

Micro-Oxygenation – Where Now?

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Australian Society of Viticulture and Oenology

Use of Gases in Winemaking Seminar, October 2002

Introduction

This paper attempts to answer the question ‘Micro-oxygenation— where now?’ Before speculating on the future, the present must be understood, so the paper will first define what is currently known, by briefly examining some of the chemistry thought to be involved and discussing some of the results achieved so far, clearing up the misconceptions surrounding micro-oxygenation, and then suggesting possibilities for the future.

Micro-oxygenation is one of the most controversial and misunderstood areas of modern winemaking. Its proponents claim that it can bring about seemingly miraculous changes in wine while its detractors claim it does not work or worse, that it destroys wine.

One school of thought maintains that it is just a useful remedy for improving sub-standard wines, while others declare that it should be used on the best wines to make them even better.

What is the reality?

What is Micro-oxygenation?

Micro-oxygenation is the controlled, continuous addition of small amounts of oxygen to wine.

The process of micro-oxygenation aims to manipulate the rate and result of the oxygen-requiring reactions in wine in order to bring about desirable changes in wine texture and aroma. This is an important point—micro-oxygenation can be seen from this perspective as simply a more efficient, effective way of introducing oxygen into wine—a process with which many winemakers are thoroughly familiar.

It is controlled because the operator needs to know and be able to adjust the dosage of oxygen into the wine, in order to achieve the desired result.

It is continuous because of the need to avoid “spikes” of oxygen.

This can be contrasted to the well-known and widely used practice of aerated racking which adds oxygen to wine in large, discrete doses. This practice could conceivably be described as “macro-oxygenation” since it can significantly (even if temporarily) increase the dissolved oxygen content of wine.

In contrast, the principle upon which micro-oxygenation is based is as follows:

The dosing rate of Oxygen must always be less than the wine’s consumption of Oxygen.

In other words, there should be no increase in dissolved oxygen levels during micro-oxygenation.

However, laws are there to be broken and there is a special case where dissolved oxygen levels may temporarily increase that will be discussed later.

Why is Oxygen Good for Wines?

In recent years, New World winemakers have profoundly altered their views on the desirability of oxygen in red winemaking. The influence of the “reductionists” of the late 70’s and early 80’s has waned and a greater appreciation of the effects of early exposure of wine to oxygen has developed.

Emphasis is again given to the view of micro-oxygenation as simply a special case of oxygen addition, not a completely new, previously unknown practice. Its genesis in France is largely due to the desire to simulate the effects of barrel maturation in a more controlled way.

Oxygen has been shown to provide significant benefits in winemaking:

- *During Fermentation*

Oxygen is necessary for healthy and viable yeast cells. In particular, it promotes synthesis of sterols/fatty acids in yeast cell walls.

- *For White Wines*

Oxygen can interact with lees to increase the apparent weight and mouthfeel of wines, especially those stored in barrel.

- *For Red Wines*

Much research and practical experimentation has shown the integral role that oxygen plays in the polymerisation of polyphenolic compounds, especially in the early stages of maturation. Polymerisation can produce stable forms of anthocyanins that resist decolorisation by sulfur dioxide and provide better colour stability at wine pH. It can also result in coloured forms (pigmented polymers) that are stable over time.

- *For Improving Aromatic Profile*

Winemakers have found that repeated aerated rackings can diminish excessively green, herbaceous characters

- *For Removing Reductive Characters*

Exposure to air, usually via racking, can help remove unpleasant reductive, sulfidic characters from wine.

Winemakers also need to be aware of oxygen’s destructive power:

- *During Fermentation*

It is generally accepted that there is little risk of oxidation during fermentation. However, some aromatic and delicate white wines such as Riesling and Sauvignon Blanc may lose some volatile compounds with over-enthusiastic oxygen sparging.

- *For White Wines*

Oxygen can promote browning in colour and the loss of positive aromatics.

- *For Red Wines*

Too much oxygen can help bring about the formation of large molecules (pigmented polymers) with high molecular weight that are unable to stay in solution. This causes precipitation of polyphenolic material, leaving wines dry and harsh to the taste and with reduced colour intensity.

The Role of Oxygen

In red wines, oxygen can stimulate polymerisation of anthocyanins and tannins. This has the effect of reducing the amount of free anthocyanins and increasing the amount of condensed anthocyanins. Importantly, these condensed forms are generally coloured at wine pH.

For example, at pH 3.40, 60% of polymerized anthocyanins are coloured but only 20% of free anthocyanins.

The effect of oxygen on phenolics and anthocyanins in wine has been thoroughly investigated by Ribereau-Gayon and Glories (1986). Much of the following information is from their investigation. Note that this discussion is not about flavour modifications that may be brought about by oxygen. Its focus is on the possible chemistry of oxygen introduction to wine. However, one might assume that altering the polymerisation of wine compounds would affect the flavour and mouthfeel. There are some major challenges here for wine researchers. The AWRI tannin project has indicated that 'stable red pigments correlate with quality (wine grade) but structures, colour properties and sensory (mouthfeel) properties largely remain to be established and put in context'.

The effect of aeration on anthocyanin levels, colour intensity and polymerisation is shown in Tables 1 and 2 (adapted from Ribereau-Gayon and Glories, 1986).

Table 1 shows that aeration causes a decrease in anthocyanin content and an increase in colour intensity. Note that the proportion of anthocyanins condensed with tannins increases with exposure to oxygen, as does the amount of polymerised pigment.

Table 1. Evolution of colour in red wine under different conditions

Conditions of storage	Anthocyanins (mg/L)	Colour intensity index*	Polymerised pigments	PVP index **
Tank	340	0.63	56	34
Aerated tank	240	0.72	66	45
Wooden barrel	240	0.75	64	47

(*pigments other than free anthocyanins, i.e. those that do not react with SO₂)

(**proportion of anthocyanins condensed with tannins)

Table 2 shows that it is the free anthocyanins that are mainly responsible for the observed decrease in anthocyanin levels. Overall, the coloured forms increase even though there is a drop in total anthocyanins.

Table 2. The Effect of Aeration on Anthocyanin levels

	Free Anthocyanins		Condensed Anthocyanins		Total Anthocyanins		Colour intensity**
	1*	2	1	2	1	2	
Non aerated tank	27	243	49	61	76	304	0.67
Aerated tank	18	171	78	33	96	204	0.72
Wooden barrel	16	146	96	22	112	168	0.83

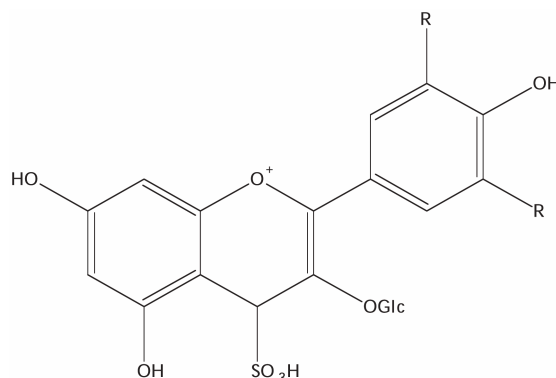
(*1. Coloured forms; 2. Colourless forms)

(** = A₄₂₀ + A₅₂₀)

The Effect of Sulfur Dioxide Addition

Sulfur dioxide addition has a negative effect on red wine colour. The aromatic nature of the anthocyanin's C ring is destroyed by the reaction of the anthocyanin with sulfur dioxide to form a bisulfite addition compound. This results in a colourless molecule (Figure 1). Furthermore, the reactive site at C4 is blocked from further activity. Polymerisation cannot occur.

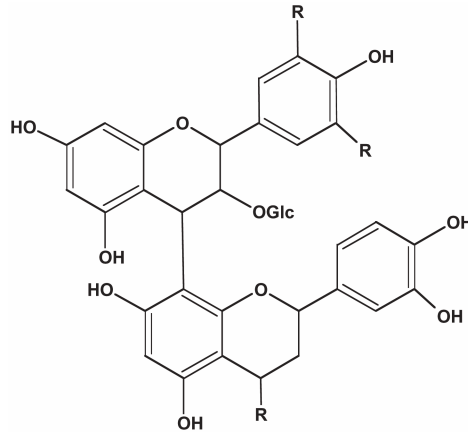
Figure 1. Colourless bisulfite addition compound showing blocked site at C4.



Because the C4 bond of the anthocyanin is involved in bond formation, polymeric forms are not reactive toward sulfur dioxide nor are they responsive to changes in pH.

Polymeric pigments show increased stability in comparison with monomeric anthocyanins. A generalised pigmented tannin comprising an anthocyanin (malvidin) and a phenolic molecule (catechin) is shown in Figure 2.

Figure 2. Pigmented tannin, showing C4-C8 linkage.



The Role of Acetaldehyde

Acetaldehyde plays a significant part in the early polymerisation reactions between anthocyanins and other phenolic compounds via the well-known “Baeyer reaction”. In this reaction, it acts as a “bridge” between anthocyanin and flavanol compounds. This is shown in Figure 3.

Acetaldehyde is formed from yeast metabolism during fermentation and from coupled oxidation of ethanol by phenolic compounds during maturation.

It can have a profound effect on colour in red wines. This is shown in Table 3 (adapted from Ribereau-Gayon and Glories, 1986). Note that anthocyanin levels decrease while condensation and tannin size (dialysis index) increase with acetaldehyde addition. If too much acetaldehyde is added, polymers increase to such a size that precipitation occurs and colour intensity decreases.

Figure 3. Acetaldehyde bridging reaction.

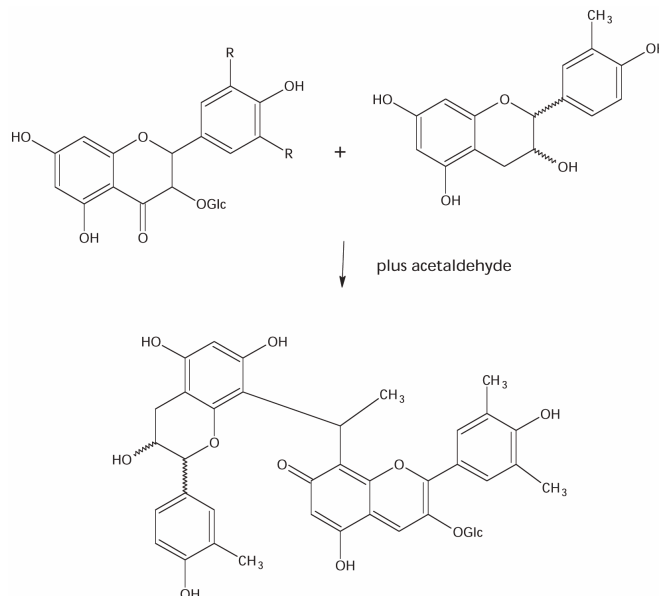


Table 3. Acetaldehyde addition to Cabernet Sauvignon for 3 months

Acetaldehyde mg/L		Anthocyanins (mg/L)	C.I.	PVP index	g/L	Tannins Dialysis index
Added	Consumed					
19	0	189	0.88	52	2.2	17
106	56	98	0.96	78	2.1	27
302	105	49	0.67	82	1.5	33

The Goals of Micro-oxygenation

If this evidence is accepted for the beneficial role of oxygen in early stages of red wine maturation, a better understanding of the goals of micro-oxygenation and how well or otherwise they have been achieved is gained.

Note that the evolution of desirable tannin structures in the mouthfeel of micro-oxygenated wines has barely been discussed. The changes that may occur in this area are very difficult to verify analytically—indeed they are impossible for winemakers to measure with the tools available to them. Nonetheless, sensory assessment has often shown significant changes in a treated wine's tannin profile. There is speculation that the process of polymerisation and condensation produces a softening and rounding of what would otherwise be hard and aggressive tannins but measurement of what is going on cannot yet be done. Other papers in these proceedings will outline their experiences and sensory results in this area.

When is Micro-oxygenation Used?

Prior to Malolactic Fermentation

Monomeric and oligomeric anthocyanins are more unstable in the early phase of wine maturation. Thus, from the micro-oxygenator's point of view, it makes sense to begin the process as early as possible in the life of a wine. The early addition of oxygen is intended to stimulate polymerisation and increase colour stability. This is an area of great interest to winemakers and it is here that the question of "micro-oxygenation-where now?" may begin to be answered.

Winemakers who have used the technique in this way report significant differences in the organoleptic assessment of the treated wines. Most noticeable is an increased awareness of the ability of wines to consume large amounts of oxygen without deleterious effects.

After MLF and SO₂ Addition

Reactions are slower and less "significant" after sulfur dioxide addition because of its ability to bind with acetaldehyde and quench oxygen.

This phase can be likened to a barrel replicate effect. In this way, micro-oxygenation is used to simulate the slow oxidation that takes place in barrel. It has been calculated that wine in barrel will receive approximately 30 ml of oxygen per annum if raked and topped regularly, or 2.5 ml/litre/month if averaged over the year. If oxidation is slow and moderate, only the most

oxidisable or reducing substances are oxidised and therefore protect the other important flavour and aroma compounds in wine.

How Much Oxygen is Required?

Wines vary in their response to, and need for, oxygen. There is no recipe that can be used to achieve a desired result.

Results so far show that wines that lack colour and structure (that is, are lacking the essential substrates for polymerisation) cannot take much oxygen. These wines are best not treated at all. If they are micro-oxygenated, it is best done after MLF and with low rates.

Wines with plenty of colour and structure can take large amounts of oxygen. The best wines for micro-oxygenation are those with large amounts of anthocyanin and phenolic material. There is very little risk of these wines drying out.

It has been found that any practice or condition which diminishes phenols leads to a diminution of the effects of micro oxygenation, i.e.:

- Early fining, especially with proteins and PVPP
- Laccase activity (from botrytis)
- Dilution

On the other hand, anything aimed at reinforcing of the structure will increase the effect:

- Use of oenological tannins and/or oak “alternatives”
- Blending in press wine

It should be noted that these additives should not be seen as complete replacements for grape-derived phenolic material. Excellent grapes will always be preferred to mediocre fruit with a cocktail of additives designed to replace what careless viticulture could not provide.

Monitoring

Wines must be monitored during micro-oxygenation. This can be time-consuming, especially in the pre-MLF phase. Monitoring of the following parameters is suggested:

1. Dissolved oxygen

There should be no discernible increase in dissolved oxygen levels if micro-oxygenation is conducted properly, with an appropriate oxygen flow rate.

2. Free sulfur dioxide, if present

There should be no significant decrease in free sulfur dioxide levels during micro-oxygenation. However, it is important to understand that this does not mean the flow rate is correct; simply that it is not too high.

3. Temperature

This is an important and often misunderstood parameter. In the experience of the author, micro-oxygenation works best between 14–17 o C.

If the temperature is too low, oxygen solubility is increased and reaction rates are decreased. This results in an increase in dissolved oxygen. If the temperature is too high, reactions occur more rapidly.

4. Turbidity

In general, wines should have some degree of clarification for successful micro-oxygenation. Wines should be below 200 NTU's and ideally below 100 (lees have a well-known affinity for oxygen). Of course, wines above these levels can be successfully treated but more effort is required to monitor.

5. Tasting

Tasting micro-oxygenated wines is not intuitive. Some training and exposure to treated wines is valuable.

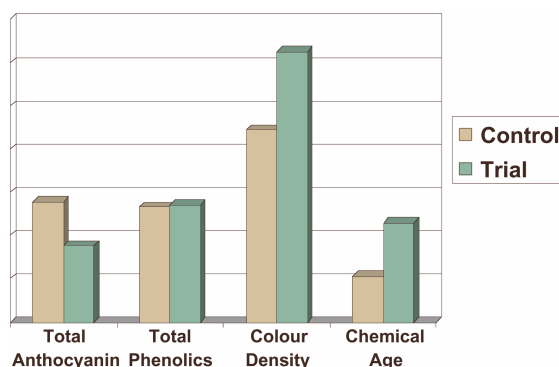
Results

Many Australian wines have been successfully treated. However, it has been difficult to persuade winemakers to retain control wines for evaluation purposes. The following examples were small batch (400 litre) trials in specially designed tanks. The flow rates were significantly higher than would normally be used.

In a recent (July 2002) tasting involving 20 regional winemakers, the treated wines were overwhelmingly preferred. Interestingly, in July 2001, a similar group overwhelmingly favoured the controls, due to the level of perceived aldehyde in the treated wines which were still being micro-oxygenated.

In figure 4, data is presented without numerical scale to allow easy comparison.

Figure 4. 2001 Central Victorian Cabernet Sauvignon

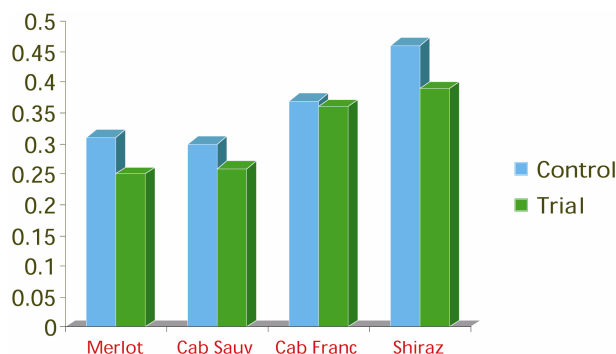


Microbiological Considerations

Many winemakers are concerned about the potential for microbial spoilage during micro-oxygenation. Volatile acidity is often mentioned as a likely problem. As far as the authors are aware, no wines treated in Australia or New Zealand have shown increased levels of V.A.

In fact, levels often drop during micro-oxygenation, as shown in Figure 5 which illustrates four wines from the 2001 South Australian vintage.

Figure 5. Volatile Acidity, 2001 Vintage (g/L)



Brettanomyces is becoming of more concern to Australian winemakers. While there is evidence to suggest that oxygen will stimulate the growth of *Brettanomyces*, no direct link can be established with micro-oxygenation. If *Brettanomyces* grows in a micro-oxygenated wine, the author suggests that there may be other, more fundamental reasons for its proliferation, including high pH, low SO₂ and stressed fermentation amongst others. Winemakers contemplating micro-oxygenation need to understand the means for controlling *Brettanomyces* and make sure that they take the necessary precautions to minimise its impact:

- If micro-oxygenating prior to MLF, ensure that no residual sugar is present in the wine. *Brettanomyces* loves sugar! Thus, a sluggish, difficult-to-ferment wine may not be a good choice for micro-oxygenation unless the winemaker is absolutely sure of its status, both chemical and micro-biological.
- Ensure prompt and stringent pH control.
- Add SO₂ at crush (50 ppm).
- Ensure sulfur dioxide levels in finished wines are adequate. Levels of 80 ppm total SO₂ have been quoted as inhibiting *Brettanomyces* growth.
- Do not micro-oxygenate at high temperatures.
- Do not micro-oxygenate wines made from unsound fruit. There is evidence that diseased fruit can considerably increase spoilage organism load.

In other words, if sensible winemaking practices that are designed to control *Brettanomyces* are followed, micro-oxygenation should not present any problems.

What Next?

- *Use of lysozyme to delay MLF*

Lysozymes show some promise for the more adventurous winemaker who wishes to treat wines as much as possible before malo-lactic fermentation.

Lysozyme degrades the cell walls of gram-positive bacteria such as *Oenococcus*, *Pediococcus* and *Lactobacillus* and thus reportedly delays or inhibits MLF. This allows more time for micro-oxygenation before sulfur dioxide addition.

- *Higher flow rates*

As users become more comfortable with the technique, flow rates will increase as they seek more and more structural effects. Levels as high as 300 ml/litre/month have been used overseas. In Australia, rates of 80 ml/litre/month are not uncommon.

- *Co-pigmentation, tannin measurement and monitoring*

A greater appreciation of grape and wine polyphenolics is now evident in the wine industry. The importance of anthocyanin/condensed tannin polymerisation is now well recognised and the role of co-factors such as quercetin, caftaric acid and isovitexin is being promoted as fundamental to maximum colour extraction. However, co-pigmentation does not of itself confer colour stability. Micro-oxygenation has been shown to do so and, allied with a more complete understanding of the role of endogenous and exogenous tannins and their use, provides winemakers with the ability to more closely control the style of wine produced.

- *Redox potential*

Better understanding of the influence of redox potential on the way in which a particular wine responds to oxygen could allow even more precision in micro-oxygenation. It is already known that wines with high phenol content generally have a low redox potential and a high capacity for oxygen—remembering that the lower the redox potential, the more a wine is protected from oxidation. However, quantifying this concept in any meaningful way has yet to be done.

- *Use on super-premium and icon wines*

As users see the effects of micro-oxygenation, they will increasingly use it on better wines. Many winemakers are cautious and wish to trial the technique on less expensive wines before they commit to serious decisions. Once satisfied that it is not a dangerous technique if properly monitored, they understand the potential benefits for high quality wine.

- *Replacement of barrels*

There are significant cost savings to be made by replacing barrels with maturation in tanks complemented by the addition of barrel alternatives such as staves or planks. Table 4 shows some indicative costings (\$A).

Table 4. Cost Comparison of barrels vs. staves for maturation of wine.

	New Barrel + 3 y.o.	Barrel 3 y.o.	Tank + new stave		Tank + stave 2 fill	
			#1	#2	#1	#2
Purchase	2.22	0.88	1.04	0.33	0.52	0.17
Labour	0.01	0.01	0.002	0.002	0.002	0.002
M/ox			0.02	0.02	0.02	0.02
Total	2.23	0.89	1.06	0.35	0.54	0.19

Future Technology

Micro-oxygenation is still a new winemaking technique. As it becomes more widespread, developments of the process are likely. These may include:

- *Barrel micro-oxygenation, using very low flow rates.*

In a somewhat broadened form, this is already happening. At the recent Romeo Bragato conference in Christchurch, New Zealand, Nigel Tibbits from Kumeu River Wines explained his approach using regular doses of oxygen to current vintage reds. Strictly speaking, this periodic addition should be called “meso-oxygenation” but if refinements are made that allow continuous oxygen input into barrels, this may negate the need for racking. The advantage here, apart from an obvious labour-saving one, is the greater degree of control available. To quote Mr Tibbits: ‘It is extremely difficult to gauge the amount of oxygen absorbed by red wine during a normal racking, because of the large number of variables involved and the resultant effects on the wine can be haphazard. Monthly oxygenation of the wine, on the other hand, has a very similar effect to racking but with a far greater degree of control.’

- *Small tank micro-oxygenation*

– using specially-designed equipment that can avoid the potential problem of oxygen accumulation in the headspace of small tanks, which is a feature of the current technology.

- *New approaches to oxygen introduction into wine.*

These could include such techniques as diffusion where oxygen moves through a “membrane” of material with a known diffusion coefficient.

Previous micro-oxygenation technology has been based on controlling the bulk flow of oxygen to the wine and dispersing the oxygen into the wine in the form of fine bubbles—the finer the better. The technology described here exploits the principle of controlled diffusion across a membrane, described by Fick's Law:

$$dM/dt = m A/x \Delta P$$

(where M is mass of diffusing gas, t is time, m is permeability, A is membrane area, x is membrane thickness and DP is pressure differential of the diffusing gas).

For a given gas and the right combination of membrane structure and size, this allows simple pressure control of diffusion rate. Since the process does not involve the presence of bubbles, there is no minimum head to allow dissolution, so the process can be adapted to vessels of any size or shape—including barrels.

This technology has been patented and is in the process of commercialisation. The first industry trials are now taking place in Heathcote, Victoria.

Conclusion

Micro-oxygenation is a well-defined process for improving wine quality. The chemistry underpinning the technique is not clearly understood at this stage but many winemakers are persuaded of its value by the empirical evidence of treated vs. control wines.

An understanding of the role of oxygen in anthocyanin/phenolics interaction in wine is useful when considering which wines to treat and at what rates.

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This article is sourced from the Australian Society of Viticulture and Oenology, Use of Gases in Winemaking Seminar Proceedings, October 2002, p.18.

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