

Sacrificial yeast cultures for SO₂ reduction

Occasionally winemakers find themselves in situations where there are small amounts of sulfur dioxide (SO₂) in white grape juice that could impede primary fermentation or inhibit a coinoculated malolactic fermentation, yet the concentration is not high enough to consider removal with hydrogen peroxide. This can occur due to over-additions of SO₂ during harvest, transport, crushing and/or pressing. In this column AWRI Oenologist Ben Cordingley investigates the use of yeast additions to remove small amounts of free SO₂ from juice before the main inoculation is conducted, commonly known as a sacrificial yeast culture.

When would winemakers consider using a sacrificial culture?

The use of a *Saccharomyces cerevisiae* sacrificial yeast culture could be considered for reducing relatively small quantities of free SO₂ in the range of 10-15 mg/L from juice before primary fermentation, or when wanting to reduce the free SO₂ to nearly zero in a sparkling base wine prior to secondary fermentation.

For larger SO₂ reductions, hydrogen peroxide (H₂O₂) addition is the preferred method as it rapidly oxidises free SO₂ to sulfate. One advantage is the reduction in the total SO₂ achieved through this process, whereas sacrificial yeast cultures are unlikely to significantly reduce the total SO₂ concentration. Hydrogen peroxide can cause oxidation and mousiness if calculations and additions are not performed correctly. Another alternative is to blend high SO₂ juice parcels with other parcels containing no SO₂.

How are sacrificial yeast additions performed?

The helpdesk has heard variable reports of how this technique is performed in practice. Generally, a yeast addition occurs at 10, 25 or 50% of the usual inoculum rate, added to the juice about half a day before adding the 'proper' inoculum (made at the usual rate). Alternatively, some winemakers have simply added greater than normal inoculum rates when commencing primary fermentation.

How does it work?

At typical grape must pH, the majority of free SO₂ exists in the bisulfite ion form (HSO₃⁻) with a small portion in the molecular form (SO₂). The molecular form can diffuse through yeast cell membranes where at the higher intracellular pH it is mostly converted to bisulfite, which can disrupt intracellular metabolic processes and thus inhibit microbial growth. *S. cerevisiae* yeast

Figure 1. Free and total SO₂ concentration reduction in white juice treated with 20, 50, 100 and 200% recommended inoculum rates

Treatment	Free SO ₂ (mg/L)	Total SO ₂ (mg/L)	Free SO ₂ (mg/L)	Total SO ₂ (mg/L)	Free SO ₂ (mg/L)	Total SO ₂ (mg/L)	Free SO ₂ (mg/L)	Total SO ₂ (mg/L)	Free SO ₂ (mg/L)	Total SO ₂ (mg/L)
Target total SO ₂ addition rate to juice ¹	-	0	-	20	-	30	-	40	-	100
Free and total SO ₂ concentration after SO ₂ addition	0	0	10	21	15	30	23	41	68	98
Free and total SO ₂ concentration measured 3 hours after different yeast addition rates ²										
No yeast addition	0	0	7	19	13	30	17	39	51	96
1 x 10 ⁶ cells/mL (20% inoculum rate)	0	0	5	19	12	29	17	38	51	96
3 x 10 ⁶ cells/mL (50% inoculum rate)	0	0	2	19	11	29	17	38	50	95
5 x 10 ⁶ cells/mL (100% inoculum rate)	0	0	0	19	10	28	16	37	50	94
10 x 10 ⁶ cells/mL (200% inoculum rate)	0	0	0	19	5	26	15	36	49	92

1. Initial juice contained no detectable free or total SO₂

2. Recommended inoculum rate for Lalvin CY3079 was 25 g/HL or 5 x 10⁶ cells/mL

have several resistance mechanisms that work together to protect against the toxic effects of SO₂:

- Efflux pumps that actively export bisulfite from the yeast cell
- Acetaldehyde production that strongly binds to bisulfite, yielding a much less toxic bound sulfone form and a reduction in free SO₂ concentration (Divol *et al.* 2012).

S. cerevisiae strains vary in their resistance to SO₂; thus, there will likely be differences in their ability to reduce free SO₂ concentrations. The amount of acetaldehyde produced is also influenced by parameters such as grape variety, nutrient status, temperature and free SO₂ concentration (Jackowetz *et al.* 2011).

Is there really a sacrifice?

Acetaldehyde is an intermediate in the yeast fermentation pathway and is produced during active metabolism in living yeast cells, thus the term 'sacrificial yeast cultures' is likely a misnomer. It is not clear how past SO₂ exposure impacts yeast health and fermentation kinetics.

Does it work in practice?

To provide practical information to wineries attempting small SO₂ reductions using yeast, potassium metabisulfite was added to aliquots of sterile filtered 2020 McLaren Vale Chardonnay juice to give SO₂ concentrations ranging from 0 to 100 mg/L. Within each of these SO₂ treatments, varying additions of yeast were inoculated into the juice at 20°C, ranging from no addition up to double (200%) the recommended rate specified by the manufacturer (Lallemand 2017). Lalvin CY3079 was selected due to its widespread use in white ferments and reported low to moderate SO₂ production. Treatments were performed in triplicate except for the 30 mg/L SO₂ addition rate treatment, which was performed in duplicate. Free and total SO₂ concentrations were analysed by discrete photometric analyser three hours post-yeast addition. After 24 hours the free and total SO₂ concentrations were analysed a second time (one replicate) but these results were unchanged from the three hour

time point so were not included. Results are presented in Figure 1.

How much free SO₂ was reduced using sacrificial yeast cultures?

Half of the manufacturer's recommended yeast addition rate (3 x 10⁶ cells/mL) was required to reduce the free SO₂ from 10 mg/L down to near zero after three hours at 20°C. A wine with an initial free SO₂ of 15 mg/L required double the manufacturer's addition rate (10 x 10⁶ cells/mL) to achieve a reduction down to 5 mg/L after three hours. When the initial free SO₂ concentration was more than 15 mg/L, there was no significant reduction resulting from the addition of yeast cultures at the rates trialled. No measurable SO₂ was produced by the yeast in this trial. Total SO₂ concentrations did not significantly decrease even with high yeast addition rates, suggesting that acetaldehyde produced by yeast was responsible for binding free SO₂ where significant reductions in the free SO₂ concentration were observed.

What is recommended for SO₂ removal?

For most situations, hydrogen peroxide additions performed correctly are the most effective way to reduce the free SO₂ in juice or wine; however, blending or using a small initial yeast addition can reduce small amounts of SO₂ in juice (up to 15 mg/L).

For further information about SO₂ removal or other technical winemaking or viticulture questions, contact the AWRI helpdesk on (08) 8313 6600 or helpdesk@awri.com.au

References

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- Lallemand Inc. 2017. Lalvin CY3079™ technical datasheet. Accessed November 2021, from www.lallemandwine.com **CW**



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