# High power ultrasonics: a new and powerful tool for removing tartrate deposits and killing viable *Brettanomyces* cells in barrels

Andrew Yap[1] and Warwick Bagnall[1]

## INTRODUCTION

Oak products constitute the greatest cost element in wine production, after the cost of grapes (Corsey 2006). An estimated seven million wine barrels are in use worldwide. Shortened barrel life – reduced not only by microbial contaminants and taints, but also by excessive tartrate build-up – is also a major problem for winemakers, because barrels typically represent their largest recurring plant and equipment capital expenditure item. The control of microbiological contamination during barrel ageing and, in particular, the spoilage yeast *Brettanomyces*, is one of the major oenological issues identified by winemakers all over the world. Spoilage of wine in barrel generally results in loss of value through downgrade in quality and loss of wine. Further financial loss is due to the fact that the wine has already received 75% of its processing before spoilage occurs.

During red wine maturation, potassium bitartrate crystals, yeast lees and microbial cells deposit on the inner surface of the barrel. If tartrate deposits are not adequately removed during the cleaning process, they increase in thickness and hardness over time (Yap *et al.* 2007a). Dead and living microbial cells and spores migrate into the pores of the wood by capillary action and, thus, impregnate and clog the pores. Microbial biofilms may also form on surfaces wherever microbial cells can survive. Pseudomycelia formation by *Brettanomyces* assists in the organism's ability to penetrate the pores of oak wood.

[1]Cavitus Pty Ltd, PO Box 260, Crafers, SA 5152
Email: ayap@cavitus.com

High power ultrasound (HPU) has become an efficient tool for large-scale commercial applications only in the last six to eight years. The mechanism of HPU and its application in food processing have been recently reviewed (Patist and Bates 2008). The energy released from cavitation (collapse of high-energy micro-bubbles) creates shockwaves that transfer kinetic energy, acoustic streaming and vibration. The energies generated by HPU disintegrate solids and remove layers of solid material or dirt from surfaces and porous interior structures; kill micro-organisms on surfaces in liquids and in porous interior structures; and reduce particle size and prevent undesirable matter adhering to solid surfaces. Ultrasound also provides an efficient method of stressing cells and detaching them from solid surfaces. In the food and pharmaceutical industries, HPU is currently employed to remove biofilms from surfaces of equipment and stainless steel lines (Bates, pers. comm.). Recent trials in Australian and Californian wineries successfully demonstrated that HPU is an effective tool in extracting colour, anthocyanins, flavour and tannin from red must. Formal sensory assessment of the wines made from Pinot Noir, Sangiovese, Shiraz and Merlot grapes found that HPU-treated wines have palate weight, and flavour, textural and structural complexity greater than that of untreated (control) wines.

Current practices and methods employed for cleaning and disinfection of barrels have been reviewed by Yap *et al.* (2007b). They are clearly deficient, as evident by the rampant spread of the insidious spoilage yeast *Brettanomyces* in all wine-producing countries.

Validation trials by the Australian Wine Research Institute (AWRI), the University of Adelaide and University

Figure 1. A barrel being sonicated using Cavitus' HPU barrel cleaning and disinfection system. Ultrasound is delivered by a sonotrode inserted through the bung-hole into a barrel filled with water at 60°C. Tartrate removal and destruction of spoilage micro-organisms occurs simultaneously. Water after cleaning is filtered and reused up to 100 times.

of South Australia in 2007 and 2008 showed that barrel cleaning and disinfection by HPU is superior to that of high-pressure hot water (HPHW) operating under parameters typically used in industry (1000psi/6900kPa and 60°C for 5 minutes). HPU not only removed all tartrate deposits but also killed all (100%) viable *Brettanomyces* cells on the surface and up to a depth of 4mm in the wood (Yap 2008; Yap *et al*. 2008).

Ultrasound energy did not affect the internal structure of oak wood (up to a depth of 8mm), as determined by computer X-ray tomography.

In 2008 and 2009, trials in Australia, New Zealand and California compared HPU and HPHW technologies for barrel cleaning and disinfection. This paper presents data from trials in two large Californian wineries, comparing the efficacy of HPU and HPHW for tartrate removal and disinfection (inactivation of viable *Brettanomyces* yeast cells). The advantages HPU has over current technology and the benefits it will bring to the wine industry will be discussed.

## EQUIPMENT AND BARRELS USED IN THE TRIALS

The application of HPU to barrels to remove tartrate deposits or kill viable *Brettanomyces* cells was carried out with Cavitus' HPU Beta prototype cleaning and disinfection system (pat. pend).

The system comprises components for ultrasound production (comprising a 4kW ultrasonic generator, transducer and sonotrode), a reverse osmosis (RO) water production unit, hot water heater, storage tank and filtration unit (for recycling water). The semi-automatic system allows one barrel to be filled with RO water at 60°C, another treated with HPU (Figure 1) and a previously sonicated barrel emptied, simultaneously. The sonication times used in the trials varied from 5-12 minutes, depending on the age of the barrel and other factors, such as the history of the barrels, type of cleaner previously used to clean the barrels, and the length of time the barrels were left in a dry state post-rinsing.

The Tom Beard barrel washer, manufactured by the Tom Beard Company, California (www.tombeard.com), was used by both wineries for HPHW cleaning. The pressure employed by the wineries varied and ranged from 120-300psi (830-2070kPa) and water temperature ranged from 60-82°C, respectively.

Barriques made from French and American oak and representing four different ages were used in the trials. Barrels of the same age had similar usage history and were made by the same cooperage with timber from the same source.

## TARTRATE REMOVAL FROM BARRELS BY HPU AND HPHW TREATMENTS

The efficacy of the removal of tartrate deposits by the two treatments, HPU and HPHW, from the interior surface of barrels was examined with 40 red wine barrels representing three different ages (2007, 2005 and 2004) from the two wineries. On the day of the trial, wine was racked from each barrel and the empty barrel given a 20-second rinse with low pressure ambient temperature water via a static spray head. Barrels of the same age were then randomly divided into two sets and one headstave of each barrel was removed. The interior surfaces of the barrel and headstaves were examined with the naked eye by the winemaker representing the winery and the lead author of this paper. The thickness and texture of the tartrate deposit, and the total surface area covered by solids (tartrates and yeast lees) were recorded. The appearance of the deposit and its distribution on the barrel surface were recorded with a digital camera. After replacing the headstaves, one set of barrels from each age was cleaned by HPU and the other by HPHW. Following the cleaning process by the two technologies, the headstave of each barrel was again removed and the interior of the barrel and headstaves examined as before. Data from three representative cleaning trials are presented in Figures 2 and 3 (see page 32). Trials carried out in Winery A with 2007 and 2005 barrels employed HPHW at 120psi/830kPa and 60°C for two minutes, followed by ozonated water (2.0mg/L ozone) spray for one minute, while at Winery B, HPHW at 300psi/2070kPa and 82°C was applied for three minutes, followed by ozonated water (3.5mg/L ozone) spray for four minutes. The 2007 barrels were sonicated for five minutes at 60°C, and 2005 and 2004 barrels for eight minutes at 60°C.

# Grape Colour and Flavour Extraction

- Continuous high-volume process (post-crushing)
- Releases more colour and flavour compounds from skins and flesh
- Applicable to both red and white grapes
- Significantly improves wine quality (colour, flavour, texture, structure, palate weight)
- Ideal for grapes with low colour pigments and flavour
- Eliminates cold-maceration and extended maceration
- Reduces primary fermentation and fermenter capacity requirement
- Improves opportunities for upgrading, blending and earlier processing
- Increases purchasing and harvesting choices (timing, quality, pricing)

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Demonstrations of Cavitus' HPU Mobile Barrel Cleaner will take place in the Barossa Valley in late October; Adelaide Hills, Clare Valley, Mount Benson, Padthaway, Coonawarra, Geelong, Macedon Ranges, Mornington Peninsula, Yarra Valley, North-East, Central and Western Victoria, Langhorne Creek and McLaren Vale in November; and New South Wales, Western Australia and Tasmania in Q2 and Q3 2010.

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before

after

Cavitus is the leading developer and solutions provider of HPU (high-power ultrasonics) applications for food and beverage processing.

# Wine Barrel Cleaning and Disinfection

- Simultaneous cleaning and disinfection
- Removes up to 99.9% tartrate from surfaces, pores, cracks and blisters
- 100% Brettanomyces kill on surface and up to 4 mm below surface; up to 5 log kill elsewhere
- Optimal oak transfer and oxygenation
- Lower costs - 4 litres water per clean, no chemicals, less power
- Competitive cycle time vs conventional high pressure hot water and ozonation
- Longer barrel life
- Improves barrel inventory condition and value
- Attractive pay-back and cash savings
- Static or mobile options

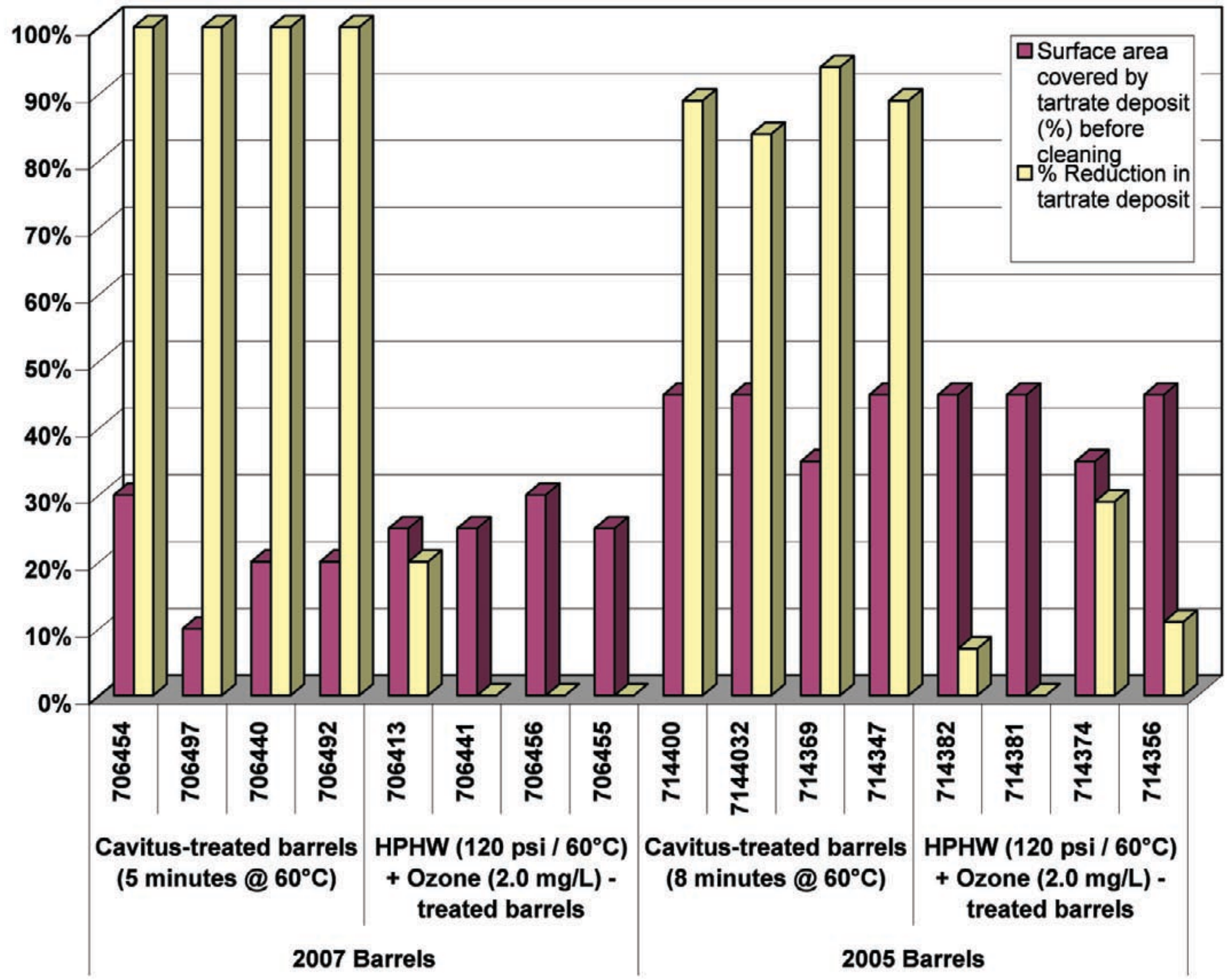


Figure 2. Removal of tartrate deposits by HPU and HPHW from the surface of 2007 and 2005 red wine barrels.

The tartrate deposits in the 2005 and 2004 barrels were hard to very hard, whilst those in the 2007 barrels were soft to hard. HPU treatment consistently removed more tartrate than HPHW. Initial area covered by tartrate deposits in 2007 barrels ranged from 10-30%. Following HPU cleaning, all tartrates were removed (100% reduction), whereas no reduction occurred in three barrels and up to 20% in the other HPHW-cleaned barrel (Figure 2). In the 2005 barrels, tartrates were reduced by 84-94% in HPU-cleaned barrels, compared with 0-29% in HPHW-cleaned barrels (Figure 2). HPU gave 70-100% reduction in tartrates in 2004 barrels compared with 25-75% by HPHW (Figure 3).

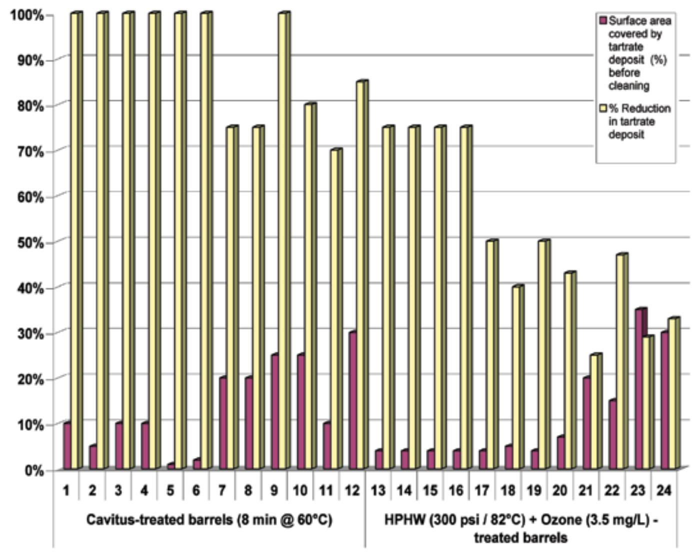


Figure 3. Removal of tartrate deposits by HPU and HPHW from the surface of 2004 red wine barrels.

## DESTRUCTION OF VIABLE *BRETTANOMYCES* CELLS IN INFECTED BARRELS BY HPU AND OZONE TREATMENTS

The efficacy of ozone at two concentrations (3.5 and 7.6mg/L) was compared with that of HPU in Winery B. Ozone was applied immediately following cleaning by HPHW at 82°C. Barrels containing wine with 4-ethylphenol (4-EP) and 4-ethylguaiacol (4-EG) were selected. The presence of viable *Brettanomyces* cells in the wines was confirmed by Scorpion Microbial Assay (SMA) (Culbert *et al*. 2008). After the wine was removed the barrels were rinsed with water at ambient temperature via a static spray head for about one minute. To facilitate the removal of viable *Brettanomyces* cells from the walls of and pores in the staves of each barrel, pre- and post-HPU and HPHW/ozone treatments, the following procedure was applied: 500mL of a sterilised alcoholic saline solution (12% v/v alcohol; pH4.2) was aseptically introduced into the empty barrel.

The bung hole was sealed with a sterilised silicon bung and air removed to create a vacuum (see Figure 4), after which the barrel was gently rolled for 20 seconds. The solution was then collected aseptically for microbiological testing. The number of viable *Brettanomyces* cells present was determined by plating (using YM + cycloheximide and Apple Juice Rogosa + cycloheximide media) and SMA.

In the first trial, 24 barrels comprising eight of each of three different ages (2007, 2006 and 2005) were used. The barrels were divided into two sets of 12 barrels. Each set comprised 4 x 2007, 4 x 2006 and 4 x 2005 barrels. One set was treated with HPU at 60°C for eight, 10 and 12 minutes for the 2007, 2006 and 2005 barrels, respectively. The other set was treated with HPHW at 300psi at 82°C for three minutes, followed by ozonated water (3.5mg/L) spray for four minutes. Following HPU treatment, water was removed from the barrels by draining for several minutes, while ozone-treated barrels were given a quick rinse with cold water, before microbiological sampling. The results of the tests before and after HPU and HPHW treatments are given in Table 1.

The infected barrels chosen for the trial contained large populations of viable *Brettanomyces* cells. Initial viable cell numbers present, as determined by SMA, ranged from 80,640 to 4.7m cells/mL. Barrels treated by HPU showed a dramatic reduction in cell numbers, viz.99.96-100%, as determined by plating and 94.2-99.3% (by SMA). Barrels treated with ozone after being subject to 82°C heat gave no reduction in viable cell numbers in eight out the 12 barrels, but gave a 21.2-47.1% kill in three barrels (as determined by SMA).


Figure 4. Air being evacuated from the barrel to enhance the extraction of viable *Brettanomyces* cells from the surface and pores of the staves by sterilised alcoholic (12% v/v) saline solution.

Table 1. Destruction of viable *Brettanomyces* cells by HPU and ozone in 2007, 2006 and 2005 infected red wine barrels.

| Barrels treated with HPU | | | | Barrels treated with HPHW (300psi / 82°C for 3 min, following by ozonated water (3.5mg/L) for 4 min | | | |
|---|---|---|---|---|---|---|---|
| Barrel ID | SMA cells/mL | SMA % reduction | Plating % reduction | Barrel ID | SMA cells/mL | SMA % reduction | Plating cfu/mL |
| CAV101 | 135,750 | 94.2 | 99.997 | OZ101 | 253,530 | 47.1 | TNTC[b] |
| CAV102 | 154, 150 | 97.3 | NA[a] | OZ102 | 218,640 | 0 | TNTC |
| CAV103 | 4,698,260 | 99.0 | 99.999 | OZ103 | 359,880 | 34.8 | TNTC |
| CAV104 | 1,166,160 | 96.7 | NA | OZ104 | 245,410 | 0 | TNTC |
| CAV105 | 591,440 | 97.4 | 99.96 | OZ105 | 80,640 | 0 | TNTC |
| CAV106 | 314,970 | 99.1 | 99.97 | OZ106 | 243,130 | 0 | TNTC |
| CAV107 | 67,950 | 97.8 | NA | OZ107 | 347,810 | 0 | TNTC |
| CAV108 | 141,000 | 99.3 | NA | OZ108 | 108,840 | 21.1 | TNTC |
| CAV109 | 144,880 | 98.6 | 99.997 | OZ109 | 118,070 | 0 | TNTC |
| CAV110 | 311,810 | 97.2 | NA | OZ110 | 189,440 | 0 | TNTC |
| CAV111 | 383,790 | 98.3 | 100.0 | OZ111 | 130,790 | NA | 99.94% kill |
| CAV112 | 167,739 | 99.3 | 99.998 | OZ112 | 411,520 | 0 | TNTC |

Note:
[a] Data not available
[b] Too numerous to count

In the second trial, a higher concentration of ozone (7.6mg/L) was used. Six barrels infected with *Brettanomyces* (4 x 2007 and 2 x 2005) were treated with HPHW at 300psi at 82°C for three minutes, followed by ozonated water for four minutes. Initial cell numbers ranged from 194,900 to 496,320 cells/mL. Data available for only three of the six barrels showed a 1-log reduction (by SMA). These data show that treatment of HPHW-cleaned barrels is more effective at a higher concentration of ozone (7.6mg/L compared with 3.5mg/L).

## DISCUSSION AND CONCLUSION

Previous studies over the past five years by the authors on more than 500 barrels ranging in age from one to nine years found that the application of HPHW resulted in a highly variable degree of cleanliness of the barrels. Data presented in this paper from Californian trials correlate well with the data from the above studies and those of the AWRI, the University of Adelaide and University of South Australia on the effectiveness of HPU for barrel cleaning and disinfection (Yap *et al.* 2008).

Although ozone is widely used in the USA, NZ and some European countries, its effectiveness as a barrel sanitiser is only anecdotal. There is a lack of scientific information about the efficacy of ozone for killing *Brettanomyces*. The concentration of ozone commonly used in wineries in California and New Zealand range from 1.5-4mg/L. These doses are likely to be sub-lethal, according to data from these trials. A concentration of 3.5mg/L has been shown to

**The merits of ultrasonic treatment of barrels are compelling, namely:**

- reduction of inventory degradation and waste
- extension to barrel life due to more uniform cleaning, such that the maximum quantity of oak flavour compounds are accessible, spoilage micro-organisms cannot survive to adversely impact upon wine flavour and reduce the life of the barrels, and the maximum amount of air can infuse the wine via stave capillary action
- lower input costs, resulting from the use of hot water at lower temperature that does not require the later addition of chemicals for disinfection purposes, thus saving electricity and chemicals
- reduction in Occupational Health and Safety (OH&S) risk and workplace injury, since the technology uses a lower temperature and no chemicals
- improvement in environmental costs, because HPU technology eliminates the use of chemicals and the need for their special disposal
- lower operational costs from reducing the two-step washing and disinfection process to one step
- decrease in brand risk — the risk results from consumers' growing concerns about the use of additives in foods — since the technology reduces the need for chemical use amongst winemakers to stabilise wine and contain microbiological populations.

be ineffective, while up to 7.6mg/L (applied as a spray wash for four minutes following HPHW treatment at 82°C) is required to reduce viable *Brettanomyces* cells by 1-log in some infected barrels. It should also be noted that there are also health issues related to the use of ozone in the winery environment and it readily oxidises oak wood and, thus, decreases the amount of oak volatiles that can be imparted to wine during the maturation cycle.

Barrels cleaned by HPHW do not realise the full flavour potential of the barrel because the most desirable flavours that are contained within a thin layer (2-3mm) of toasted wood at the barrel surface are either obscured by unremoved tartrates or destroyed. Shaving, dry-ice blasting and HPHW indiscriminately destroy the toast layer. Several studies are in progress to compare the impact of HPU and HPHW on the retention of flavour compounds by the barrel. Data from a recently completed 12-month study at a Barossa Valley winery in South Australia has established that barrels cleaned by HPU retained more toasted oak flavour compounds than barrels cleaned by HPHW (1000psi at 60°C for eight minutes). This study also showed that there was a higher concentration of furfural in Shiraz wines stored in one-year-old barrels cleaned by HPU (these data will be published at a later date). Due to their desirability and scarcity in oak barrels (Garde-Cerdan and Ancín-Azpilicueta 2006), furfural (flavour threshold is 14,000μg/L in water/alcoholic solution) is an ideal performance indicator to compare the impact of the two cleaning techniques. It has been shown that complete removal of tartrate deposits from the surfaces of staves (Yap 2008), and residues and microbial cells from the pores and cracks can be achieved by ultrasonic action during the cleaning process. Thus, the most likely reason for the increase in furfural yield and extraction rate in the HPU-treated barrels is increased availability of furfural compounds and increased rate of diffusion due to the removal of inhibiting layers of tartrate on the surfaces and the pores. This may shorten the time necessary for the wines to attain the desired oak maturation characteristics, i.e., shorter maturation time and possibly extending the useful life of the barrel. Another explanation for the lower furfural concentration in HPHW-cleaned barrels is the removal of toasted compounds due to damage to or removal of the thin toast layer caused by jets of high pressure hot water, as suggested by the presence of patches of oak surfaces with furry appearance post-HPHW cleaning.

High-power ultrasonics technology offers a solution to the age-old problems in the wine industry of microbial contamination and spoilage of product and barrels, which have traditionally resulted in value losses at the cellar door in the order of 12-15% per bottle. Together, these problems are estimated to cost the wine industry more than US$1 billion annually. Energy costs and water usage for HPU applications are low. Energy costs are approximately 1kWh per barrel and cleaning trials in wineries showed that an average of 4L of water is required to clean a barrel. In comparison, HPHW technology is energy- and water-intensive.

## SUMMARY OF KEY FINDINGS

HPU energy was more effective in removing tartrate deposits and solid residues from the interior surface of barrels than HPHW. All tartrate deposits were removed from 2007 barrels by HPU treatment compared with 0-20% reduction by HPHW.

HPU was superior to HPHW in the destruction of viable *Brettanomyces* cells in infected barrels. HPU gave 99.9-100% kill, as determined by the plating method and 1- to 2-log kill by SMA. In contrast, ozone at 3.5mg/L, combined with HPHW treatment at 82°C, gave no reduction of viable cells (by SMA) present in eight out of 10 barrels infected with *Brettanomyces*. Ozone at 7.6mg/L gave improved kill – up to 1-log kill in only three out of six barrels infected with *Brettanomyces*.

## ACKNOWLEDGEMENTS

The Californian winery trials involved considerable planning and their execution required the co-operation of the program leaders, winemakers, cellar masters, barrel masters, microbiologists, laboratory and cellar personnel, engineers and others. The authors would like to thank all of them for their willingness to participate and goodwill, and Dr Terry Lee for assisting in the preparation of this paper and for critical comments.

*More information about Cavitus' HPU barrel cleaning and disinfection systems, static or mobile, can be obtained from www.cavitus.com, or from Andrew Yap, director of oenology and industry marketing.*

*Andrew Yap was formerly senior lecturer in oenology and wine microbiology at The University of Adelaide and the former Roseworthy Agricultural College. He is currently director of oenology and industry marketing of Cavitus Pty Ltd and honorary lecturer in wine science at The University of Auckland. Warwick Bagnall is senior engineer of Cavitus Pty Ltd and has considerable experience in the application of HPU for food and beverage production over the past 10 years.*

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