>
</tr>
<tr>
<td>3</td>
<td>8.2</td>
<td>6.06 %</td>
<td>0.5</td>
</tr>
<tr>
<td>3.6</td>
<td>25</td>
<td>1.6 %</td>
<td>0.4</td>
</tr>
<tr>
<td>3.6</td>
<td>31</td>
<td>1.6 %</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>0.64 %</td>
<td>0.16</td>
</tr>
<tr>
<td>4</td>
<td>78</td>
<td>0.64 %</td>
<td>0.5</td>
</tr>
</tbody>
</table>

At pH = 3, mol. SO₂ = 6.06 % ⇒ 8.2 mg/l of free SO₂ is sufficient
At pH = 4; mol. SO₂ = 0.64 %, ⇒ 78 mg/l of free SO₂ is sufficient
Playing with the timing of MLF

Co-fermentation/ Co-inoculation vs. Sequential

Yeast & ML
Pros and Cons of Early Inoculation

PROS
• Time Savings
• No Alcohol inhibition
• Temperature from AF
• Flavor Implications

CONS
• Watch for SO2 (free&bound)
• Yeast Compatibility
• VA Production from Metabolism of Sugars
  – (not with V22, homofermentative strain)
What does the RESEARCH show?

(King and Beelmann 1986)

NO INFLUENCE OF *O. Oeni* on AF
Tosi, E\textsuperscript{1}. & G. Zapparoli \textsuperscript{2}  
Dipartimento di Biotecnologie, Università degli Studi di Verona, Verona, Italy  
*The Australian & New Zealand Grapegrower & Winemaker*  
(February 2007, pp 71-77)
• As we know, aldehyde is especially responsible for green apple and vegetative sensations.

• In co-inoculation we have a very early consumption of acetaldehyde,
  – peak levels of acetaldehyde are significantly lower compared to a sequential inoculation
  – Same concept is valuable for other aldehydes and ketones.

• This explanation is based on results of our research collaboration with Ramón Mira de Orduna.
What about HIGH PH REDS (high risk)?
# Experimental set-up - 2005

## Cabernet Sauvignon (RSA) (initial pH 3.77)

<table>
<thead>
<tr>
<th>AF</th>
<th>MLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lalvin ICV D254</td>
<td>MBR Alpha Lalvin VP41</td>
</tr>
<tr>
<td>Lalvin Rhone 2056</td>
<td>MBR Alpha Lalvin VP41</td>
</tr>
</tbody>
</table>

**Moment of inoculation:**

- **A:** co-inoculation yeast and bacteria
- **B:** Inoculation of bacteria before the end of AF residual sugars < 30 g/L
- **C:** Inoculation after AF

**Control:** Spontaneous MLF

All trials in duplicate

**Analysis:** FT-IR - malic acid degradation

HPLC - determination of biogenic amines
Speed of MLF

- With coinoculation, MLF much faster
MALOLACTIC FERMENTATION
DIFFERENT TIMING OF INOCULATION vs SPONTANEOUS MLF
MONTEPULCIANO D’ABRUZZO-VINTAGE 2006 C.Di Meo

- MLF MUCH FASTER
- NO SIGNIFICANT INCREASE IN VOLATILE ACIDITY!
Improved nutrient formulations for *Oenococcus oeni*
Bacteria Nutrition

• How do you know what you need?

• Difficult as no “NOPA” for bacteria.....

• Focus on positives and make life as comfortable as possible!
Nutrient requirements for *O. oeni*

**Nitrogen**
- Only in organic form (a-amino acids, peptides)
- NO AMMONIUM SALTS

**Vitamins**
- (nicotinic acid, biotin, thiamine, pantotenic acid, etc.)

**Trace elements**
- (Mg, Mn & K)

**IMPORTANT**
- Important for the function of the bacteria
- Absence of inhibitory compounds: SO₂, pesticides, medium chain fatty acids etc.
WINE
SITUATIONS OF NUTRIENT DEFICIENCY

- Nitrogen limitation in the must + a short yeast autolysis = limited release of amino acids by yeast... for the nutrition of the bacteria
- Post-fermentation procedures (clarification, filtration, etc.)
- The yeast strain used for alcoholic fermentation

All these factors influence how “malolactic friendly” the wine is after alcoholic fermentation
# Table of compatibility with MLF

<table>
<thead>
<tr>
<th>Level of compatibility</th>
<th>5++</th>
<th>5+</th>
<th>4</th>
<th>3+</th>
<th>3</th>
<th>2+</th>
<th>2</th>
<th>1</th>
<th>No information</th>
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<tr>
<td>Yeast strains</td>
<td>QA23</td>
<td>2056</td>
<td>EC1118</td>
<td>SIMI White</td>
<td>V1116 (K1)</td>
<td>C or R7</td>
<td>ALB</td>
<td>ALB</td>
<td>C1108</td>
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<tr>
<td>AMH ICV D254 DV10 R2 ICV D21 2323 WAM FC9</td>
<td>2323</td>
<td>2226</td>
<td>Opale</td>
<td>Enoferm M1</td>
<td>T73</td>
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<tr>
<td>W15 W 4U BRL97 RHST</td>
<td>2056</td>
<td>2056</td>
<td>Syrah</td>
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<td>VRB QD145 SLO</td>
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<tr>
<td>CSM Cross evolution</td>
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<td>Syrah</td>
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<td>GHM MCS BM45 CGC62</td>
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<td>RC212 RQ15 BM4x4 CK</td>
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<td>ICV D80 228 BA11</td>
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<td>VN ICVD47</td>
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<td>43</td>
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<td>T73</td>
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</tr>
</tbody>
</table>
Number of essential nutrients for four wine LAB determined with the single omission technique. Each strain was grown in a simplified version of the chemically defined medium.
NUTRITIONAL NEEDS

Growth yield Oenococcus oeni strain Lalvin 31 on a synthetic medium lacking the indicated amino acid (Data are expressed as the percentage of OD 600 nm)

<table>
<thead>
<tr>
<th>Amino acid eliminated</th>
<th>% delta DO control</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td></td>
</tr>
<tr>
<td>Pro</td>
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</tr>
<tr>
<td>Gly</td>
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<tr>
<td>Lys</td>
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<tr>
<td>Thr</td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td></td>
</tr>
<tr>
<td>His</td>
<td></td>
</tr>
<tr>
<td>Trp</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td></td>
</tr>
<tr>
<td>Asp</td>
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</tr>
<tr>
<td>Met</td>
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<td>Arg</td>
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<td>Cys</td>
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<tr>
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<td>Tyr</td>
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<td>Phe</td>
<td></td>
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<tr>
<td>Ser</td>
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</tr>
</tbody>
</table>
NUTRITIONAL NEEDS

Growth yield Oenococcus oeni strain ALPHA on a synthetic medium lacking the indicated amino acid (Data are expressed as the percentage of OD 600 nm)
NUTRITIONAL NEEDS:

Kinetics of malic acid degradation in a 2003 Cabernet Sauvignon (alcohol 13%vol, T-SO₂ 35 ppm, pH 3.68) after direct inoculation with ML starter cultures with (+) and without (-) addition of ML nutrient Opti'Malo Plus.
<table>
<thead>
<tr>
<th><strong>Comparisons</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rehydration Nutrients</strong></td>
</tr>
<tr>
<td>• Acti-ML</td>
</tr>
<tr>
<td>– Special inactivated yeast</td>
</tr>
<tr>
<td>• Amino Acids</td>
</tr>
<tr>
<td>– Peptides</td>
</tr>
<tr>
<td>– Vitamins</td>
</tr>
<tr>
<td>– Growth factors</td>
</tr>
<tr>
<td>– Cellulose</td>
</tr>
</tbody>
</table>
Nutrient Need

*O. oeni* strain

<table>
<thead>
<tr>
<th>VP41</th>
<th>ALPHA</th>
<th>PN4</th>
<th>Elios 1</th>
<th>Inobacter</th>
<th>Lalvin31</th>
<th>Beta</th>
</tr>
</thead>
<tbody>
<tr>
<td>Will benefit.</td>
<td>Will benefit.</td>
<td>Will benefit.</td>
<td>Will benefit.</td>
<td>Obligatory in the pied-de-cuve step, but also beneficial in the base wine</td>
<td>ALWAYS</td>
<td>ALWAYS especially in low pH conditions</td>
</tr>
<tr>
<td>Especially in difficult conditions, high alcohol, low pH</td>
<td>Especially in difficult conditions, high alcohol, low pH</td>
<td>Especially in difficult conditions</td>
<td>Especially in difficult conditions</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Nitrogen need:  
- **++**
- **+**
- **+/-**
Bacteria sensory contribution to the MLF
pH & aroma in wine

Limestone Coast

Clare Valley

AF ~ 550 kg vinimatic Lalvin Rhone 2056
18-20°C

pH 3.3

MLB 1  MLB 2  MLB 3  Non-MLF Control

MLF duration: 20 days

Post-MLF pH adjustment → pH 3.5

15L, triplicate, 20°C

pH 3.7

MLB 1  MLB 2  MLB 3  Non-MLF Control

MLF duration: 12 days

Post-MLF pH adjustment → pH 3.5

Peter Costello

Eveline Bartowski, AWRI, 2009
Wine pH affects *O. oeni* metabolism

Limestone Coast

**pH 3.3**
- No MLF
- MLF (3)

**pH 3.7**
- No MLF
- MLF (3)

Eveline Bartowski, AWRI, 2009
Malolactic fermentation

- Reduction in wine acidity

More than just pH change

Eveline Bartowsky, AWRI, Neustadt 2010
In function of the wine type and the wine style
1 - 4 mg/L = impacts on complexity
> 5 - 7 mg/L = undesired buttery sensations

Rankine et al., 1969

Perception threshold

- Chardonnay 0.2 mg/L
- Pinot Noir 0.9 mg/L
- Cabernet Sauvignon 2.8 mg/L

Martineau & Henick-Kling, 1995

(threshold > red wines)

from Bartowsky & Henschke, 2004
Diacetyl - management during winemaking

O. oeni strain

Diacetyl concentration

Exp. Strain
C22L9

VP41
Only attacks citric acid after completion of malic acid.

Elios 1
Medium producer

Alpha
Medium producer

Lalvin 31
Medium to low producer

Beta
In sequential inoculation: high producer

PN4
Early attack of citric acid (mid MLF)
Diacetyl (mg/L) from Bartowsky & Henschke, 1999

Days after induction of MLF

Viable cells (log10 CFU/mL)

Malic & citric acid (g/L)

FORMATION DURING MLF

Cabernet Sauvignon, 2000
Barossa & Eden Valley blend
Strain III
(12.5 % alcohol)

from Bartowsky & Henschke, 1999
Diacetyl - management during winemaking

- **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
- **Sulfur (SO₂)**: binds to diacetyl - sensorially inactive
- **Aeration**: air - higher, anaerobic - lower
- **Contact with yeast lees**: long contact - lower
- **pH**: lower pH may favour

Eveline Bartowsky, AWRI, Trier 2008
Buttery aroma - Diacetyl

1 - 4 mg/L = enhance flavour complexity
> 5 - 7 mg/L = undesirable buttery aroma

Eveline Bartowsky, AWRI, 2004
In conclusion, diacetyl & MLF regime ...

- Buttery character
  - Diacetyl
  - Easily manipulated by the winemaker
    - *O. oeni* strain
    - Length of MLF
    - Temperature
    - Yeast (& bacterial) lees contact
- Co-inoculation of MLF with AF
  - Can reduce the time of malic acid metabolism
  - Can be used to modulate the aroma and flavour of wine
  - Length of MLF can influence the chemical composition of wine

MLF inoculation regime can be used as a means to influence the wine style.

Eveline Bartowski, AWRI, Neustadt 2010
In conclusion, fruity character ...

• Changes in ester concentration is dependent upon several factors
  – *O. oeni* strain
  – Wine composition
  – MLF conditions
  – Viticultural region
  – Vintage

• Increase in total fruit berry concentration translates to an increase in berry related sensory descriptor terms

• MLF can be used to enhance the fruity berry characteristics of Cabernet Sauvignon

• ML strains behave similarly in other red varieties regarding fruity berry characters

Eveline Bartowski, AWRI, Neustadt 2010
THANK YOU

For your attention!

www.lallemandwine.com
www.scottlab.com

and there is a (free) APP for that!
Effective $\text{SO}_2$ and $\text{pH}$

Molecular $\text{SO}_2$ (%) = \( \frac{100}{10^{\text{pH}-1.81} + 1} \)

<table>
<thead>
<tr>
<th>pH</th>
<th>Free $\text{SO}_2$ mg/l</th>
<th>% molecular $\text{SO}_2$ mg/l</th>
<th>Total molecular $\text{SO}_2$ mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>25</td>
<td>6,06 %</td>
<td>1,54</td>
</tr>
<tr>
<td>3,6</td>
<td>25</td>
<td>1,6 %</td>
<td>0,4</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>0,64 %</td>
<td>0,16</td>
</tr>
</tbody>
</table>
**Effective SO$_2$ and pH**

Molecular SO$_2$ (%) = 100 / [10$^{pH-1.81}$ + 1]

<table>
<thead>
<tr>
<th>pH</th>
<th>Free SO$_2$ mg/l</th>
<th>% molecular SO$_2$ mg/l</th>
<th>Total molecular SO$_2$ mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>8,2</td>
<td>6,06 %</td>
<td>0,5</td>
</tr>
<tr>
<td>3,6</td>
<td>31</td>
<td>1,6 %</td>
<td>0,5</td>
</tr>
<tr>
<td>4</td>
<td>78</td>
<td>0,64 %</td>
<td>0,5</td>
</tr>
</tbody>
</table>

At pH = 3, mol. SO$_2$ = 6,06 % $\Rightarrow$ 8,2 mg/l of free SO$_2$ will inhibit MLF
At pH = 4; mol. SO$_2$ = 0,64 %, $\Rightarrow$ 78 mg/l of free SO$_2$ will inhibit MLF
Environmental Factors that Impact Malolactic Fermentation

<table>
<thead>
<tr>
<th></th>
<th>Good</th>
<th>Moderate</th>
<th>Difficult</th>
<th>Extreme</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcohol (%)</strong></td>
<td>&lt;13</td>
<td>13 - 15</td>
<td>15 - 17</td>
<td>&gt;17</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>&gt;3.4</td>
<td>3.1 - 3.4</td>
<td>2.9 - 3.1</td>
<td>&lt;2.9</td>
</tr>
<tr>
<td><strong>Free SO₂ (mg/L)</strong></td>
<td>&lt;8</td>
<td>8 - 12</td>
<td>12 - 15</td>
<td>&gt;15</td>
</tr>
<tr>
<td><strong>Total SO₂ (mg/L)</strong></td>
<td>&lt;30</td>
<td>30 - 40</td>
<td>40 - 60</td>
<td>&gt;60</td>
</tr>
</tbody>
</table>
Molecular SO$_2$ (mg) based on 1 mg free SO$_2$ dependant of pH and different alcohol levels

<table>
<thead>
<tr>
<th>Alcohol %vol</th>
<th>2.80</th>
<th>2.90</th>
<th>3.00</th>
<th>3.10</th>
<th>3.20</th>
<th>3.30</th>
<th>3.40</th>
<th>3.50</th>
<th>3.60</th>
<th>3.70</th>
<th>3.80</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.078</td>
<td>0.063</td>
<td>0.051</td>
<td>0.041</td>
<td>0.033</td>
<td>0.026</td>
<td>0.021</td>
<td>0.017</td>
<td>0.013</td>
<td>0.011</td>
<td>0.008</td>
</tr>
<tr>
<td>1</td>
<td>0.081</td>
<td>0.066</td>
<td>0.053</td>
<td>0.043</td>
<td>0.034</td>
<td>0.027</td>
<td>0.022</td>
<td>0.017</td>
<td>0.014</td>
<td>0.011</td>
<td>0.009</td>
</tr>
<tr>
<td>2</td>
<td>0.085</td>
<td>0.069</td>
<td>0.055</td>
<td>0.044</td>
<td>0.036</td>
<td>0.029</td>
<td>0.023</td>
<td>0.018</td>
<td>0.015</td>
<td>0.012</td>
<td>0.009</td>
</tr>
<tr>
<td>3</td>
<td>0.089</td>
<td>0.072</td>
<td>0.058</td>
<td>0.047</td>
<td>0.037</td>
<td>0.030</td>
<td>0.024</td>
<td>0.019</td>
<td>0.015</td>
<td>0.012</td>
<td>0.010</td>
</tr>
<tr>
<td>4</td>
<td>0.093</td>
<td>0.075</td>
<td>0.061</td>
<td>0.049</td>
<td>0.039</td>
<td>0.031</td>
<td>0.025</td>
<td>0.020</td>
<td>0.016</td>
<td>0.013</td>
<td>0.010</td>
</tr>
<tr>
<td>5</td>
<td>0.097</td>
<td>0.078</td>
<td>0.063</td>
<td>0.051</td>
<td>0.041</td>
<td>0.033</td>
<td>0.026</td>
<td>0.021</td>
<td>0.017</td>
<td>0.013</td>
<td>0.011</td>
</tr>
<tr>
<td>6</td>
<td>0.100</td>
<td>0.081</td>
<td>0.066</td>
<td>0.053</td>
<td>0.043</td>
<td>0.034</td>
<td>0.027</td>
<td>0.022</td>
<td>0.017</td>
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</tr>
<tr>
<td>7</td>
<td>0.104</td>
<td>0.085</td>
<td>0.069</td>
<td>0.055</td>
<td>0.044</td>
<td>0.036</td>
<td>0.028</td>
<td>0.023</td>
<td>0.018</td>
<td>0.014</td>
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</tr>
<tr>
<td>8</td>
<td>0.109</td>
<td>0.088</td>
<td>0.072</td>
<td>0.058</td>
<td>0.046</td>
<td>0.037</td>
<td>0.030</td>
<td>0.024</td>
<td>0.019</td>
<td>0.015</td>
<td>0.012</td>
</tr>
<tr>
<td>9</td>
<td>0.113</td>
<td>0.092</td>
<td>0.075</td>
<td>0.060</td>
<td>0.048</td>
<td>0.039</td>
<td>0.031</td>
<td>0.025</td>
<td>0.020</td>
<td>0.016</td>
<td>0.013</td>
</tr>
<tr>
<td>10</td>
<td>0.118</td>
<td>0.096</td>
<td>0.078</td>
<td>0.063</td>
<td>0.050</td>
<td>0.040</td>
<td>0.032</td>
<td>0.026</td>
<td>0.021</td>
<td>0.017</td>
<td>0.013</td>
</tr>
<tr>
<td>11</td>
<td>0.122</td>
<td>0.100</td>
<td>0.087</td>
<td>0.066</td>
<td>0.053</td>
<td>0.042</td>
<td>0.034</td>
<td>0.027</td>
<td>0.022</td>
<td>0.017</td>
<td>0.014</td>
</tr>
<tr>
<td>12</td>
<td>0.127</td>
<td>0.104</td>
<td>0.084</td>
<td>0.068</td>
<td>0.055</td>
<td>0.044</td>
<td>0.035</td>
<td>0.028</td>
<td>0.023</td>
<td>0.018</td>
<td>0.014</td>
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<tr>
<td>13</td>
<td>0.132</td>
<td>0.108</td>
<td>0.088</td>
<td>0.071</td>
<td>0.057</td>
<td>0.046</td>
<td>0.037</td>
<td>0.030</td>
<td>0.024</td>
<td>0.019</td>
<td>0.015</td>
</tr>
<tr>
<td>14</td>
<td>0.138</td>
<td>0.113</td>
<td>0.091</td>
<td>0.074</td>
<td>0.060</td>
<td>0.048</td>
<td>0.039</td>
<td>0.031</td>
<td>0.025</td>
<td>0.020</td>
<td>0.016</td>
</tr>
<tr>
<td>15</td>
<td>0.143</td>
<td>0.117</td>
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<td>0.050</td>
<td>0.040</td>
<td>0.032</td>
<td>0.026</td>
<td>0.021</td>
<td>0.016</td>
</tr>
</tbody>
</table>

The quantity of molecular SO$_2$ can be calculated for each mg of free SO$_2$ with the help of a factor to be taken from the table above in relation to the wine pH and wine alcohol content. E.g. for a wine with 11 %vol and pH 3.1, which has 8 mg/L free SO$_2$ the mol. SO$_2$ will be:

$$8 \text{ mg/L free SO}_2 \times 0.65 = \boxed{5.2} \text{ mg/L molecular SO}_2$$

The quantity of molecular SO$_2$ to protect the white wines from oxidations is > 0.50 mg/L the quantity to inhibit malolactic bacteria between 0.30 and 0.50. (O.oeni most sensitive)
INFLUENCE OF THE TEMPERATURE

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Total-SO$_2$ (mg/L)</th>
<th>Free SO$_2$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0°C</td>
<td>412</td>
<td>68</td>
</tr>
<tr>
<td>15°C</td>
<td>412</td>
<td>85</td>
</tr>
<tr>
<td>30°C</td>
<td>412</td>
<td>100</td>
</tr>
</tbody>
</table>

At higher temperatures – liberation of more free SO$_2$ from bound SO$_2$ = higher antiseptical effect.

The antiseptically power of free SO$_2$ = molecular SO$_2$ (active form) is only a small fraction of it.

Similar effect for alcohol = at higher temperatures increasing toxic effect of ethanol
## Additive effects: Alcohol & Temperature

<table>
<thead>
<tr>
<th>Alcohol content of wine (% v/v)</th>
<th>Temperature (°C) Which should not be exceeded</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower than 14.5%</td>
<td>28</td>
</tr>
<tr>
<td>Higher than 14.5%</td>
<td>23</td>
</tr>
</tbody>
</table>
Volatile phenol content after MLF (wines inoculated pre-ML for the ‘control’)
Table 2. Volatile phenol (μg/L) 4-ethylphenol (EP) and 4-ethylguaiacol (EG) produced (Experiment 2, PN1 Wine)

<table>
<thead>
<tr>
<th>LACTIC ACID BACTERIA</th>
<th>No ML inoculation</th>
<th>MBR® 1</th>
<th>MBR® 2</th>
</tr>
</thead>
</table>

**Brettanomyces inoculation**

<table>
<thead>
<tr>
<th>Temp.</th>
<th>pH</th>
<th>Pretreatment</th>
<th>No ML inoculation</th>
<th>MBR® 1</th>
<th>MBR® 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>14°C – pH 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>148 / 353</td>
<td>41 / 119</td>
<td>68 / 178</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>28 / 68</td>
<td>30 / 82</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>14°C – pH 3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>191 / 387</td>
<td>4 / 22</td>
<td>&lt;1 / 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3 / 10</td>
<td>&lt;1 / 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18°C – pH 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>152 / 357</td>
<td>79 / 148</td>
<td>68 / 143</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;1 / &lt;1</td>
<td>&lt;1 / &lt;1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18°C – pH 3.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>185 / 398</td>
<td>&lt;1 / &lt;2</td>
<td>&lt;4 / &lt;6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;1 / &lt;1</td>
<td>&lt;1 / &lt;1</td>
<td></td>
</tr>
</tbody>
</table>
Influence of phenolic compounds deriving from wine grapes or enological tannins on vitality and activity of *O. oeni*

Collaboration with University of Bordeaux (Prof. Aline Lonvaud)

a) Investigation of different polyphenolic fraction from different grape varieties

b) Investigation of different enological tannins (grape seed tannins, grape skin tannins, oak tannins….)

c) Investigation of nutrient formulations to overcome the inhibition

d) Investigation how to use the stimulating effect of some fractions
• Positive impact of nutrient addition containing mannoprotein fraction

Rapid growth in presence of Opti’Red in comparison to tanin Z only