



FERMENTATION MANAGEMENT - SPECIFIC CASE: YEAST / BACTERIA CO-INOCULATION



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1- What is co-inoculation?

Traditionally, fermentation management is characterised firstly by a yeast inoculation, followed by a bacterial inoculation after completion of alcoholic fermentation (AF) (see the technical booklet: “Good MLF management”).

Yeast / bacteria co-inoculation is an alternative to this classic scheme. It consists of inoculating bacteria into the must at the start of AF. When yeast activity starts to slow down, the bacteria take over and, ideally, malo-lactic fermentation (MLF) commences rapidly (figure 1). Co-inoculation can be applied to most vinification procedures (traditional in reds, or in white fermentations).

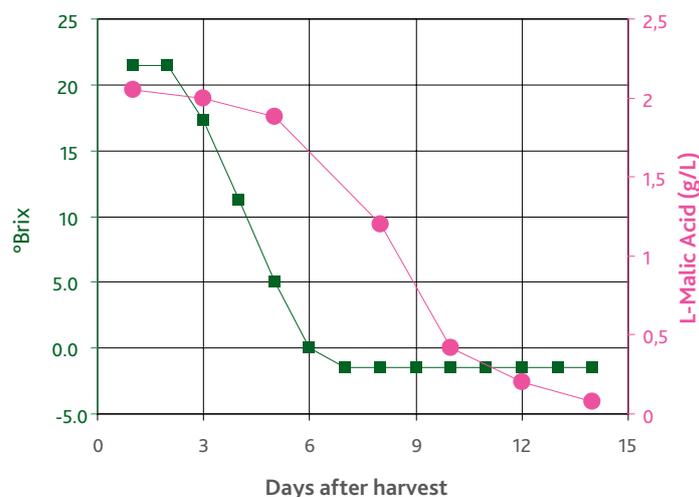


Figure 1: Example of fermentation kinetics during a co-inoculation..

The application of **co-inoculation at the end of AF** is also commonly used: towards 0°Brix, if the fermentation curve is clear-cut, bacteria can be added.

2- Why carry out co-inoculation?

While there appears to be a variety of practical motivations (time saving for “primeur” wine production, safety requirements in atypical situations, making use of the heat of fermentation), this technique has two main objectives:

Promoting malolactic bacterial implantation

When added late after AF, the bacteria find themselves in a medium which is more hostile to their development (ethanol content, competition with other microorganisms, inhibitor compounds synthesised by the yeasts such as SO₂, medium-chain fatty acids...). **Co-inoculation facilitates implantation and expression of the selected bacteria**, with advantage in more extreme conditions: a must with a high sugar content is less inhibitory than the corresponding wine which will have a high alcohol content, and the bacteria adapt progressively.

From a gustatory point of view, despite the fact that the appreciation of transformations which are usually perceived during sequential fermentation can be modified in co-inoculation, this technique does not induce sensory differences. On the contrary, because **the development of contamination organisms is forestalled**, and the fact that the associated spoilage risks (volatile phenols, volatile acidity, biogenic amines, acetaldehyde), are limited, the finished wine demonstrates remarkable purity and considerable crispness.

Dominating the microbial ecosystem throughout the vinification period

The use of selected yeast and bacterial strains allows the detrimental metabolisms of indigenous flora to be avoided. This is all the more important for bacteria, as indigenous strains can produce biogenic amines, which are detrimental to the consumer’s health.

The natural progression of indigenous fermentations leaves a microbiological void between yeast population decline and that of the bacteria (figure 2, graph 1). **This microbiological void is conducive to spoilage organism development, such as *Brettanomyces*.**

The sequential usage of selected bacterial strains already allows this latent period to be considerably reduced, but with co-inoculation, this microbiological void is totally avoided (see figure 2, graph 2). In the case of controlled co-inoculation, ***Brettanomyces* cannot develop as the microbial ecosystem is never in void status.** Moreover, fermentations are completed earlier, which allows the wine to be protected sooner by post-fermentative sulphiting.

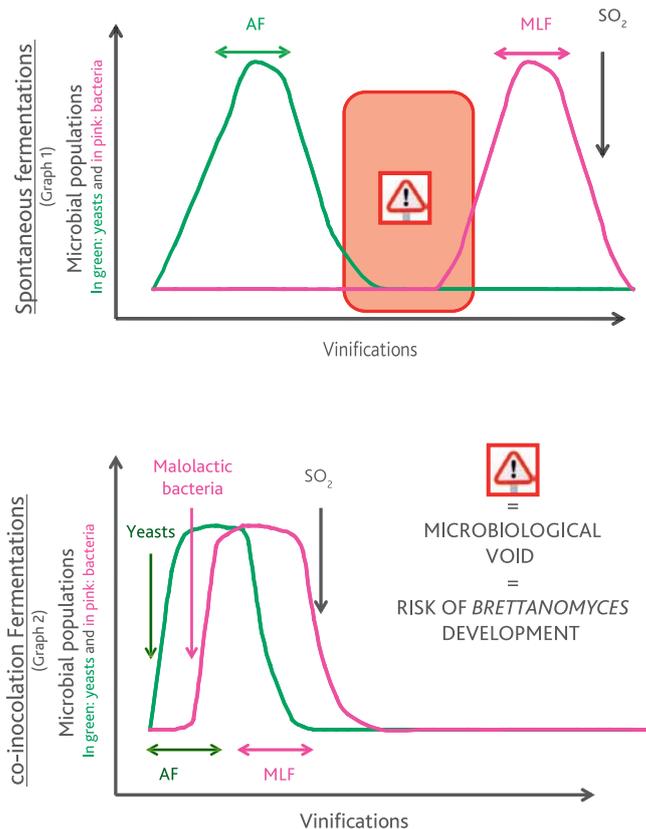


Figure 2: Microbic waves and microbiological void during fermentations.

3- What are the key points of MLF management in co-inoculation?

AF management constitutes the primary element for successful MLF (see technical booklets «Good MLF management» and «Good fermentation activator management»). This control is essential in the case of co-inoculation, as any decline in yeast activity before completion of the primary fermentation can lead to serious alteration of the wine by undesirable products of bacterial sugar metabolism.

In addition to maintaining fundamental regulations of hygiene, sulphiting and temperature control, **it is advisable to rapidly implant a yeast strain which is adapted to the conditions of the medium and to provide it with all elements necessary to its growth and survival.**

3-1 Sulphiting

Do not exceed 8 g/hL at vatting. Carry out an SO₂ measurement before all bacterial inoculation: bacteria can only be added if the free SO₂ content is equal to 0 mg/L.

3-2 Choice of the yeast strain for AF

In all cases, co-inoculation must only be implemented with selected yeasts and under no circumstances during AF which is carried out by indigenous yeasts.

In co-inoculation, the yeast strain and the bacterial strain must coexist in perfect harmony. Understanding the interactions between these two microorganisms is essential. Research is in progress in order to improve understanding of these phenomena and to establish a range of Co-Inoculation Compatible (CIC®) strains.

3-3 Management of fermentation activator provision

(see technical booklet: «Good fermentation activator management»)

To guarantee an active and clear-cut AF finish, it is advisable to adhere to the following guidelines:

- **rehydrate the ADY (active dry yeast) in the presence of Dynastart® (30 g/hL).**
- **add Thiazote® to the yeast** to be calculated according to the assimilable nitrogen deficiency, the potential alcohol, and the AF temperature).
- **make up, if necessary, with ammonium phosphate after a loss of 5-6°Brix (20 to 30 g/hL to be calculated according to the abovementioned factors).**

3-4 AF temperature management

Temperature is an important factor for AF progress and completion, as is the case for MLF. As yeasts and bacteria are sensitive to temperature and its variations, it is essential to maintain the temperature below **28°C** in the liquid phase and to avoid thermal shocks. For example, red fermentations cooled through must-chiller may not be suitable for co-inoculation, as the must would go from ferment temperature down to 10°C and bacteria will not handle such a thermal shock.

3-5 Bacterial inoculation

Bacterial inoculation is applied 48 hours after the start of AF, only if the free SO₂ content is equal to 0 mg/L. To guarantee fast MLF start-up, it is advised to inoculate the bacteria at 1 g/hL. Certain specific vinification techniques (Beaujolais maceration for example) allow successful inoculation at half dosages. A direct inoculation strain is preferable: **Lactoenos SB3®**, (CIC® strain).

For **co-inoculation towards the end of AF** (supply at 0°Brix), ideally use a rapid re-acclimation bacterium (24h): **Lactoenos 450 PreAc®** (CIC® strain).

Red: incorporate the bacteria under the cap.

White: incorporate the bacteria at the top of the tank.

In both cases, it is not necessary to carry out a homogenising pumping over during bacteria addition; mixing will occur naturally through fermentation activity.

During bacterial inoculation and in the following 12 hours, no oxygen addition must be carried out.

4- Analytical follow-up

To ensure successful co-inoculation, regular analytical follow-up is recommended:

Phase	Analyses to be carried out
Must	Sugars content, TA, pH, Malic acid, Free SO ₂ , Total SO ₂ , assimilable nitrogen
From 10°Brix Up to MLF completion	Malic acid + VA, twice / week
24 h after inoculation	Microbial population control using epifluorescence microscopy



LAFFORT

l'œnologie par nature

BP 17 - 33072 bordeaux cedex
Tél : 05 56 86 53 04 / Fax : 05 56 86 30 50
www.laffort.com



SARCO

11 Rue A. Bergès - 33270 Floirac
Tél : 05 56 86 53 04 / Fax : 05 56 86 30 50
www.sarco.fr