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Confidential report for:

Bactest

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Report on:

Application of Speedy Breedy to determine the microbiological quality of raw orange juice

Work performed by Campden BRI (Chipping Campden) Limited
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1 INTRODUCTION:

Bactest has developed an instrumental method for detection of microorganisms. The Speedy Breedy system offers a rapid test for the detection of microbiological contaminants based on changes in pressure caused by microbial respiration. The system can detect minor changes in negative or positive pressure and so has application to detection of many different bacterial species with different respiration patterns.

Previous tests done on behalf of the Client has shown the potential of the system to detect a range of clinical microorganisms and microbial populations in water samples. Studies have shown equivalent or faster detection times than other rapid growth detection systems and thus the Speedy Breedy shows promise for the detection of microbial populations in foods and drinks.

The aim of the studies reported here was to investigate the potential application of Speedy Breedy to determine the yeast population in orange juice.

Detection times in the Speedy Breedy were compared to plate count results obtained using conventional ISO standard methods in order to determine the correlation between the two approaches.

The data provided in this report is intended to provide demonstration data that the Speedy Breedy can be used to determine the microbiological quality of orange juice. Users of the system would need to demonstrate it was fit for purpose for their own products as they would have to do for any analytical method.

2 EXPERIMENTAL APPROACHES

2.1 Product

UHT orange juice was be used for these trials to ensure absence of non-target organisms.

Samples of juice were taken for the first set of trials on 7/8/13 and the remaining juice was placed in the refrigerator at 4-6°C and used for subsequent test days. The testing was done over the period 7/8/13 to 15/8/13.

2.2 Organisms

Saccharomyces cerevisiae CRA 16224 and *Zygosaccharomyces bailii* Y107 were used.

Prior to each experiment, the culture was grown in Malt Extract Broth at 25°C for 48-72hr. The numbers of cells present were estimated microscopically using a haemocytometer and an equal concentration of the two cultures were added together to make a cocktail.

2.3 Experimental matrix

Five sets of samples were analysed at different contamination levels level to reach a final level of between 100 and 10^6 cfu/ml yeasts. A 300ml sample of orange juice was inoculated with the yeasts

cocktail to achieve the desired level. 5x1ml samples were analysed for levels of yeasts using conventional ISO methods and 5x 50ml samples of inoculated juice were analysed in the Speedy Breedy system.

2.4 Microbiological analysis

Juice samples were analysed for yeasts using the conventional standard tests methods and the Speedy Breedy system using empty vessels containing 50ml juice. The yeasts were enumerated using the method below and the Speedy Breedy chambers were set to run at 25°C for 5 days but was stopped once a significant event was recorded.

Organism	Test method	Method Summary*
Yeast and mould enumeration for products with aw>0.95	TES-MB-197	Spread plate with DRBCA agar. Incubation at 25°C for 2 and 5 days

2.5 Analysis of results

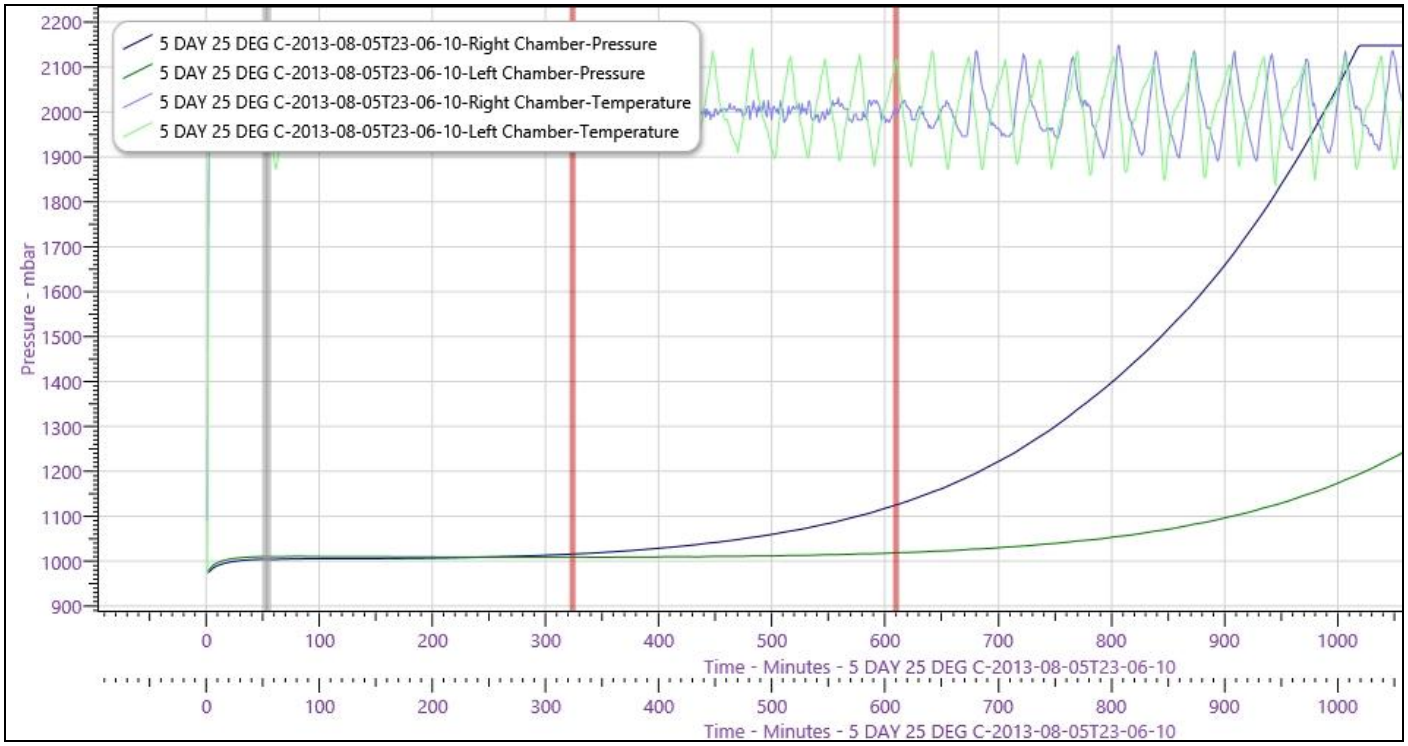
For the conventional test, the numbers of cells per gram of product were calculated.

For the Speedy Breedy, the time at which a significant event was registered was recorded as the detection time (DT) in minutes. This was converted to DT in hours.

The $\log_{10}$ number of cfu/g were plotted against the $\log_{10}$ DT in hours and the correlation was calculated.

An example of the Speedy Breedy output for yeasts in orange juice is shown in Figure 1. The graph is characteristic of all the graphs for yeasts where the change is pressure was positive throughout incubation.

Figure 1: Speedy Breedy Graph for yeasts in orange juice



3 RESULTS AND DISCUSSION:

Table 1 contains the data for the juice samples as cfu/ml, detection time in minutes and detection time in hours. This is also shown in Figures 2 as $\log_{10}$ cfu/ml versus $\log_{10}$ detection time in hours.

For samples with high levels of orange juice i.e. $>10^6$ cfu/ml, the detection was immediate. The pressure change began as soon as the samples were placed in the chamber.

For lower levels of yeasts (10^2 to10^5 cfu/ml) there was an excellent correlation between plate counts and detection time (R-sq = 0.96).

Table 1: Data for orange juice inoculated with yeasts

Samples	cfu/ml	DT (min)	DT (hr)
1a	$4.10x10^6$	immediate	-
1b	$2.70x10^6$	immediate	-
1c	$1.70x10^6$	immediate	-
1d	$3.90x10^6$	43	0.72
1e	$2.90x10^6$	immediate	-
2a	$4.30x10^5$	343	5.72
2b	$4.10x10^5$	340	5.67
2c	$4.40x10^5$	331	5.52
2d	$5.50x10^5$	324	5.40
2e	$5.30x10^5$	333	5.55
3a	$2.50x10^4$	651	10.85
3b	$2.70x10^4$	693	11.55
3c	$4.50x10^4$	627	10.45
3d	$2.20x10^4$	610	10.17
3e	$1.90x10^4$	619	10.32
4a	$5.60x10^3$	$1.2 x10^3$	19.45
4b	$5.10x10^3$	$1.1 x10^3$	19.43
4c	$4.50x10^3$	$1.0 x10^3$	17.48
4d	$5.30x10^3$	$1.1 x10^3$	18.92
4e	$4.10x10^3$	$1.0 x10^3$	18.02
5a	472	$1.4 x10^3$	24.77
5b	445	$1.5 x10^3$	25.50
5c	490	$1.4 x10^3$	24.55
5d	327	$1.4 x10^3$	23.82
5e	454	$1.4 x10^3$	24.12

Detection times of around 24hr were observed for the lowest level of 10^2 cfu/ml which is markedly shorter than the 2-5 days required for the conventional count. In addition, the detection occurred in the juice sample itself therefore not requiring the use of a selective yeast broth, although it is possible that the detection could be further improved by the development of a yeast broth.

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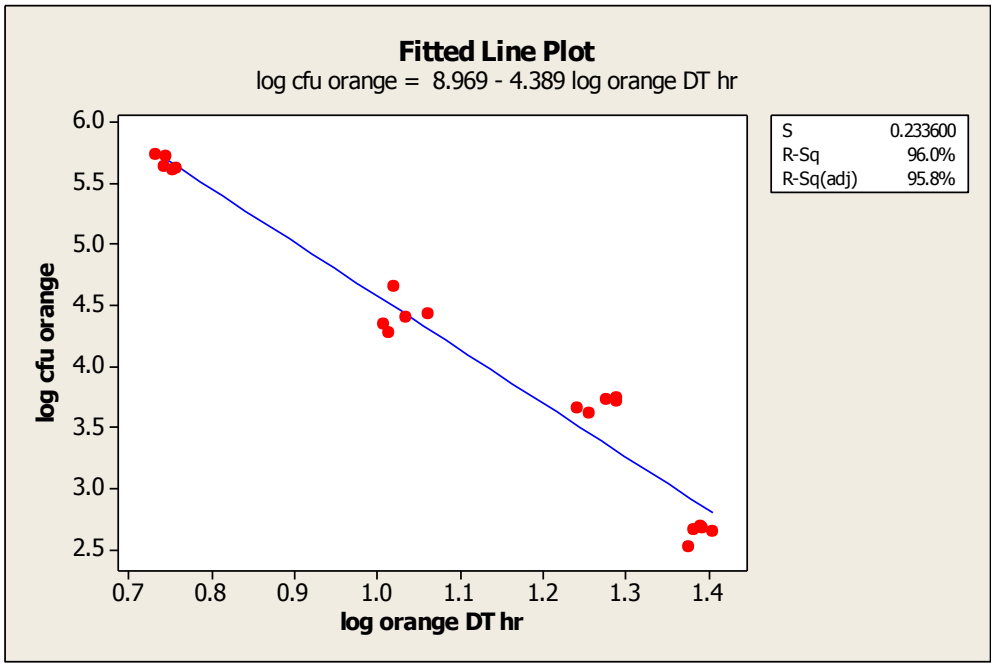
Figure 3: Log cfu/ml yeasts versus log DT (hr)



4 CONCLUSIONS

- The Speedy Breedy has shown excellent correlation between log cfu/ml and detection time for yeasts in orange juice (r =0.96).
- There is good agreement in the detection times between replicates at all levels.
- The data shows promise for use as a quantitative tool for determination of levels of yeasts in fruit juice or as a screening tool where absence of detection in 24h would equate to $<10^2$ cfu/ml.
- Speedy Breedy was very fast compared with current techniques, taking only a few hours to detect positive contamination compared with up to 5 days for plate counts. At low levels of contamination results were available in just 24 hours and immediately for high levels.
- Speedy Breedy can be used at the site of juice processing, removing the need for samples to be shipped to a laboratory, further reducing the time to achieve a result.
- Detection occurred in the juice sample itself therefore not requiring the use of a selective yeast broth, although it is possible that the detection could be further improved by the development of a yeast broth.

In summary, all micro-organisms tested in this project were detectable by the Speedy Breedy respirometer technology and detection was more rapid than by traditional microbiology in all cases by a considerable degree.

This new methodology was also found to be very sensitive and able to detect low cell concentrations.

The Speedy Breedy staff provided excellent training and technical support. The device was easy to use.