Microbiological stability of wine packaging in Australia and New Zealand

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One of the last processing steps that takes place before a wine reaches a consumer is the packaging process. Whether a wine goes into a cask, bottle or other vessel, the decisions and processes carried out at the point of packaging will have a significant impact on the characteristics of the wine when it is consumed. Ensuring that wines are free from microbes that can grow in the packaged product is one of the key considerations of the packaging process, and one of the more difficult to manage. This article presents knowledge gained through the conduct of microbiological audits of packaging processes across the Australian and New Zealand wine industries. It includes a brief overview of industry practices, some common misconceptions regarding the microbiology of bottling and an audit case study.

INTRODUCTION

Packaging is an integral step in the wine production process that strongly influences the integrity of the wine that reaches consumers. The packaging process can have a significant impact on a wine's longevity, including its microbiological stability. If microbial contamination occurs during packaging it may not only result in off-flavours but can also cause hazes and deposits, both of which negatively affect consumer perception. Each year the AWRI helpdesk is contacted about packaging-related microbial spoilage issues such as filter failures, refermentation in bottle and sporadic yeast or bacterial growth. The worst cases of such problems can result in costly product recalls and brand damage.

In response to these issues, AWRI Commercial Services developed a microbiological audit service for wine packaging facilities. This service adopts an investigative approach to assess the efficacy of sanitisation regimes and search for any underlying issues that could lead to microbial problems. After an audit is completed, detailed information is provided to the facility describing areas of increased risk and recommendations on improvements to procedures or facilities. More than 20 packaging line audits have been conducted in Australia and New Zealand since 2011.

WHAT STEPS ARE CARRIED OUT DURING AN AUDIT?

Each packaging audit is slightly different, because it will depend on the design of the packaging line and the type of issues (if any) that have been reported. The following general steps are carried out:

- a review of past and current microbial issues
- an evaluation of current sanitisation methods and procedures
- identification of design or infrastructure risks (e.g. degraded o-rings, unused valves)
- testing of inputs into the packaging line (e.g. wine, rinse water, dry goods) for viable microbes
- swabbing of numerous points throughout the packaging line, and plating out the swabs to check for the presence of viable microbes (yeast, bacteria, or moulds).

Audits are ideally carried out directly after a full sterilisation procedure, and access is required to the internal surfaces of the bottling line to be able to conduct thorough testing. Key areas to focus on are filter integrity, streamlined piping, incoming water, routine maintenance of o-rings on valves and filler heads, vacuum lines and minimisation of bypass line use.

WHAT PROCEDURES ARE BEING USED IN INDUSTRY?

From the audits that have been conducted, it is clear that there is no single industry standard for packaging wine and this has led to widely varied practices for controlling microbial contamination. For packaging lines a common practice used to 'sterilise' the line is to flush the entire unit with 80°C hot water for 20-30 minutes, or to use steam in conjunction with caustic cleaning agents. When hot water is used for sterilisation, a minimum temperature of 80°C is critical since the heat is absorbed by the stainless steel ensuring hard-to-access points that may harbour microbial contamination (such as joints, seals and valves) are reached. Such points can be difficult to reach when using chemical cleaning/sanitation agents. Another method is to use ozone; however this is less

AT A GLANCE

- Microbiological spoilage issues occurring in wine after packaging are reported to the AWRI helpdesk each year, and can cause costly product holdbacks or recalls. Examples include refermentation of an entire batch of packaged wine and cases of sporadic yeast or bacterial spoilage.
- Audits of packaging facilities have found that packaging practices designed to avoid microbiological contaminations vary throughout industry.
- Key areas to target include: cleaning procedures, testing of inputs, water treatment, routine maintenance and implementation of QA specifications.
- Changes to procedures and infrastructure can significantly reduce occurrences of microbiological failures.

common and may also not be effective in reaching all critical points in the packaging line.

The timing of when and how often a line is 'sterilised' is again varied. In the majority of facilities that have been audited, the line is 'sterilised' every day prior to a bottling run and rinsed with water at the end of the day. Often it is standard practice for high risk products that contain residual sugar and are low in alcohol to be run at the end of a round of bottling to reduce the risk of cross contamination between products.

For the assessment of cleanliness, a common practice is to use swabs which detect the presence of ATP (a molecule associated with the presence of organic material). However, it is important to note that false positives can occur with this type of testing, as it can detect dead cellular material as well as viable microbes.

Another common practice is the storage of unused filtration units in potassium metabisulfite (PMS)/citric acid solution. While this is an effective storage medium, it must be carefully managed because the anti-microbial sulfur dioxide released from the potassium metabisulfite can deplete rapidly in the acidic solution, resulting in a citric acid solution with little anti-microbial activity. Monitoring the sulfur dioxide concentration and topping up the solution with PMS when needed can overcome this issue; however few of the facilities that were audited were found to conduct such monitoring.

'STERILE' IS NOT REALLY 'STERILE'

'Sterilisation' is a term commonly used in the wine industry to describe hot water sanitisation of a packaging line. The term 'sterile' suggests that a surface or wine is 'free from living organisms'. In general, this is a misconception since the standard environment in which wine is bottled does not allow for true sterilisation; there are always some environmental microorganisms present. Additionally, what is generally termed 'sterile filtration' in the wine industry (filtration using 0.45µm membrane filters) does not necessarily always exclude bacteria or yeast spores. Filtration is certainly effective in removing the majority of microorganisms from wine; however the wine may still become contaminated after filtration if there are areas in the packaging line after the filter where cleaning has been insufficient or biofilms have formed. Biofilms are communities of microorganisms attached to a surface that form a protective extracellular layer over time. This layer can make them resistant to sterilisation efforts and, therefore, a source of sporadic microbial contamination. Biofilms commonly form at 'dead spots' or unused valves in packaging lines - areas that are not flushed out regularly and/or where hot water cannot easily penetrate.

QUALITY ASSURANCE (QA) SPECIFICATIONS - HOW MUCH IS TOO MUCH?

QA testing for the presence of microorganisms in wine after packaging is usually conducted by taking filled bottles or casks off the line, filtering 100-200mL of wine and placing the filter onto a generic medium favourable to the growth of yeast and bacteria. Samples are left to incubate and then assessed for the number of colony forming units (cfu) of yeast or bacteria that grow. AWRI audits found that incubation times used to assess colony growth varied from three to 10 days. Shorter times could increase the risk of not picking up the presence of slow growing bacteria such as *Oenococcus oeni*.

There is also a lack of standard guidelines specifying

the limits for the maximum number of yeast, mould and bacterial counts detected that represent acceptable levels of risk. 'Pass' results have been found to vary from one up to 20 colony forming units (cfu)/100mL for yeasts and up to 50cfu/100mL for bacteria. The acceptable level of viable microorganisms in a wine depends greatly on that wine's characteristics. For example, dry red and white wines with standard alcohol and sulfur dioxide levels have lower risk of microbial spoilage and, therefore, detection of 10cfu/100mL might be considered acceptable. In contrast, 1cfu/100mL could cause serious microbial instability issues in a high-risk product with higher residual sugar and low alcohol. It is also important to keep in mind that usually only a relatively small sample volume from each bottle is subjected to QA testing for viable microorganisms and this reduces the probability that microorganisms will be detected if they are present in low concentrations. Therefore, if a facility is concerned with detecting very low levels of microorganisms, greater sample volumes need to be tested. In general if organisms are detected sporadically it can be a sign that there is an underlying microbial issue.

Results from the standard plating tests, where microorganisms are grown in optimum conditions, do not necessarily reflect whether the organism isolated will be able to grow in wine, which is quite a harsh environment from a microbe's point of view. In some facilities colonies grown during plating are then placed into a wine-like medium and observed for their ability to re-ferment – an ability which might suggest that they pose a greater risk. Fluorescence viability assays are an alternative method for the detection of microorganisms used in some facilities. This method provides a more rapid assessment of samples, but requires specialised equipment and training and has not been widely adopted in the wine industry.

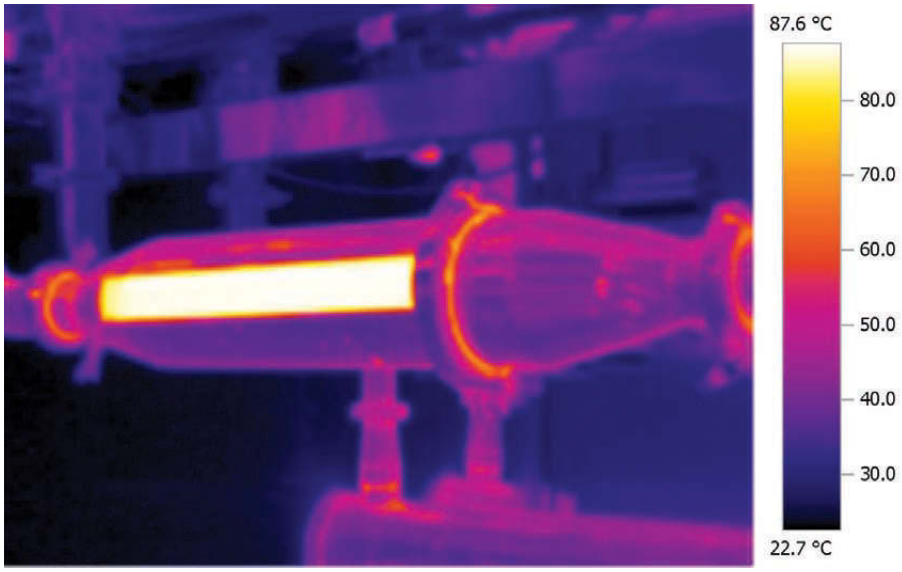

Figure 1. Example thermal image of filtration system to verify temperature of water during sanitisation.

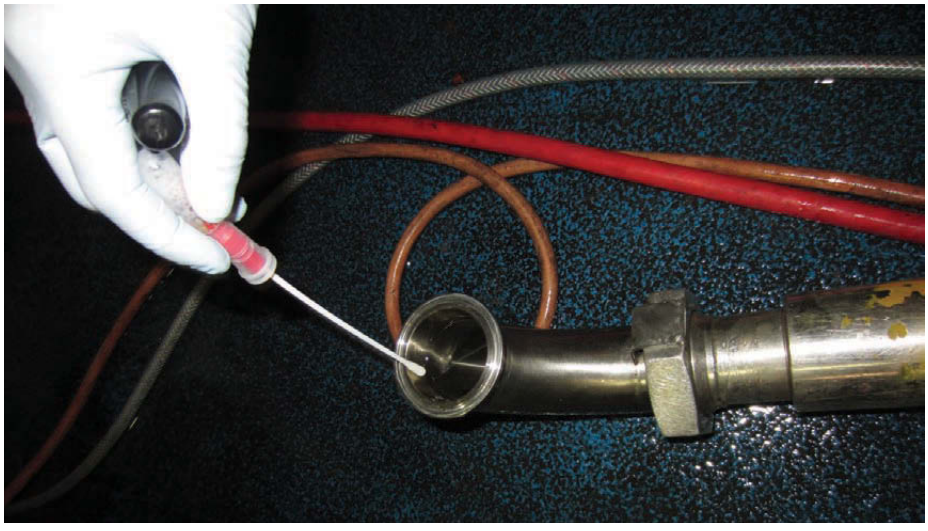

Figure 2. Swab sample being taken from bottling line piping which appears to be 'clean'.

WATER, A HIDDEN RISK

In a number of the audits undertaken wine handling and filtration met the highest standards, however, the water used for rinsing and cleaning did not undergo the same rigorous treatment. This water can come into contact with almost every surface in the packaging chain and often has totally separate filtration and sanitation regimes. If these water treatment processes are not monitored to the same level as those used for wine, the water can pose a significant risk factor for microbial contamination. Ideally all water that comes in contact with fillers and bottles should undergo filtration and monitoring immediately before use. In some facilities water is stored in tanks after it is treated until it is needed, increasing the risk of the growth of new microbial populations.

CASE STUDY

AWRI Commercial Services was contacted by a packaging facility experiencing a problem with sporadic detection of microbes in bottled wine. Increases in microbe detection were being seen at start-up and at the end of long bottling runs (more than 12 hours). This was causing samples to fail QA testing, meaning that products had to be held back. An audit was requested to identify the cause of the microbial contamination.

The first step of the audit was to review the packaging facility's procedures, practices and infrastructure, as well as the history of the issue. Existing practices were verified where possible, for example the effectiveness of the hot water sanitisation of the line was checked using a thermal imaging camera. The internal surfaces of the bottling line were examined closely and swabs were taken throughout interior surfaces of the line, particularly at critical control points.

Overall the hygiene standard of the facility was high and generally accepted practices were in place and adhered to. Thermal imaging showed that the hot water sanitisation was effective, with temperatures reaching 80°C from end to end of the line, with no visible cold spots (Figure 1).

During the audit several components were identified as probable sources of contamination. For example, Figure 2 shows a portion of a bottling line where the internal surface was swabbed after a full hot water sanitisation. The part tested appeared 'clean' on the surface but plating of the swab showed that this surface actually had a high microbial loading of yeast and bacteria not visible to the naked eye (Figure 3). It is possible that this microbial load was in the form of a biofilm and, thus, resistant to hot water sterilisation.

Figure 4 is a microscopic image of a plated swab sample showing mould filaments (known as hyphae) with yeast cells attached. Black mould is common in dark and humid packaging environments. This sample was swabbed from a filler head and shows that the yeast were able to use the mould as a 'scaffold' and release spores into the bottled product sporadically.

A mechanical failure was also found, where the o-ring for a filler head had disintegrated allowing wine to leak, potentially providing a location for microbial growth (Figure 5).

The following recommendations were made to minimise the risk of further issues:

- upgrade and re-design the filtration system to streamline it, removing leaky non-crucial valves and 'dead spots'
- purchase a unit to dispense the microbial control agent dimethyldicarbonate for use with high-risk products
- implement a decision-making system to determine the appropriate packaging regime depending on the type of wine
- consider seeking Good Manufacturing Practices (GMP) certification
- use UV light to sterilise the water used in packaging
- apply specifications for the maximum levels of viable microorganisms and minimum free sulfur dioxide concentrations in the wine holding tank prior to packaging.

A follow-up audit a year after implementation of the recommended changes found the level of microbial failures had been significantly reduced. This example shows that even in an established and well-run facility an external review using 'fresh eyes' can resolve long-running and seemingly intractable issues. The recommended changes did, however, require capital investment and significant changes in protocols which may not be possible in all facilities.

SUMMARY

A detailed independent assessment of a packaging facility can be used to investigate an existing microbial contamination problem or to take steps to prevent such problems occurring. Verifying the effectiveness of cleaning protocols, seeking out 'dead spots' in lines where microbes may gather and generally increasing awareness of the risks of spoilage can all help to improve the quality of wine packaging and reduce the occurrence of costly spoilage problems.

Figure 3. Plated swab sample showing a high microbial loading indicated by yeast and bacterial growth.

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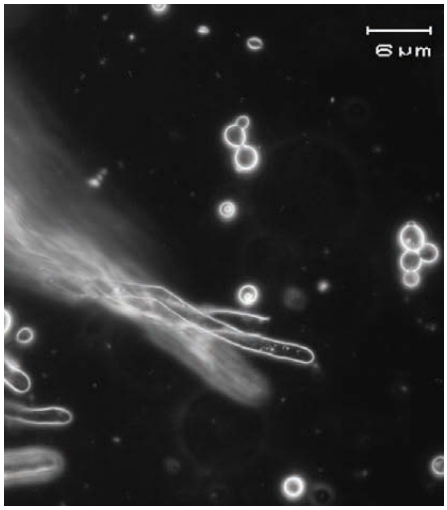

Figure 4. Example of 400x magnification microscopic image of mould with yeast attached, sampled from a bottling filler head.

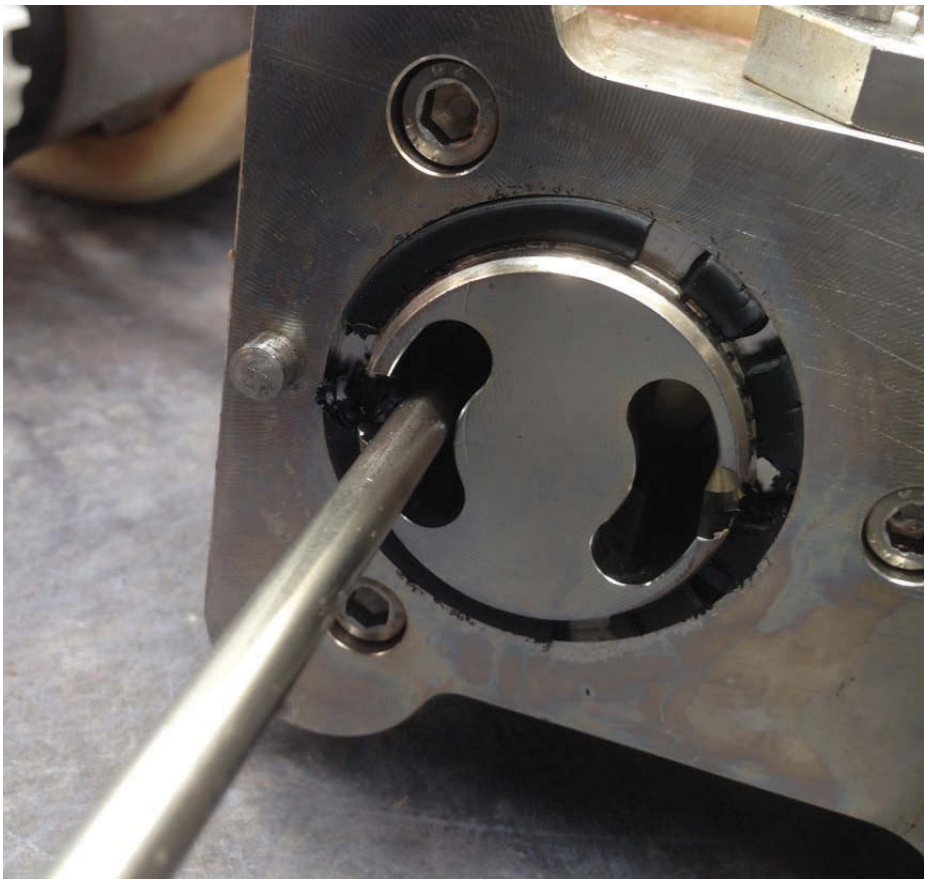

Figure 5. Example of degraded o-ring in filler head component.