Easytesters Bactiscan
Assessment of a novel biofilm detection device

Executive Summary
Bactiscan is a user friendly, robust and portable lamp allowing fluorescent material to be detected. In this study it was shown that high concentrations of bacterial or yeast cells fluoresce strongly allowing Bactiscan to detect well established biofilms with high microbial cell numbers. Rapid detection of troublesome biofilms in brewing and wine-making equipment and vessels is of great interest to these industries.

Background
The food and drink industry is greatly concerned with biofilm on surfaces in contact with the manufactured product - such as on vessel walls and pipes – as it poses the risk of microbiological contamination. The manufacturer needs an assessment of whether there is remaining biofilm on surfaces following the cleaning procedure. Material deposited as biofilm is often not recognised by eye and the current methods for testing have drawbacks. Vessel walls can be swabbed (limited area tested) and either swabs evaluated microbiologically (slow) or by ATP bioluminescence. Last rinse waters of the CIP procedure can be collected and analysed (this data can only give an indication).

Easytesters have developed a promising new instrument for the visualisation of biofilms. The Bactiscan is a portable lightsource emitting specific wavelengths that make certain biofilm components fluoresce. It can be employed to check the efficacy of CIP in vessels and pipes by detecting remaining biofilm following the CIP cleaning procedure. A procedure for the rapid visualisation of biofilm would be of great help to the industry allowing quick remedial action to be taken. Easytesters approached Campden BRI to evaluate the Bactiscan to determine its applicability for the brewing and wine industry and in particular to assess whether typical contaminant microorganisms are detected using the lamp.
Scope of Work

This project assessed Bactiscan as a possible tool for detection of micro-organisms typically encountered in surface material in brewing and wine making equipment.

Experimental

To assess whether the Bactiscan lamp detects the organisms typically encountered in biofilms associated with brewing and wine-making, a selection of representative micro-organisms were tested. These were: *Saccharomyces cerevisiae* (a production yeast strain), a ‘wild’ yeast (a contaminant yeast strain), an *Enterobacteriaceae* species (a bacteria typically associated with poor hygiene), *Lactobacillus* (an anaerobic spoilage bacteria). Additionally, Campden BRI’s in-house biofilm culture (consisting of a mix of aerobic bacteria and yeast as well as anaerobic bacteria recovered from pub dispense systems) was tested.

The individual organisms and the mixed biofilm culture were cultivated. Roughened squares of 10x10cm stainless steel were sterilised. The coupons were incubated with the microbial cultures for 3 weeks during which time the growth media was replaced regularly to allow best possible development and growth of micro-organisms on the surfaces.

Non-attached cells were rinsed off with sterile water and the steel surface analysed with the Bactiscan lamp. Photos were taken using an iphone to record the results, this being the best way to capture the image. Following photographic recording, the micro-organisms attached to the steel squares were recovered by swabbing the surface. The swabs were re-suspended in sterile diluent. A serial dilution was then performed from each re-suspended sample and an aliquot of each plated onto growth media chosen to determine the presence and respective numbers of either aerobic bacteria, yeast or anaerobic lactic acid bacteria. All tests were carried out in duplicate.

Additionally, to investigate whether Bactiscan is able to detect a conditioning layer (the pre-cursor to biofilms, prior to cell attachment) stainless steel coupons were incubated for 3 weeks with sterile beer. These surfaces were analysed using Bactiscan as described above and then swabbed for ATP detection (an indicator of hygiene/organic material).

A negative control sample, consisting of sterilised non-incubated stainless steel, was also investigated. These surfaces were also analysed by ATP bioluminescence.

Concentrated cultures of all the microorganisms were prepared to understand the appearance of the different microorganisms under the Bactiscan light when present at high concentrations. For this purpose the microbes were grown in liquid culture media for 3 to 5 (depending on the individual organism’s requirements). The fully grown samples were spun down in a centrifuge and rinsed several times with sterile water to remove any media residues. Finally, the cells were separated from the rinse water by centrifugation and a sample, taken using a sterile loop, was placed on a sterile, non reflective surface. The samples were either streaked lightly onto the surface (not visible to the naked eye) or placed onto the surface as a heap of cells (visible by eye). The images resulting from illumination of these samples with the Bactiscan lamp were captured with an iphone.

Results

Coupons were incubated for three weeks with sterile beer to check whether the Bactiscan is able to detect a conditioning layer of deposited non-microbial material. For comparison a sterilised non-incubated stainless steel square was used as a negative control. Both coupons, incubated in beer and the control, were analysed using the Bactiscan followed by analysis for ATP bioluminescence, an indicator of organic material. The ATP measurements and the photos are shown in Table 1 and Figures 1 and 2 below.

<table>
<thead>
<tr>
<th>Sample</th>
<th>ATP (Relative Light Units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Duplicate 1</td>
</tr>
<tr>
<td>Control stainless steel surface</td>
<td>21</td>
</tr>
<tr>
<td>Beer-incubated stainless steel surface</td>
<td>91</td>
</tr>
</tbody>
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*Table 1: ATP bioluminescence of control stainless steel surface (not incubated) and surface incubated for 3 weeks with sterile beer*
All ATP bioluminescence readings were low indicating that little organic material was deposited on the surfaces. In fact, the stainless steel squares incubated with beer did not show significantly higher values than the control samples. The incubated plates were rinsed with sterile water prior to the analysis to remove any unbound material whereas the control samples were not rinsed so that loose material on the control plates may have been picked up.

Indeed the images of the control plates taken with Bactiscan showed fluorescent specks that could have been sterile dust particles on the surfaces. Interestingly, duplicate 2 showed the higher number of specs and also showed the higher ATP value. A purple hue was seen on the plates which is likely to be from Bactiscan light reflecting off the surfaces.

The Bactiscan images of the beer-incubated stainless steel surfaces in Figure 2 were taken immediately post rinsing (wet state). Duplicate 1 showed very little fluorescence, this was the plate with lower ATP reading. Duplicate 2 on the other hand showed a veil-like blue/purple pattern. This pattern would indicate beer residue rather than reflection.

The stainless steel plates were incubated for periods of three weeks with the different microorganisms. All samples were analysed wet (after rinsing with sterile water) and also dry to determine whether there were any differences in fluorescence. The numbers of microbial cells attached to the surfaces were established by swabbing the plates and using traditional microbiological growth-based methods.
A considerable amount of fluorescing material was seen deposited on the surfaces incubated with the Enterobacteriaceae species. The fluorescence had a grainy appearance and a gray tinge. The graininess may be due to bacterial cells growing in clusters on the surface. Enterobacteriaceae species are known to excrete polysaccharide which aids development of biofilm. It is possible that this polysaccharide is fluorescent. Upon drying of the contaminated stainless steel plate (right photo in Figure 3) the grainy fluorescence was still visible and a drying mark (concentrated fluorescing material) was also seen at the lower edge of the dried square. The number of bacterial cells on this surface was $3.9 \times 10^6$ cells.

Figure 4 shows the images for the stainless steel squares incubated with the Lactobacillus species. The wet image showed a blue veil of fluorescence with a few small areas of higher intensity (specks). The dry image appeared more intense probably as a result of fluorescing material becoming more concentrated upon drying and/or the fluorescent light being less buffered by water. A high intensity homogeneous blue band was seen at the bottom edge of the dried surface which was most probably as a result of light reflection from the stainless steel surface. The number of bacterial cells on the surface was high at about $1.32 \times 10^7$ bacterial cells recovered from the 10x10cm surface.
The yeast cell counts recovered from the stainless steel plates following incubation for 3 weeks with this organism were below 100 cells indicating that this yeast was not able to attach and settle onto the roughened metal surface. The corresponding wet Bactiscan image showed a blue patch on the right side of the chip (see Figure 5, left side). It is not quite clear what matter is causing this fluorescence. A homogeneous band across the middle of the image was noted probably again due to reflectance. No significant fluorescence was detected once the plate was dried.

In Figure 6 the Bactiscan images for the stainless steel squares incubated for 3 weeks with a contaminant ‘wild’ yeast strain can be seen. Apart from some reflectance on the wet plate image, 4 spots of high fluorescence intensity were noted on both the wet and the dry plate. Under natural light, these locations on the plate showed clumps of yeast cells which were not rinsed off but strongly attached to the surface. The yeast culture used for plate incubation did show cell flocculation, i.e. rather than cells being homogeneously distributed in the liquid growth medium clumps of yeast were growing. Only $1 \times 10^4$ yeast cells were retrieved from the surface. This low count corresponds well with the limited deposition of yeast cells observed.
The stainless steel plate incubated for 3 weeks with the biofilm culture (a mix of aerobic and anaerobic bacteria as well as yeast) showed a blue/purple veil of fluorescence as seen in Figure 7. Only the wet plate image was taken in this case. In terms of numbers of micro-organisms deposited on the plates, the highest cell counts were obtained for the biofilm-incubated chips - $2.56 \times 10^8$ cells. These consisted, as expected of a mix of bacteria and yeast.

Concentrated cultures of all individual microorganisms were prepared and tested with the Bactiscan lamp to understand whether higher cell concentrations would yield stronger fluorescence. The concentrated cell culture were placed onto a surface in two manners: as a thin streak (not visible by eye) or as a concentrated cluster of cells (visible by eye as a heap of cell matter). Figure 8 shows the photos of these preparations taken under natural light (clusters only) and under the Bactiscan light.

**Figure 7:** Bactiscan image of stainless steel surface incubated for 3 weeks with a biofilm culture. Wet surface

**Figure 8:** Images of concentrated cell suspensions. Top: Bactiscan image of light streaks; Middle: Concentrated cells under natural light; Bottom: Bactiscan image of concentrated cells
The 5 streaks were visible under the Bactiscan lamp to varying degrees with the *Enterobacteriaceae* species streak being the faintest and the yeast *Saccharomyces cerevisiae* streak the strongest. In general, the yeast streaks appeared to show higher fluorescence than the bacterial streaks. The differences in fluorescence intensities may be due to varying cell concentration in the streaks and/or the different micro-organisms fluorescing more or less. The fluorescence image of the contaminant ‘wild’ yeast streak (second from left in Figure 8) had a granular appearance (specks), this was again due to these cells strongly flocculating and forming cell clusters that could not be spread out.

Due to the cells of the different organisms concentrating up more or less well upon centrifugation it was more difficult to obtain a heap of cells for the *Enterobacteriaceae* species and the *Candida* wild yeast as these were more liquid and less concentrated. All 5 concentrated cell clusters clearly showed fluorescence as can be seen at the bottom of Figure 8. And the more concentrated samples (contaminant ‘wild’ yeast, *S. cerevisiae* yeast, *Lactobacillus* bacteria) show the higher fluorescence.

### Summary and Conclusions

In this project Bactiscan was used to determine whether it could pick up cells of a number of bacterial and yeast species encountered in the brewing and wine-making industry and potentially causing product contamination if remaining on vessel and process equipment surfaces post cleaning. Roughened stainless steel plates were incubated for 3 weeks with either a bacterial strain (*Enterobacteriaceae* species, *Lactobacillus*), a yeast strain (wild contaminant species, *Saccharomyces cerevisiae* brewing strain) culture or a biofilm culture (mix of bacteria and yeast). This process was to allow the micro-organisms to attach to the metal surface and develop as a biofilm. Following incubation the plates were inspected with the Bactiscan and cell numbers settled on the plates determined. An un-incubated sterilised stainless steel plate was also analysed as a control and additionally a plate was incubated with beer for 3 weeks to see if beer residue would be detected.

The control sample showed a few specks of fluorescence with Bactiscan and the beer-incubated plate only showed very weak fluorescence in the pattern of a veil. Both these surfaces were swabbed and ATP bioluminescence, for the detection of organic material, measured. Both showed very low levels indicating that not much beer residue had settled on the surface.

Both stainless steel surfaces contaminated with bacteria showed fluorescence. The *Enterobacteriaceae* species gave a granular fluorescence of blue/gray colour, whereas the *Lactobacillus* produced blue/purple fluorescence which was more clearly detected when the plate was dried. The brewing yeast *S. cerevisiae* did not settle on the stainless steel surface over the 3-week incubation period and the *Candida* contaminant ‘wild’ yeast strain attached in clumps being a flocculent strain. These clumps of yeast strongly fluoresced under the Bactiscan light. Finally, the biofilm microorganisms showed as a blue/purple veil of fluorescence. Samples of concentrated cells of the microorganisms were also investigated with Bactiscan. The fluorescence for all 5 bacteria and yeast was very strong when viewed in this way.

In summary, the Bactiscan lamp is a robust device that is easy to use, needs little operator training and is portable so that it can be used wherever required in the manufacturing environment. Some training/experience would be required for the interpretation of the fluorescence produced from investigated surfaces as reflected light may be misinterpreted. In terms of detection of microorganisms, the lamp will clearly highlight a high concentration of bacteria/yeast cells as would occur in a fully developed biofilm. Early stages of biofilm formation on surfaces are more difficult to detect as the fluorescence produced is not as intense as seen in some of the cases in this study.

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