Bactest - Speedy Breedy

Assessment of the suitability of Speedy Breedy as a rapid detection method for brewing contaminants

Prepared by Karin Pawlowsky and Belen Perez
August 2013

Executive summary

In this study the new respirometer-based product (Speedy Breedy) developed by Bactest was assessed for its suitability as a rapid microbiology tool for microbial brewing contaminants. Tests were performed with a number of wild yeast species as well as aerobic and anaerobic bacteria.

All micro-organisms were successfully detected using the new methodology. Detection time was dependent on type of organism as well as cell concentration in the sample analysed. In most cases Speedy Breedy allowed a more rapid detection than traditional agar plate based methods. This was found to be particularly advantageous for the detection of anaerobic lactic acid bacteria where detection was achieved in under 2 days (at a concentration of ~2,000 cells/ml) compared to 5-7 days incubation required with traditional agar plate based methods. Some wild yeast species were also detected very rapidly (11 hours at ~3,000 cells/ml) comparing well with the 3-5 days required for the traditional analysis. Additionally, Speedy Breedy allowed effective rapid detection of low cell concentrations (2-5 bacterial cells/ml in the current tests).

Background

The brewing industry is concerned with possible microbiological contamination that could affect the quality and safety of its products. Traditional microbiological methods for the detection of possible contaminants are reliable but slow. Therefore any new technology that has the potential to speed up the detection process is of great interest. A rapid detection would allow the brewer to take more immediate corrective actions.

Bactest have developed a new promising product, ‘Speedy Breedy’, a small portable
device which consists of a cell respirometer that measures biological gas exchange. Headspace pressure in stirred sealed vessels containing microbiological cultures is monitored and pressure changes are linked to cell metabolism. Very low cell numbers can be rapidly grown to detectable levels with speed of detection being more rapid with higher microbial levels. The device works in situ, is user friendly and allows fast detection of micro-organisms in liquid samples. Bactest has approached Campden BRI to evaluate Speedy Breedy and to determine its applicability in the brewing industry.

Scope of Work

This study evaluated the capability of Speedy Breedy to detect some of the most commonly encountered brewing contaminants and compare the results with those obtained using traditional microbiological methods.

Experimental

Before the assessment was carried out, a full training on the use of the machines and the software was given by Bactest. The training was easy to follow and all the basic principles (writing protocols (programs), starting the machines, running the experiments, analysis of the data...) were well explained. Bactest also supplied all the material needed for this assessment; the vessels (for the anaerobic studies some were purged with 5% carbon dioxide/1% hydrogen in nitrogen), the Speedy Breedy machines and the reducing agents (for the production of anaerobic environments in the growth medium). Technical support was available throughout the study; the Bactest staff was always very helpful and knowledgeable.

The assessment was carried out using different species of beer contaminants (lactic acid bacteria (Lactobacillus sp., Pediococcus sp.), non-brewing contaminant ‘wild’ yeast and an aerobic spoilage bacterium (acetic acid bacteria)). The 4 micro-organisms were grown up (concentrated stock cultures) and their purity checked to ensure that they were free of other microbial contaminants. The concentration of each micro-organism in the concentrated stock cultures was determined and specific broths (WLN for acetic acid bacteria, YM-Copper for non-brewing wild yeast and NBB for lactic acid bacteria) were spiked with a calculated volume of the stock solution to give a concentration of about $5 \times 10^3$ cells/ml. This cell suspension was serially diluted to obtain 4 concentrations ($5 \times 10^3$, $10^2$, $10^1$, $10^0$ cells/ml). Volumes of 50ml of each cell-containing broth at each concentration were pipetted into the Speedy Breedy vessels. Lactic acid bacteria are facultative anaerobic microorganisms and are, when using traditional plating techniques, incubated anaerobically in anaerobic jars under a CO$_2$ enriched environment. To create similar anaerobic conditions in the Speedy Breedy vessels, these vessels were purged with 5% CO$_2$/1% H$_2$ in N$_2$. In this case, the bacterial samples were inoculated through a septum integrated into the vessel using a syringe and needle so as to maintain the internal environment in the vessels. Every experiment was carried out in triplicate for each micro-organism at each concentration.

Additionally, to create an anaerobic environment in the vessels testing for lactic acid bacteria, a reducing agent was added to the NBB broth to scavenge any oxygen present. Two possible reducing agents were tested for their suitability, sodium thioglycolate and Oxyrase. A test was carried out to determine which of these reducing agents would produce similar results compared with incubation in an anaerobic jar. Volumes of 50ml NBB broth in triplicate were supplemented with either one of the reducing agents and, as a control, 3 x 50ml volumes were not supplemented. All the broths were inoculated with the same concentration of Lactobacillus. The cultures supplemented with the reducing agents were incubated for 2 days at 25°C without shaking, while the broths containing no reducing agent were incubated in an anaerobic jar for 2 days at 25°C. Following the incubation period, all the broths were plated out and all the plates incubated for 2 days at 25°C inside an anaerobic jar. All plates showed similar numbers of colonies indicating that both reducing agents are suitable for the growth of the Lactobacillus strain tested here. It was decided to use sodium thioglycolate for the lactic acid bacteria broths employed for the Speedy Breedy tests.

Once the vessels had been filled with the cell containing broths, they were inserted into the 2 chambers of each Speedy Breedy machine and the specific protocol (program) applied. The protocols consisted of two stages. In stage 1, time is given so that stability in the system (temperature and pressure) is achieved before moving to stage 2, during which pressure is monitored for any changes occurring. The time allowed for detection (stage 2) was set as the time required for traditional agar plate incubation (i.e. 3 days for aerobic organisms and 5 days for anaerobic bacteria). The 2 protocols, based on the aerobic or anaerobic nature of the contaminants tested, that were used in this study were as follows:
Yeast and acetic acid bacteria analyses

- Stage 1 (stability): rotor speed 60 rpm; temperature 25°C; temperature stability time 10 min; pressure stability target 0.1 mbar/min; pressure stability window 10 min.
- Stage 2 (detection): rotor speed 60 rpm; temperature 25°C; time target 4320 min (3 days).

Lactic acid bacteria (Lactobacillus and Pediococcus)

- Stage 1 (stability): rotor speed 60 rpm; temperature 25°C; temperature stability time 10 min; pressure stability target 0.1 mbar/min; pressure stability window 10 min.
- Stage 2 (detection): rotor speed 60 rpm; temperature 25°C; time target 7200 min (5 days).

All the Speedy Breedy trials were run for the length of time specified in the protocols (3 days for the acetic acid bacteria and the non-brewing wild yeast and 5 days for Lactobacillus and Pediococcus) or until an event was seen. Each sample was also spread plated onto specific agar media (VWN for acetic acid bacteria, YM-Copper for non-brewing wild yeast and NBB-A for lactic acid bacteria) which were then incubated 3 days at 25°C in the case of the aerobic organisms and 5 days at 25°C in anaerobic jars for the lactic acid bacteria. After the period of incubation, the colonies on the plates were counted and the results compared with those obtained from the Speedy Breedy. Screenshots of the pressure changes in the different experiments were taken to show the behaviour of the microorganisms. These traces and the corresponding detailed data were examined for the detection of ‘events’, significant pressure change indicating metabolic activity. The criteria for an ‘event’ were a continuous pressure rise or decrease for a duration of at least 20 mins.

Results

Wild yeast (Saccharomyces diastaticus)

It was noticed, while growing the organism, that this Saccharomyces diastaticus yeast strain was forming flocs quite readily rather than staying dispersed. This made accurate spiking of the broth difficult. It was attempted to prepare concentrations of ~5x10^3, 5x10^2, 5x10^1 and 5x10^0 cells/ml.

The tests in the Speedy Breedy devices did not show any ‘events’, except for the highest yeast concentration investigated. At the highest yeast concentration (1.41x10^3 cells/ml) an event was detected after 3,230 minutes (2.2 days) and 4,078 minutes (2.8 days) for the left and right Speedy Breedy chambers respectively (see Figure 1). The ‘events’ occurred quite late into the test, not showing a great advantage compared to the traditional testing (3 day incubation). An example of a negative result is depicted in Figure 2 – no ‘event’ is detectable.

The Saccharomyces diastaticus results by traditional microbiology can be seen below in Table 1.

Figure 1: Speedy Breedy results for Saccharomyces diastaticus at a concentration of 1.41x10^3 cells/ml in YM-Cu broth

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Pressure (mbar)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1000</td>
</tr>
<tr>
<td>400</td>
<td>1200</td>
</tr>
<tr>
<td>800</td>
<td>1300</td>
</tr>
<tr>
<td>1200</td>
<td>1400</td>
</tr>
<tr>
<td>1600</td>
<td>1500</td>
</tr>
<tr>
<td>2000</td>
<td></td>
</tr>
<tr>
<td>2400</td>
<td></td>
</tr>
<tr>
<td>2800</td>
<td></td>
</tr>
<tr>
<td>3200</td>
<td></td>
</tr>
<tr>
<td>3600</td>
<td></td>
</tr>
<tr>
<td>4000</td>
<td></td>
</tr>
<tr>
<td>4400</td>
<td></td>
</tr>
</tbody>
</table>

Figure 2: Speedy Breedy results for Saccharomyces diastaticus at a concentration of 1.41x10^3 cells/ml in YM-Cu broth
Figure 2: Speedy Breedy results for *Saccharomyces diastaticus* at a concentration of 23 cells/ml in YM-Cu broth.

<table>
<thead>
<tr>
<th>Nominal cell concentration</th>
<th>Nominal cell concentration</th>
<th>Nominal cell concentration</th>
<th>Nominal cell concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>5x10^3 cells/ml</td>
<td>5x10^2 cells/ml</td>
<td>5x10^1 cells/ml</td>
<td>5x10^0 cells/ml</td>
</tr>
<tr>
<td>1.41 x 10^3</td>
<td>1.17 x 10^2</td>
<td>23</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Table 1: *Saccharomyces diastaticus* concentrations determined by plating onto YM-Cu agar plates; average of triplicates

**Wild yeast species**

Following the somewhat unexpected results with *Saccharomyces diastaticus*, it was decided to carry out an additional experiment testing 6 different wild yeast strains at the highest concentration only (~10^3 cells/ml). This would allow part of the previous test to be repeated and other less flocculent yeast strains to be investigated. These analyses were performed in duplicate.

- *Saccharomyces diastaticus*

  The Speedy Breedy results are shown in Figure 3. The pressure in both vessels increased. *Saccharomyces diastaticus* is a fermentative yeast so that an increase in pressure would have been expected. These ‘events’ occurred at around 897 minutes (15hrs, 0.6 days) and 957 minutes (16 hrs, 0.7 days) for the left and right chamber respectively. This is much faster contaminant detection than when using traditional microbiological techniques (3 days).

  The detection time (event time) was also much faster here than in the first set of experiments (~2.5 days) at the corresponding cell concentration. It is not clear why there is such a discrepancy between the results, but these latter results fit more the expected fast detection. The yeast cell concentration in the repeat test was about 10 times higher which would lead to earlier detection. Also in this latter experiment the strain was grown up in a different medium (YM rather than YM-Cu) to see whether it was possible to eliminate the flocculation effect. This may have resulted in cells being in a more active state allowing the lag phase in the Speedy Breedy vessel (in YM-Cu broth) to be shortened.

  The results from the traditional microbiology analyses can be found in Table 2. As can be seen, the yeast cell concentration was somewhat higher than expected possibly due to the anticipated difficulties with floc formation.
Figure 3: Speedy Breedy results for *Saccharomyces diastaticus* at a concentration of $2.42 \times 10^4$ cells/ml in YM-Cu broth

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Cell concentration (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Saccharomyces diastaticus</em></td>
<td>$2.42 \times 10^4$</td>
</tr>
</tbody>
</table>

Table 2: *Saccharomyces diastaticus* concentration determined by plating onto YM-Cu agar plates; average of duplicates

- **Pichia kudriavzevii (formerly Issatchenka orientalis)**

The result obtained with Speedy Breedy for this yeast can be seen in Figure 4. One of the chambers did not give a result (maybe due to a fault at setting up). Analysis of the plot showed an ‘event’ occurring around 2,452 minutes (1.7 days) into the test. This means that it took around 41 hrs for this yeast strain, at this starting concentration, to show sufficient metabolic activity to be detected in terms of a change in pressure in the culture vessel. The results by traditional microbiology analysis are reported in Table 3. Therefore, this yeast strain can be detected using Speedy Breedy at a concentration of $7.1 \times 10^3$ cells/ml in slightly less than 2 days.

Figure 4: Speedy Breedy results for *Pichia kudriavzevii* at a concentration of $7.1 \times 10^3$ cells/ml in YM-Cu broth
Table 3: *Pichia kudriavzevii* concentration determined by plating onto YM-Cu agar plates; average of duplicates

- **Pichia membranifaciens**

  The results by plating of the yeast samples on the YM-Cu medium are shown in Table 4. The results obtained with Speedy Breedy can be seen in the Figure 5. Both chambers showed a decrease in pressure with this 'event' starting around 2,907 minutes (2 days) into the test. The duplicate pressure traces are very similar in shape but the pressure values for the left vessel were shifted to slightly lower values throughout the run.

![Figure 5: Speedy Breedy results for *Pichia membranifaciens* at a concentration of 2.85x10^3 cells/ml in YM-Cu broth](image)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Cell concentration (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pichia kudriavzevii</em></td>
<td>7.1 x 10^3</td>
</tr>
</tbody>
</table>

Table 4: *Pichia membranifaciens* concentration determined by plating onto YM-Cu agar plates; average of duplicates

- **Geotrichium sp. S1 22**

  The traditional microbiology results are listed in Table 5 and the results obtained and the results from the Speedy Breedy system can be seen in Figure 6. The left vessel showed an event at around 712 minutes (12 hrs, 0.5 days) and the right vessel at about 684 minutes (11 hrs, 0.5 days). The pressure profile for this yeast showed a completely different behaviour to any of the other yeast strains tested. Pressure in the vessels decreased first, followed by a renewed pressure build up. It appears that this yeast produces gas once all the oxygen in the vessel has been depleted. The detection of this yeast by the Speedy Breedy was very fast, at approximately 11 hours after the run started.

![Table 5: Geotrichium sp. S1 22](image)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Cell concentration (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pichia membranifaciens</em></td>
<td>2.85 x 10^3</td>
</tr>
</tbody>
</table>
Figure 6: Speedy Breedy results for Geotrichium sp.S122 at a concentration of 2.95x10^3 cells/ml in YM-Cu broth.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Cell concentration (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geotrichium sp.S122</td>
<td>2.95 x 10^3</td>
</tr>
</tbody>
</table>

Table 5: Geotrichium sp.S122 concentration determined by plating onto YM-Cu agar plates; average of duplicates

- *Pichia farinosa*

The results obtained with the Speedy Breedy incubator are depicted in Figure 7. In the left vessel an ‘event’ occurred around minute 821 (14 hrs, 0.6 days) and in the right chamber around minute 807 (14 hrs, 0.6 days). This means that it takes more or less half a day for this yeast at this specific concentration to be detected with the Speedy Breedy system. This is much faster than detection by the traditional microbiological plating method (3 days). It has to be noted that the cell concentration for the *Pichia farinosa* samples were somewhat higher than for the other yeast species tested (see Table 6).

Figure 7: Speedy Breedy results for *Pichia farinosa* at a concentration of 9.2x10^3 cells/ml in YM-Cu broth.
<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Cell concentration (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pichia farinosa</em></td>
<td>$9.2 \times 10^3$</td>
</tr>
</tbody>
</table>

**Table 6:** *Pichia farinosa* concentration determined by plating onto YM-Cu agar plates; average of duplicates

- **Pichia anomala** *(also known as Candida pelliculosa, Hansenula anomala)*

The results obtained with Speedy Breedy can be seen in Figure 8. ‘Events’ occurred in the left and right chambers at about minute 898 (15 hrs, 0.6 days) and 877 (15 hrs, 0.6 days) respectively. The pressure profile indicates that after a lag phase the pressure dropped slightly followed by a steep increase to quite high pressure indicating gas-release into the vessel headspace. Speedy Breedy was able to detect this yeast at this specific starting concentration within 15hrs, much quicker than is possible by traditional detection via growth on agar plate.

**Figure 8:** Speedy Breedy results for *Pichia anomala* at a concentration of $6.05 \times 10^3$ cells/ml in YM-Cu broth

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Cell concentration (cfu/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pichia anomala</em></td>
<td>$6.05 \times 10^3$</td>
</tr>
</tbody>
</table>

**Table 7:** *Pichia anomala* concentration determined by plating onto YM-Cu agar plates; average of duplicates

From these experiments, testing 6 different yeast species using Speedy Breedy, it can be deduced that different yeast species exhibit differences in their gas uptake and production/release. Some yeast strains showed an increase in pressure, due to the release of gas (*Saccharomyces diastaticus, Pichia anomala*) whereas other strains showed a decrease (*Pichia kudriavzevi, Pichia membranifaciens, Pichia farinosa*) and *Geotrichium* sp. S122 showed a sharp drop in pressure followed by an increase. Four of the yeast strains showed metabolic activity resulting in a detectable pressure change in less than a day, but other strains required up to 2-3 days to be detected by pressure change.

In conclusion, we have seen that Speedy Breedy can be advantageous compared to traditional microbiological agar plating as it generally detects yeast significantly more rapidly (at the specific concentration tested here: $10^3$-$10^4$ cells/ml).
**Acetic acid bacteria (Acetobacter oxydans)**

The results of the experiments with the acetic acid bacterium *Acetobacter oxydans* can be seen in Figures 9 to 14 for the Speedy Breedy system and Table 9 for traditional microbiology. The Speedy Breedy detection times at the different concentrations are tabulated in Table 8. As would have been expected, the higher the bacterial concentration, the less time Speedy Breedy needed to detect metabolic activity in terms of pressure change. All concentrations, even 2 cells/ml, were detected in less than 2 days. The highest concentrations (1.09 x 10^3 cells/ml) were detected in more or less 1 day. Thus, for this acetic acid bacteria strain, Speedy Breedy represents a faster detection method than the traditional plating technique requiring 3 days of incubation.

Interestingly, the pressure plots for *Acetobacter* at the highest concentrations (1.09 x 10^3 and 50 cells/ml) showed a steep drop in pressure after which it rose again, possibly indicating that the bacteria is producing and releasing gas once anaerobic conditions prevail. For the lower bacterial concentrations tested only the decrease in pressure was observed, possibly due to insufficient time over the run of the test to show the renewed increase.

![Figure 9: Speedy Breedy results for acetic acid bacteria at a concentration of 1.09 x 10^3 cells/ml in WLN broth](image1)

![Figure 10: Speedy Breedy results for acetic acid bacteria in WLN at a concentration of 1.09x10^3 cells/ml in the left chamber and 50 cells/ml in the right chamber](image2)
Figure 11: Speedy Breedy results for acetic acid bacteria at a concentration of 50 cells/ml in WLN broth

Figure 12: Speedy Breedy results for acetic acid bacteria at a concentration of 13 cells/ml in WLN broth
Figure 13: Speedy Breedy results for acetic acid bacteria in WLN broth at a concentration of 13 cells/ml in the left chamber and 2 cells/ml in the right chamber.

Figure 14: Speedy Breedy results for acetic acid bacteria at a concentration of 2 cells/ml in WLN broth.
<table>
<thead>
<tr>
<th>Cell concentration determined on WLN agar plates (cfu/ml)</th>
<th>Time to detection by Speedy Breedy (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.09 \times 10^3$</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>50</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>13</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>44 ± 1</td>
</tr>
</tbody>
</table>

Table 8: Speedy Breedy ‘event’ detection times for acetic acid bacteria in WLN broth

<table>
<thead>
<tr>
<th>Nominal cell concentration $5 \times 10^3$ cells/ml</th>
<th>Nominal cell concentration $5 \times 10^2$ cells/ml</th>
<th>Nominal cell concentration $5 \times 10^1$ cells/ml</th>
<th>Nominal cell concentration $5 \times 10^0$ cells/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.09 \times 10^3$</td>
<td>50</td>
<td>13</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 9: Average acetic acid bacteria concentration determined (average of triplicates) by plating onto WLN agar plates

**Lactic acid bacteria**

- *Lactobacillus brevis*

The results obtained using traditional microbiology techniques can be found in Table 11. The Speedy Breedy results are shown in Figures 15 to 20. At the beginning of each run, a steady drop in pressure is noted before rising again. This initial decrease in pressure is most likely due to the reducing agent scavenging any residual oxygen in the vessel headspace. The pressure increase is probably a result of the anaerobic micro-organisms producing gas as they metabolise the broth nutrients. This effect was taken as the occurrence of an ‘event’. The Speedy Breedy detection times for this *Lactobacillus* species at the various concentrations are displayed in Table 10. Once again, the samples with the highest concentrations ($2.33 \times 10^7$ cells/ml) were detected earlier than the ones containing the lower concentrations. Detection was very rapid; just 27hrs at the highest concentration ($2.33 \times 10^7$ cells/ml) and only 48hrs for the very low concentration of 3 cells/ml. This represents a great advantage compared to the long incubation time required for anaerobic agar plates (5 days).

Figure 15: Speedy Breedy results for *Lactobacillus brevis* at a concentration of $2.33 \times 10^3$ cells/ml in NBB+sodium thioglycolate broth
Figure 16: Speedy Breedy results for *Lactobacillus brevis* at a concentration of $1.37 \times 10^2$ cells/ml in the left chamber and $2.33 \times 10^3$ cells/ml in the right chamber in NBB+sodium thioglycolate broth.

Figure 17: Speedy Breedy results for *Lactobacillus brevis* at a concentration of $1.37 \times 10^3$ cells/ml in NBB+sodium thioglycolate broth.
Figure 18: Speedy Breedy results for *Lactobacillus brevis* at a concentration of 20 cells/ml in NBB+sodium thioglycolate broth

Figure 19: Speedy Breedy results for *Lactobacillus brevis* at a concentration of 3 cells/ml in the left chamber and 20 cells/ml in the right chamber in NBB+sodium thioglycolate broth
Figure 20: Speedy Breedy results for *Lactobacillus brevis* at a concentration of 3 cells/ml in NBB+sodium thioglycolate broth.

<table>
<thead>
<tr>
<th>Cell concentration determined on NBB agar plates (cfu/ml)</th>
<th>Time to detection by Speedy Breedy (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.33 x 10^3</td>
<td>27 ± 2</td>
</tr>
<tr>
<td>1.37 x 10^2</td>
<td>37 ± 0</td>
</tr>
<tr>
<td>20</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>3</td>
<td>48 ± 3</td>
</tr>
</tbody>
</table>

Table 10: Speedy Breedy ‘event’ detection times for *Lactobacillus brevis* in NBB+sodium thioglycolate broth

<table>
<thead>
<tr>
<th>Nominal cell concentration 5x10^3 cells/ml</th>
<th>Nominal cell concentration 5x10^2 cells/ml</th>
<th>Nominal cell concentration 5x10^1 cells/ml</th>
<th>Nominal cell concentration 5x10^0 cells/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.33 x 10^3</td>
<td>1.37 x 10^2</td>
<td>20</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 11: *Lactobacillus brevis* concentration determined (average of triplicates) by plating onto NBB agar plates

- *Pediococcus damnosus*

The results obtained using traditional microbiology techniques can be found in Table 13. The Speedy Breedy results are shown in Figures 21 to 26. This lactic acid bacterium shows a different headspace pressure profile than the *Lactobacillus* species. As before there is initially a slight decrease in pressure probably due to the reducing agent scavenging oxygen. The pressure then rises but only slightly compared to the significant rise observed for the *Lactobacillus*. For *Pediococcus* a pressure drop finally occurred (only seen at the highest concentration, 1.81x10^3 cells/ml) maybe due to gases being absorbed again. The ‘events’ for this organism appeared about 12 hours later than for *Lactobacillus brevis* at the corresponding concentrations (see Table 12) – 1.6 to 2.6 days rather than 1.1 to 2 days. However, this detection time is still significantly faster than time for detection by traditional plating technique requiring a 5 day incubation period.
Figure 21: Speedy Breedy results for *Pediococcus damnosus* at a concentration of $x10^3$ cells/ml in NBB+sodium thioglycolate broth

Figure 22: Speedy Breedy results for *Pediococcus damnosus* at a concentration of $x10^2$ cells/ml in the left chamber and $x10^3$ cells/ml in the right chamber in NBB+sodium thioglycolate broth
Figure 23: Speedy Breedy results for *Pediococcus damnosus* at a concentration of $x10^2$ cells/ml in NBB+sodium thioglycolate broth.

Figure 24: Speedy Breedy results for *Pediococcus damnosus* at a concentration of $x10^1$ cells/ml in NBB+sodium thioglycolate broth.
Figure 25: Speedy Breedy results for *Pediococcus damnosus* at a concentration of $10^0$ cells/ml in the left chamber and $10^1$ cells/ml in the right chamber in NBB+sodium thioglycolate broth.

Figure 26: Speedy Breedy results for *Pediococcus damnosus* at a concentration of $10^0$ cells/ml in NBB+sodium thioglycolate broth.
## Summary and conclusions

In this study the Speedy Breedy respirometer developed by Bactest was investigated as a potential tool for the rapid detection of contaminant micro-organisms encountered in a brewing environment.

A number of wild yeast species as well as an aerobic and two anaerobic bacteria were cultivated and several cell concentrations analysed for their cell counts by traditional microbiology, via growth on agar plates, as well as by Speedy Breedy.

At concentrations $\sim 3 \times 10^3$ to $\sim 2 \times 10^4$ cells/ml the yeast species tested here (*Saccharomyces diastaticus*, *Pichia kudriavzevii*, *Pichia membranifaciens*, *Geotrichium sp. S122*, *Pichia farinosa*, *Pichia anomala*) were detected within 11-49 hours. Agar based methods require an incubation period of 3-5 days for detection of aerobic micro-organisms (including yeast). Thus, Speedy Breedy is able to detect the presence of yeast more quickly at this cell concentration, in some cases in less than a day. Not only were the different yeast species detected at different times (relating to the first change in pressure in the Speedy Breedy vessel) but they also exhibited different pressure profiles indicating that the yeast species differed in their metabolism and excretion of gas.

Speedy Breedy allowed the aerobic bacterium, *Acetobacter oxydans*, to be detected within 26 hours at a concentration of 1,000 cells/ml. Even at the very low concentration of 2 cells/ml only 44 hours were required for detection – less than the time required for detection by traditional methods.

Lactic acid bacteria (including *Lactobacillus* spp. and *Pediococcus* spp.) are facultative anaerobes, i.e. they preferentially grow in an anaerobic environment. When using traditional agar plate-based methods, the plates are incubated in anaerobic jars in a CO$_2$ enriched environment. To be able to test these bacteria in the Speedy Breedy vessels, the vessels were purged with 5% carbon dioxide/1% hydrogen in nitrogen and a reducing agent added to the broth medium to scavenge oxygen present. A preliminary test in the laboratory showed that the reducing agent-containing broth supported the growth of *Lactobacillus* similarly well to the same medium without reducing agent but incubated in an anaerobic jar. Both lactic acid bacteria, *Lactobacillus brevis* and *Pediococcus damnosus*, were detected by Speedy Breedy in just over one day at a concentration of $\sim 2,000$ cells/ml. At the lowest concentration investigated detection was still rapid (48-62 hours). This represents a significant advantage compared to the very slow detection by traditional methods (5-7 days).

In summary, all micro-organisms tested in this project were detectable by Speedy Breedy and detection was more rapid than by traditional microbiology in all cases. This was seen to be particularly advantageous for the detection of anaerobic organisms such as lactic acid bacteria for which detection

### Table 12: Speedy Breedy ‘event’ detection times for *Pediococcus damnosus* in NBB+sodium thioglycolate broth

<table>
<thead>
<tr>
<th>Nominal cell concentration 5x10^2 cells/ml</th>
<th>Nominal cell concentration 5x10^3 cells/ml</th>
<th>Nominal cell concentration 5x10^4 cells/ml</th>
<th>Nominal cell concentration 5x10^5 cells/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>$1.81 \times 10^3$</td>
<td>$3.23 \times 10^2$</td>
<td>27</td>
<td>5</td>
</tr>
</tbody>
</table>

### Table 13: Average *Pediococcus damnosus* concentration determined (average of triplicates) by plating onto NBB agar plates

<table>
<thead>
<tr>
<th>Nominal cell concentration determined on NBB agar plates (cfu/ml)</th>
<th>Time to detection by Speedy Breedy (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.81 x 10^3</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>3.23 x 10^2</td>
<td>41 ± 1</td>
</tr>
<tr>
<td>27</td>
<td>53</td>
</tr>
<tr>
<td>5</td>
<td>62 ± 1</td>
</tr>
</tbody>
</table>
normally requires 5-7 days. Speedy Breedy allowed detection of *Lactobacillus brevis* and *Pediococcus damnosus* within 27-38 hours (at a concentration of ~2,000 cells/ml). This new methodology was also found to be very sensitive and able to detect very low cell concentrations (2-5 bacterial cells/ml). The Speedy Breedy staff provided excellent training and technical support. The device was easy to use.