Sacrificial yeast cultures for SO_2 reduction

Occasionally winemakers find themselves in situations where there are small amounts of sulfur dioxide (SO_2) 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 SO_2 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 SO_2 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 SO_2 in the range of 10-15 mg/L from juice before primary fermentation, or when wanting to reduce the free SO_2 to nearly zero in a sparkling base wine prior to secondary fermentation.

For larger SO_2 reductions, hydrogen peroxide (H_2O_2) addition is the preferred method as it rapidly oxidises free SO_2 to sulfate. One advantage is the reduction in the total SO_2 achieved through this process, whereas sacrificial yeast cultures are unlikely to significantly reduce the total SO_2 concentration. Hydrogen peroxide can cause oxidation and mousiness if calculations and additions are not performed correctly. Another alternative is to blend high SO_2 juice parcels with other parcels containing no SO_2.

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 SO_2 exists in the bisulfite ion form (HSO_3^-) with a small portion in the molecular form (SO_2). 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 SO_2 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 SO_2
2. Recommended inoculum rate for Lalvin CY3079 was 25 g/HL or 5 x 10^6 cells/mL

have several resistance mechanisms that work together to protect against the toxic effects of SO_2:

- 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 SO_2 concentration (Divol *et al.* 2012).

S. cerevisiae strains vary in their resistance to SO_2; thus, there will likely be differences in their ability to reduce free SO_2 concentrations. The amount of acetaldehyde produced is also influenced by parameters such as grape variety, nutrient status, temperature and free SO_2 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 SO_2 exposure impacts yeast health and fermentation kinetics.

Does it work in practice?

To provide practical information to wineries attempting small SO_2 reductions using yeast, potassium metabisulfite was added to aliquots of sterile filtered 2020 McLaren Vale Chardonnay juice to give SO_2 concentrations ranging from 0 to 100 mg/L. Within each of these SO_2 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 SO_2 production. Treatments were performed in triplicate except for the 30 mg/L SO_2 addition rate treatment, which was performed in duplicate. Free and total SO_2 concentrations were analysed by discrete photometric analyser three hours post-yeast addition. After 24 hours the free and total SO_2 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 SO_2 was reduced using sacrificial yeast cultures?

Half of the manufacturer's recommended yeast addition rate (3 x 10^6 cells/mL) was required to reduce the free SO_2 from 10 mg/L down to near zero after three hours at 20°C. A wine with an initial free SO_2 of 15 mg/L required double the manufacturer's addition rate (10 x 10^6 cells/mL) to achieve a reduction down to 5 mg/L after three hours. When the initial free SO_2 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 SO_2 was produced by the yeast in this trial. Total SO_2 concentrations did not significantly decrease even with high yeast addition rates, suggesting that acetaldehyde produced by yeast was responsible for binding free SO_2 where significant reductions in the free SO_2 concentration were observed.

What is recommended for SO_2 removal?

For most situations, hydrogen peroxide additions performed correctly are the most effective way to reduce the free SO_2 in juice or wine; however, blending or using a small initial yeast addition can reduce small amounts of SO_2 in juice (up to 15 mg/L).

For further information about SO_2 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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Jackowetz, J.N., Dierschke, S., Mira de Orduña, R. 2011. Multifactorial analysis of acetaldehyde kinetics during alcoholic fermentation by *Saccharomyces cerevisiae*. Food Res. Int. 44(1): 310-316.

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