The use of epifluorescence versus plating to monitor the effect of different parameters on microorganisms in wine

W.J. du Toit[1], I.S. Pretorius[1,2] and A. Lonvaud-Funel[3]

[1]Department of Viticulture and Oenology, Institute for Wine Biotechnology, Stellenbosch University, Victoria Street, ZA 7600 Stellenbosch, South Africa, [2]The Australian Wine Research Institute, Waite Road, Urrbrae, SA 5064 Adealide, Australia, and [3]Faculté d'Oenologie, 351, Cours de la Libération, 33405 Talence, France
E-mail: wdutoit@sun.ac.za

The monitoring of the number of micro-orgranisms in wine is crucial for the wine producer. Traditional counting methods include microscopic enumeration and plating on selective media, which measures the culturability of the cells. The use of epifluorescence microscopy is, however, a method, which can measure both culturability and viability in wine. This method distinguishes between live and dead cells. Research showed that little difference existed between plating and epifluorescence numbers to enumerate lactic acid bacteria in wine. However, a difference exists between these two methods to distinguish between acetic acid bacteria numbers in wine. Plating counting numbers were lower than plate numbers for *Acetobacter pasteurianus* in wine under anaerobic conditions. This difference was, however, negated by the addition of oxygen to the wine. SO_2 additions lowered the culturability of *A. pasteurianus* at dosages higher than 0.35mg/L molecular SO_2, but higher dosages were required to lower epifluorescence intensity, which is an indication of viability. *Brettanomyces bruxellensis* culturability was inhibited at lower dosages, but total cell numbers according to epifluorescence microscopy were affected at higher molecular SO_2 dosages. Epifluorescence microscopy and plating also showed that *B. bruxellensis* was drastically affected after 120 min after molecular SO_2 addition and it's culturability after only 30 min. An exposure time of 5 min to molecular SO_2 reduced the cell's viability drastically and 45 min completely inhibiting the viability after two days. The bonded form of sulphur dioxide did not affect both micro-organisms.
Epifluorescence microscopy can thus be used as a quick alternative to assess micro-organisms numbers and culturability in wine. This technique has both advantages and disadvantages over traditional enumeration methods, which will also be discussed.