

A grayscale microscopic image showing various yeast cells and long, branching hyphae, likely representing the microorganisms involved in malolactic fermentation (MLF).

MLF choices in Sparkling wine production

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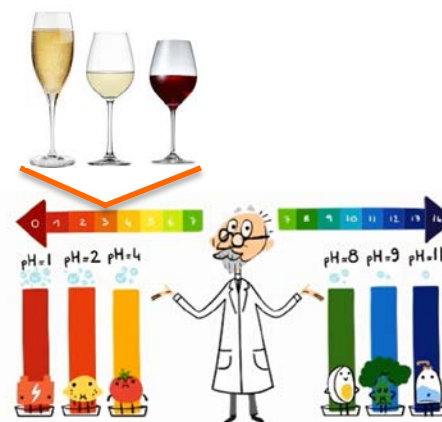
Sparkling Wine Symposium,
William Angliss College, Melbourne
26 June 2018

MLF in sparkling wine

- Style desired
 - Reduce acidity of the base wine
 - Provide microbial stability to the base wine
 - Don't want MLF during 2° fermentation



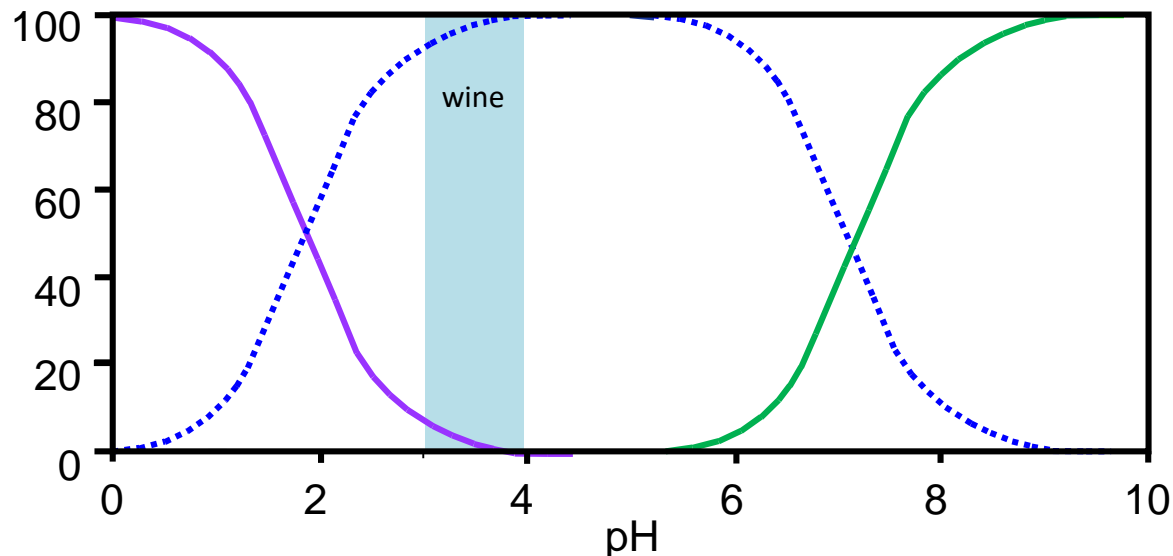
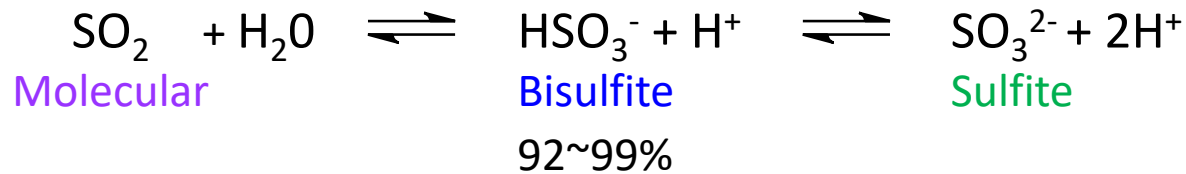
- Challenges
 - Base wine has high acidity
 - SO₂ is more effective at low pH



Sulphur dioxide



In solution SO_2 exists in 3 main forms in equilibrium



A molecular SO_2 level of 0.4 ppm (equivalent to a free SO_2 level of 20 ppm at 3.50 pH) will kill wild yeast without adversely affecting *Saccharomyces*.

Sulphur dioxide

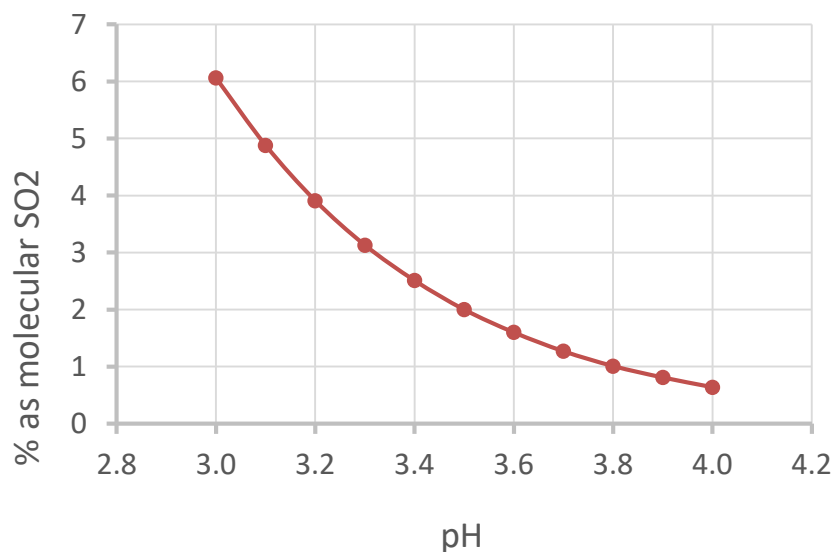


- Antimicrobial & antioxidant
- Binds to carbonyls (particularly acetaldehyde), sugars, colour & phenolics
- In wine we talk about
 - Molecular, Free, Bound & Total SO₂

SO ₂ form	Definition	
Molecular SO ₂	SO ₂	Antimicrobial
Free SO ₂	Mol SO ₂ + bisulfite HSO ₃ ⁻	Antioxidant
Bound SO ₂	Includes both strongly & weakly bound forms	Contributes to total SO ₂
Total SO ₂	Free + Bound SO ₂	

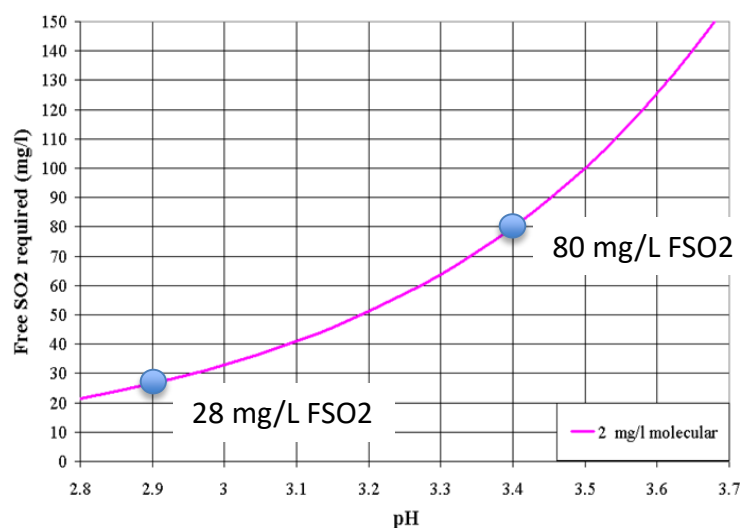
Interplay of Free SO₂, Molecular SO₂ and different pH levels in wine

Percent of the Free SO₂ that is present as molecular SO₂ at different pH levels in wine.



- More mol SO₂ at lower pH

Concentration of Free SO₂ required for 2 mg/L mol SO₂ at different wine pH.



- Less FSO₂ is required for equivalent of 2 mg/L mol SO₂ at pH 2.9 than at pH 3.4

➤ *Lower pH = higher mol SO₂ = free SO₂ is more effective at lower pH*

Challenges for MLF in Sparkling wine

- Base wine has high acidity
- SO₂ is more effective at low pH
- Alcohol is not usually a problem
- Malic acid – high
 - Lactic acid can be inhibitory to bacteria



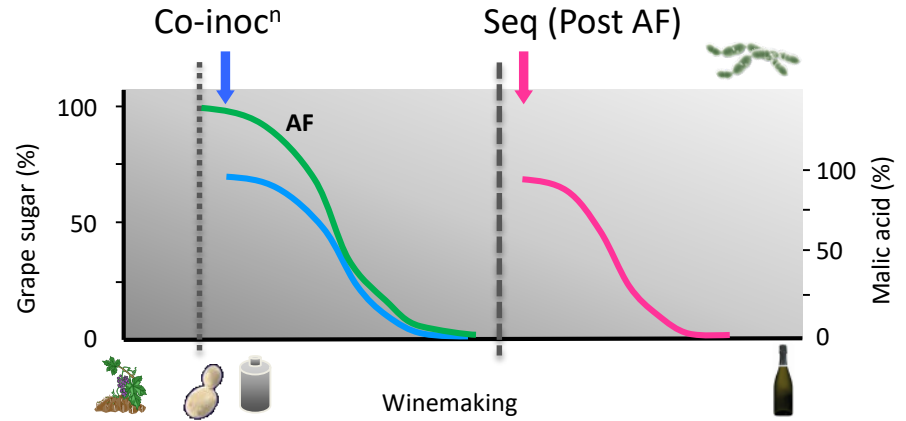
MLF options

- Timing of inoculation
- Bacteria culture preparation

Timing of inoculation

- **Co-inoculation**

- Adaptation to juice/wine
- Completed sooner



- **Sequential inoculation**

- Managing only MLF (bacteria)

- ❖ High malic acid content

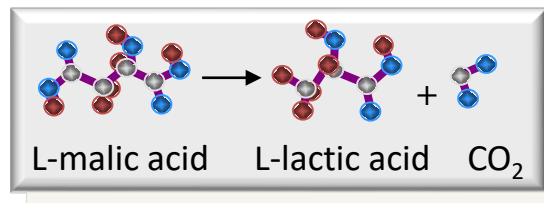
- As MLF progresses, lactic acid produced
 - *Lactic acid can be inhibitory to bacteria*

Malic acid tolerance / Lactic acid sensitivity

Factors that impact MLF	Easy	Moderate	Difficult	Extreme
Initial level of malic acid (g/L)	2 - 4	4 - 5 or 1 - 2	5 - 7 or 0.5 - 1	>7 or <0.5

2-4 g/L malic acid
ideal for MLF
efficiency

>4 (5+) g/L malic acid
can be difficult to get
to completion



Inhibition of the bacteria
by increasing concenⁿ of
L-lactic acid derived from
the MLF itself

<1 g/L malic acid
not as inductive
to MLF

<0.8 g/L malic acid
can be difficult to
restart MLF

~ 3 g/L lactic acid
can be inhibitory
to LAB

1 g malic acid → *in practice* = 0.5-0.55 g lactic acid
(in theory = 0.67 g lactic acid)

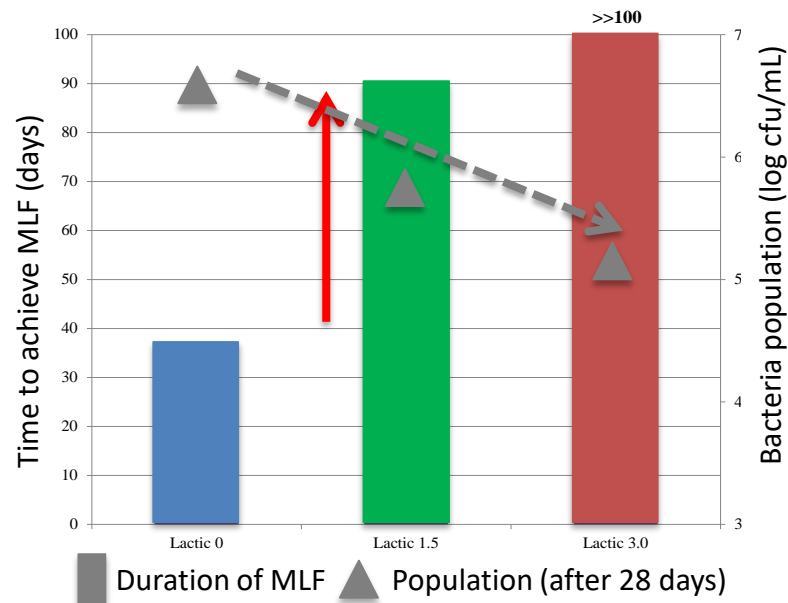
winemaking / www.lallemmandwine.com

Impact of addition of L-lactic acid before MLF



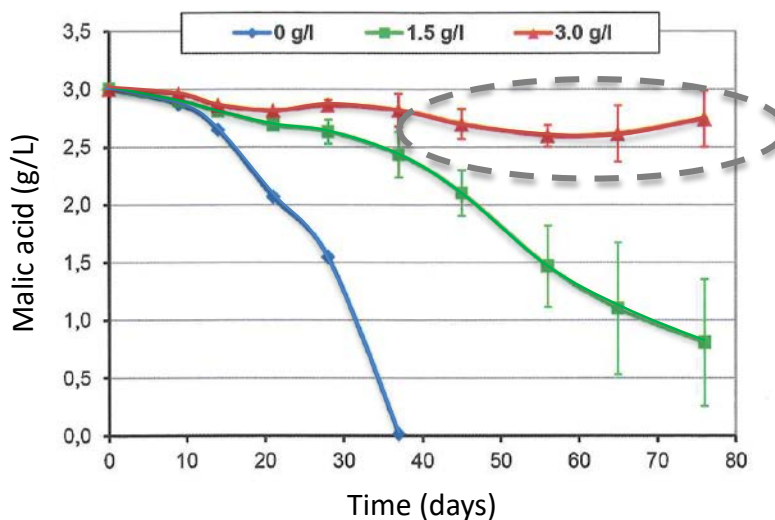
Pinot noir

Alcohol	12.3%
pH	3.25
Malic acid	3 g/L
Temp	16 °C



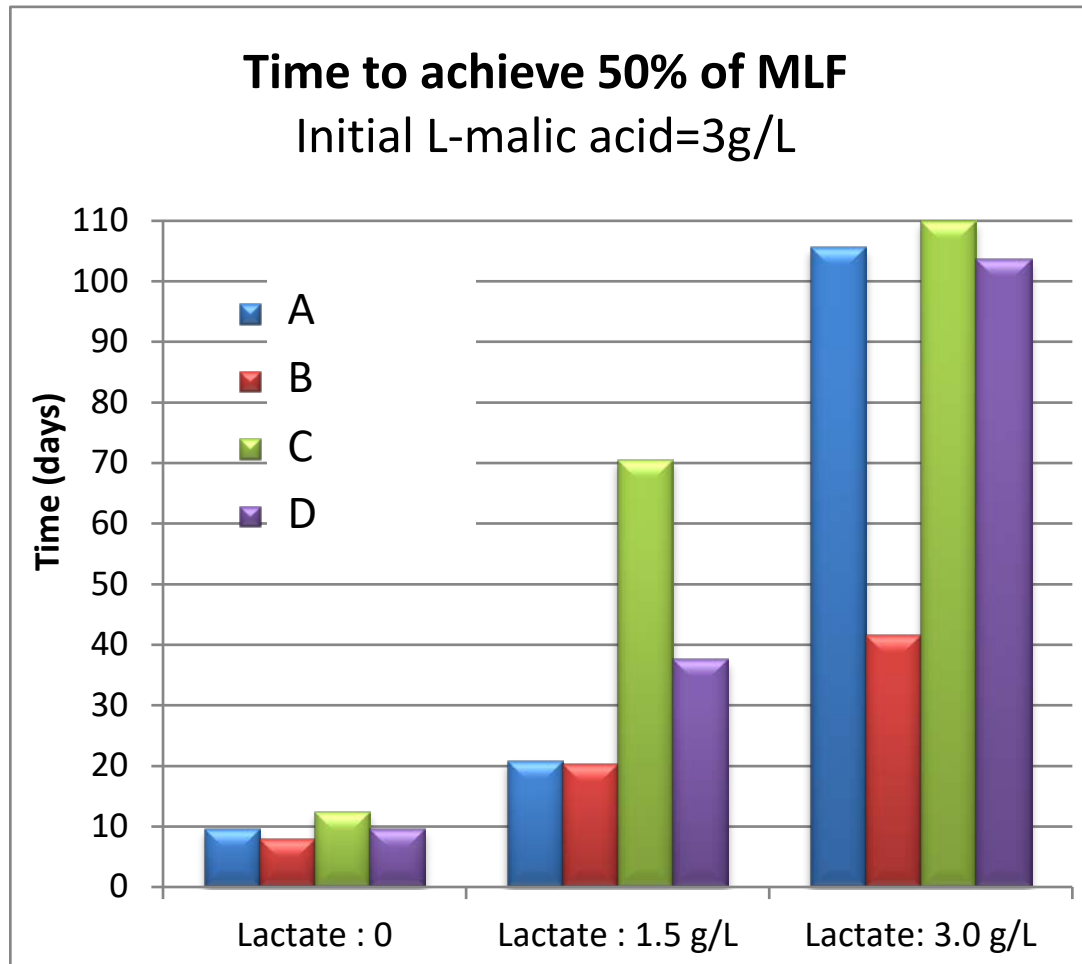
➤ Addition of 1.5g/L greatly increases the time to achieve MLF.

➤ Bacteria viability decreases with higher lactic acid concentrations



➤ Addition of 3g/L induces a high loss of viability which leads to stuck MLF.

Sensitivity of bacteria strain to L-lactic acid



Chardonnay

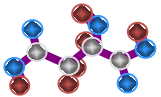
Alcohol	12.5%
pH	3.25
Malic acid	3 g/L
Temp	16 °C



- Strain variation in lactic acid sensitivity/resistance

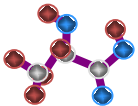
Malic acid tolerance / Lactic acid sensitivity

Malic acid



- Increasing concentration of malic acid increases the speed of malic acid degradation, but of course also increases the duration of MLF.
- Some strains more suitable than others for high malic acid content

Lactic acid



- The presence of L-lactic acid in the wine inhibits the implantation and growth of the inoculated wine LAB resulting in an inhibition of MLF.
- An initial content of L-lactic acid in the range of 1.5 g/L strongly slows MLF, but a content of 3.0 g/L fully inhibits MLF.

➤ *Problems inducing MLF by inoculation with selected wine bacteria may be encountered in wines with a partial MLF.*

Bacteria starter culture

- **Ideal**

- Direct inoculation

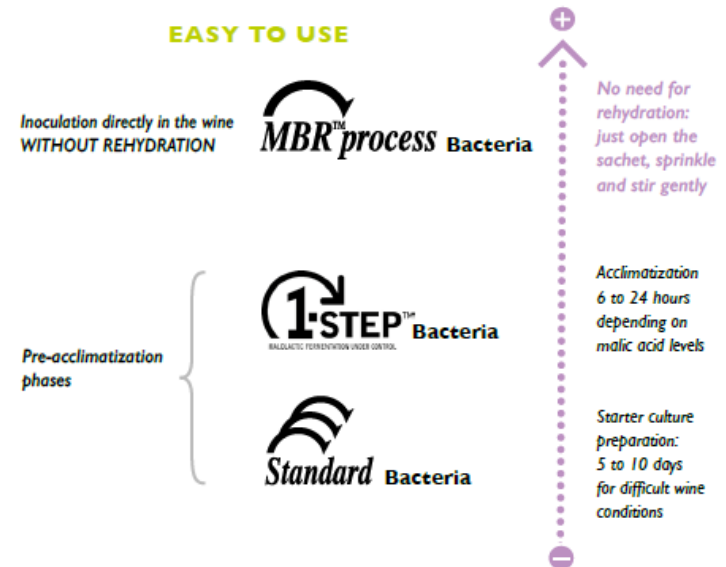
- **Reality**

- Acclimatisation

- **The real world in relation to Sparkling wine!**

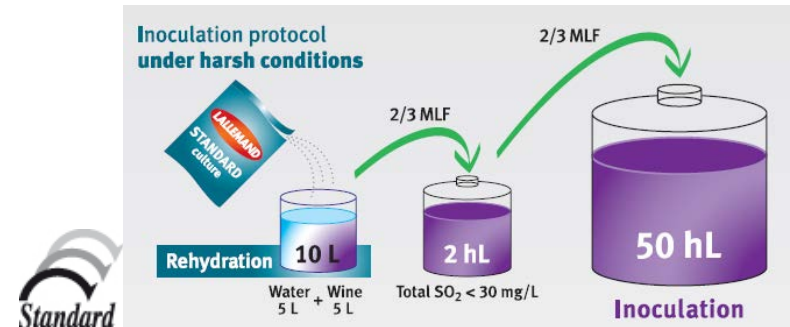
- Starter culture preparation

➤ *Balance between pH (& mol SO₂) and starter culture readiness*



Bacteria starter culture

- Direct inoculation
 - Not always able to have the ‘magic bullet’ answer
- Preparation of starter (Standard)
 - Ready bacteria for the juice/wine



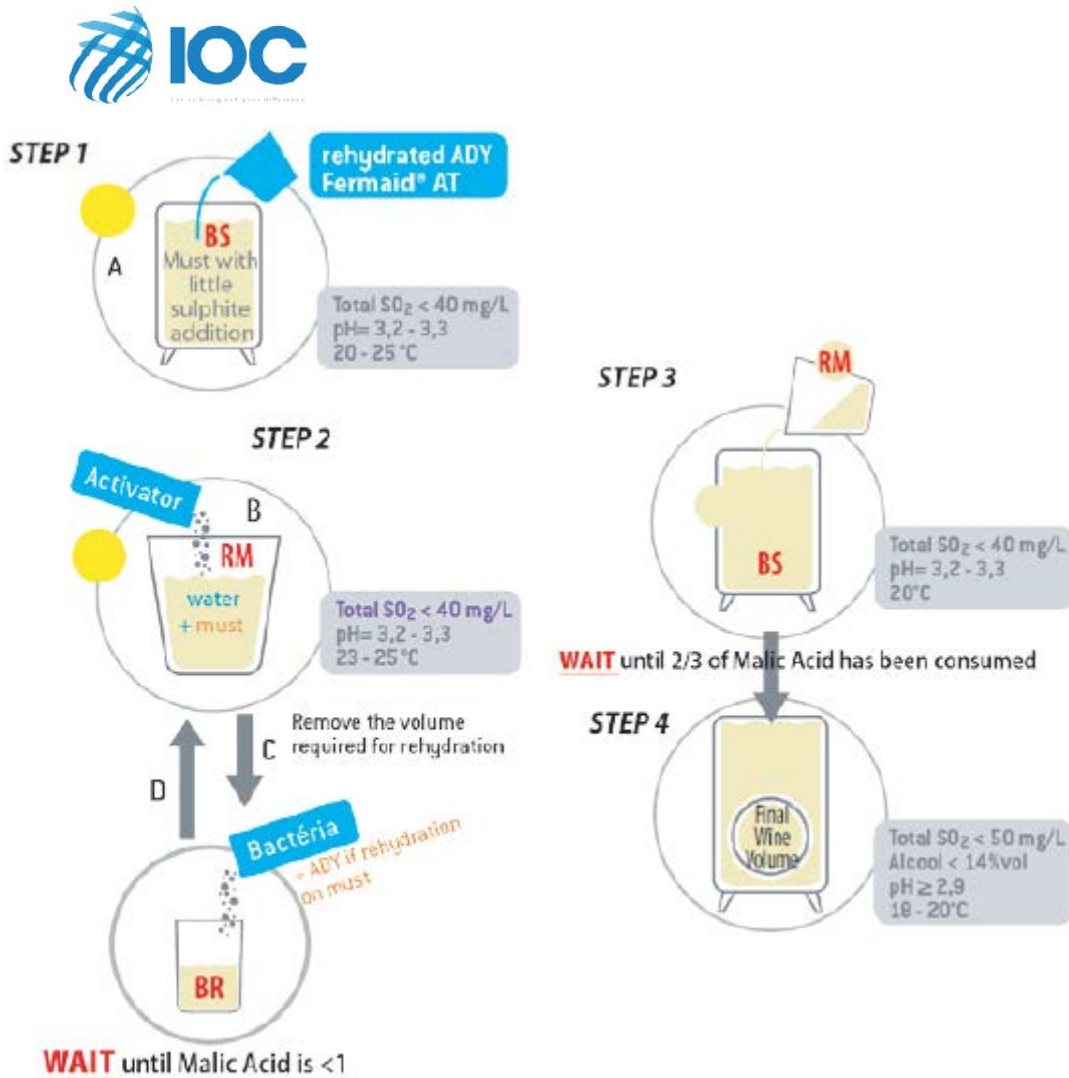
IOC Inobacter



- This *Oenococcus oeni* strain has been selected from Champagne by CIVC (International Champagne Committee)
 - Each batch is rigorously controlled by the CIVC
- IOC Inobacter is specific for sparkling wine production
 - pH tolerance >2.9
 - Alcohol tolerance up to 14% v/v
 - SO₂ tolerance up to 50 mg/L Total SO₂
 - Low production of volatile acidity
 - No production of biogenic amines
 - *Excellent tolerance of this strain to extreme wine conditions enables an effective malic acid metabolism*
 - Can be used as Co-inoculation with yeast or inoculated after AF as Sequential




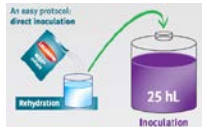


IOC Inobacter



- Step-wise build up of the bacterial culture
- Acclimatisation to the wine conditions
- Actively metabolising malic acid

➤ *Bacteria are prepared for the juice /wine*

In summary

- Consider SO₂ usage with MLF need 
- Ideally, direct inoculation 
 - Not always possible to have a direct inoculation because of specific challenges with sparkling base wine
 - Low pH & SO₂ translate to higher mol SO₂
- Select the best bacterial strain for your wine 
 - pH tolerance
- High bacteria cell numbers are crucial for implantation & MLF efficiency (>10⁶ cells/mL)

➤ *Even though perhaps not the most desired option, patience with building up the culture is the best way to ensure that the bacteria are ready for your sparkling base wine*

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