Campden BRI group:
Campden BRI (registered no. 510618)
Campden BRI (Chipping Campden) Limited (registered no. 3836922)
Campden BRI (Nutfield) (registered no. 2690377)

Registered office:
Station Road ◆ Chipping Campden ◆ Gloucestershire ◆ GL55 6LD ◆ UK

Confidential report for:

Bactest

FAO: Annie Brooking/Derek Price
St. Johns Innovation Centre
Cowley Road
Cambridge
CB4 OWS

Report on:

Application of Speedy Breedy to determine the microbiological quality of raw meat, orange juice, milk: Summary report

Work performed by Campden BRI (Chipping Campden) Limited
Report number: MB/REP/130657-4 version2 ◆ Issue date: 2nd October 2013
This version supersedes any previous versions

Contact details:

Gail Betts ◆ Microbiology ◆ Campden BRI (Chipping Campden) Limited
gail.betts@campdenbri.co.uk ◆ Tel: +44(0)1386 842071 ◆ Fax: +44(0)1386 842100
We value your opinion: **http://www.campdenbri.co.uk/campdenbri/fdbck.php**

Report issued and authorised by:

Campden BRI (Chipping Campden) Limited
Dr Gail Betts ◆ Manager, Microbiological Safety and Spoilage Section

Our ref: MB-REP-130657-4v2.doc
Page count: 8

[DC: RA-T-9-002: 09/13 (4) : R/AJR]

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 microbial populations in a range of food and drink products. The following combinations were tested:

Aerobic plate count in milk
Yeasts count in orange juice
Aerobic plate count in meat
Coliforms in meat

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.

Full experimental details and results are given in the individual product reports.

2 EXPERIMENTAL APPROACHES

There were two types of test carried out in this study:

1. Naturally present organisms
2. Inoculated organisms

For naturally present organisms in milk and meat, a sample of product was purchased and tested over a few days in order to allow the levels of naturally present organisms to increase in time. The mix of organism within the total microbiological population may also have changed with time.

For inoculated trials, a strain of *E. coli* in the case of coliforms in meat and two strains of yeasts in the case of orange juice, were cultured in the laboratory and inoculated into the product at pre-determined levels prior to analysis.

For each combination of product and organisms 5 different sets of samples were analysed in quintuplet making a total of 25 tests.

The levels of microorganisms present per gram of product were evaluated using conventional ISO methods. The detection times in the Speedy Breedy were obtained using the following conditions.

Product	Organisms	Vessel	Test conditions
Milk	Aerobic plate count	Empty vessel containing milk	30°C/48hr
Milk	Aerobic plate count	TSB	30°C/48hr
Meat	Aerobic plate count	TSB	30°C/48hr
Meat	Coliforms	MacConkey	37°C/48hr
Orange juice	Yeasts	Empty vessel containing juice	25°C/5days

3 RESULTS AND DISCUSSION

The outputs from the Speedy Breedy showed different changes in vessel pressure between the different products. Figure 1 to 4 show a typical graph from each of the different trials. The milk graphs showed curves where there was a decrease in pressure throughout the incubation showing that oxygen was being used. The curves for the coliforms in meats was similar. For the TVC in meats whilst the initial part of the curve showed a decrease in pressure, the latter part of the curve showed an increase in pressure showing a switch in dominant organisms or in respiration pathway. For the yeasts there was an increase in pressure caused by the fermentative respiration of these organisms.

In order to determine whether there was a relationship between microbial levels and detection time, the $\log_{10}$ number of cfu/g were plotted against the $\log_{10}$ DT in hours as shown in Figure 5 to 8.

It is apparent that when the type of organism present in the product is controlled as in the case of coliforms in meat and yeasts in orange juice, there is an excellent correlation between cfu/g and detection time. The time to detection microbial respiration rate is consistent between samples and it is possible to use the calibration graph as a quantitative indication of levels of organisms present.

For samples where the type and level of microorganisms is not controlled as in the case of naturally present samples, the agreement between levels and detection time is not so consistent. The rate or growth, respiration rates and pathways may vary for mixed groups of organisms but the data could be used as a screening tool, i.e. no detection time in 24h means that the levels of cfu/g are below a minimum threshold.

Based on the studies done to date, the Speedy Breedy is an easy to use convenient system for measuring time to detection of microbial populations as an indication of contamination levels. It appears to be better suited to the use of selective media/test conditions which target for a defined group of organisms, e.g. yeasts or coliforms where the respiration patterns appear to be more consistent.

Speedy Breedy was fast compared with current techniques, in many cases taking only a few hours to detect positive contamination compared with two to five days for plate counts.

Speedy Breedy can also be used at the site of food processing, removing the need for samples to be shipped to a laboratory, further reducing the time to achieve a result. Based on the results, Speedy Breedy would therefore represent a suitable screening tool for rapid on-site testing.

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.

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.

Campden BRI (Chipping Campden) Limited – part of the Campden BRI group
Station Road◆Chipping Campden◆Gloucestershire◆GL55 6LD◆UK
Providing services under an ISO 9001 registered quality management system

Figure 1: Speedy Breedy Graph for milk and milk in TSB

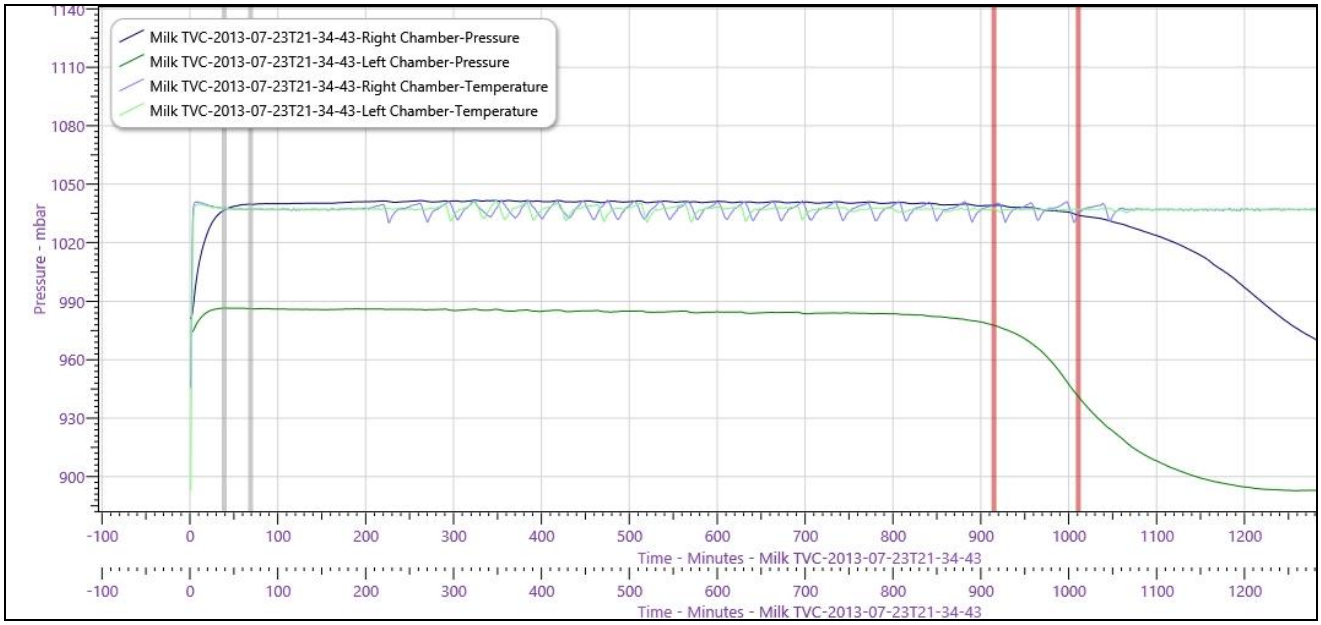

Figure 2: Speedy Breedy Graph for APC in meat

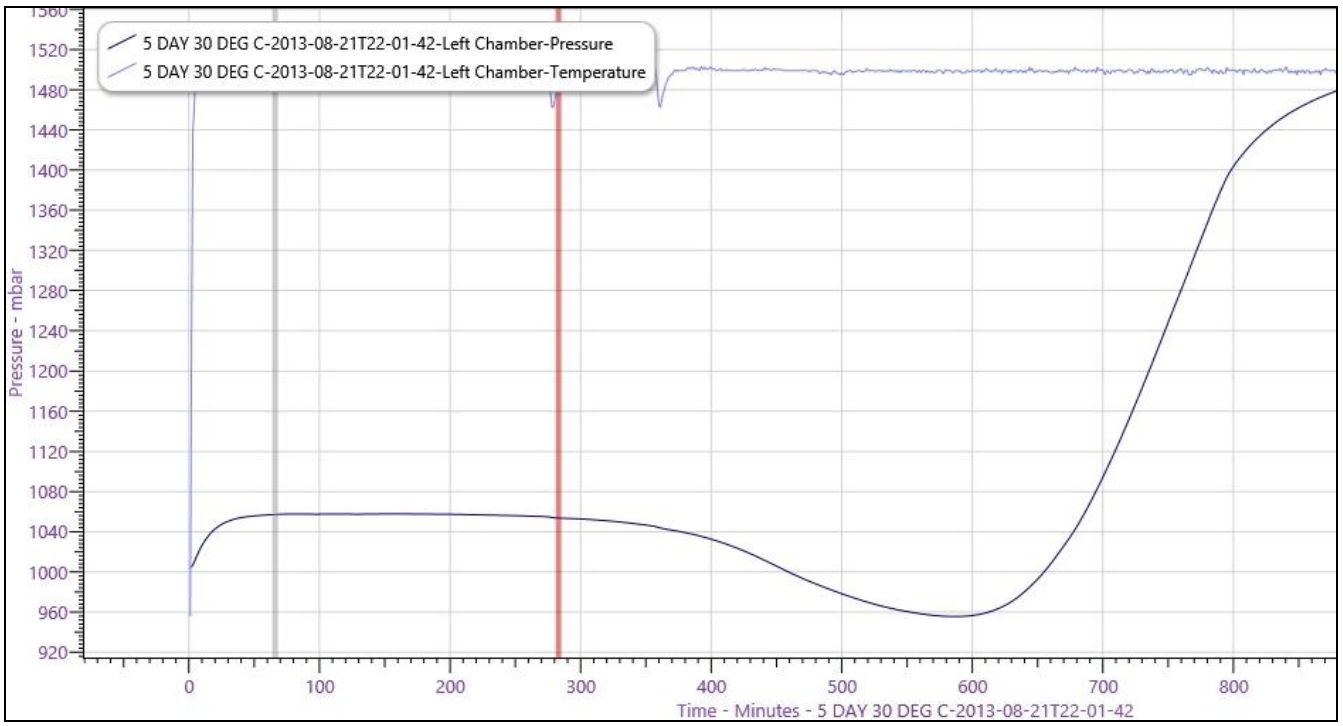

Figure 3: Speedy Breedy Graph for coliforms in meat

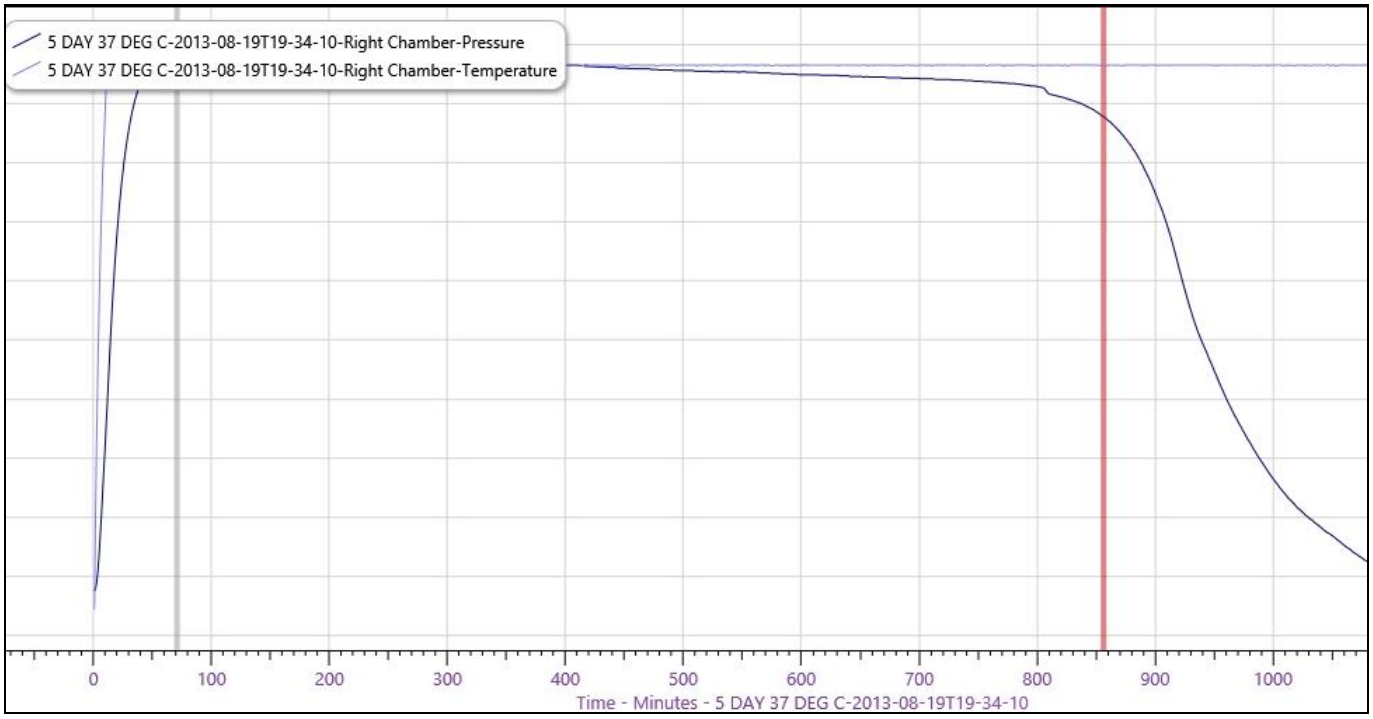

Figure 4: Speedy Breedy Graph for yeasts in orange juice

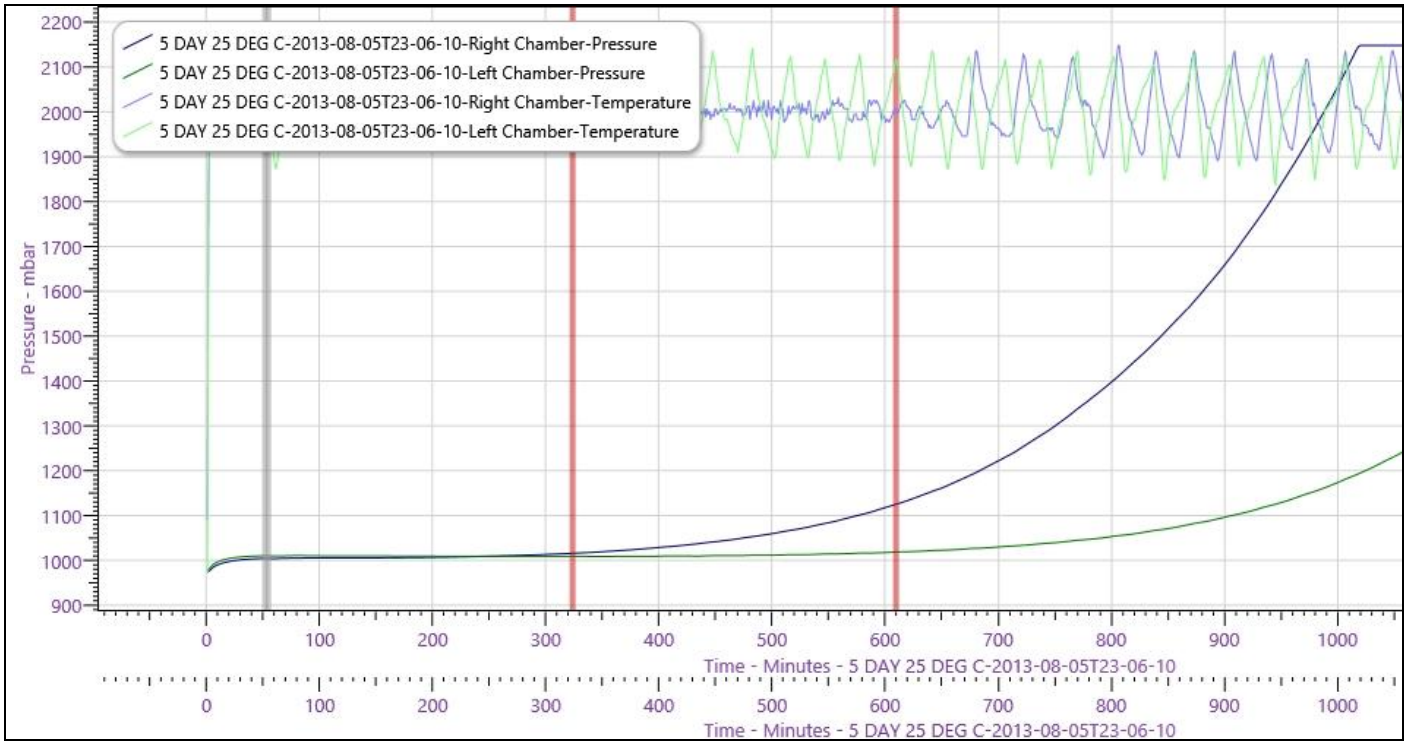

Figure 5: Log cfu/ml milk versus log DT (hr)

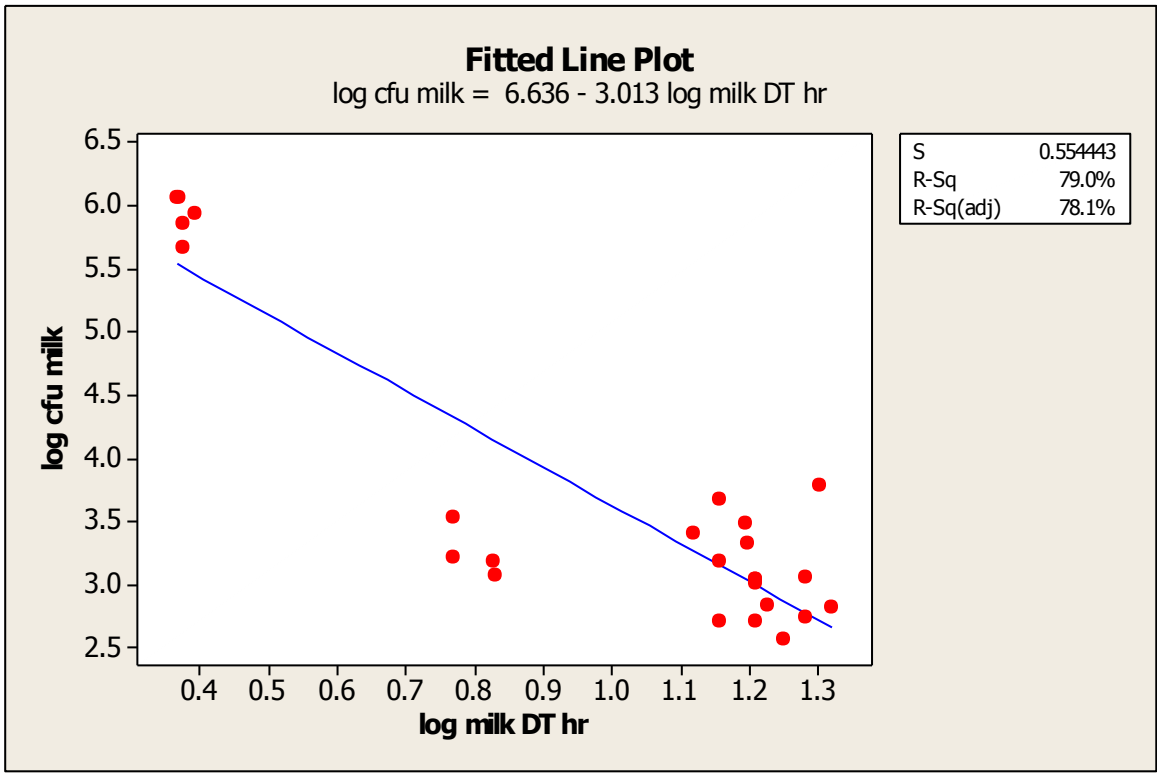

Figure 6: Log cfu/g APC in meat versus log DT (hr)

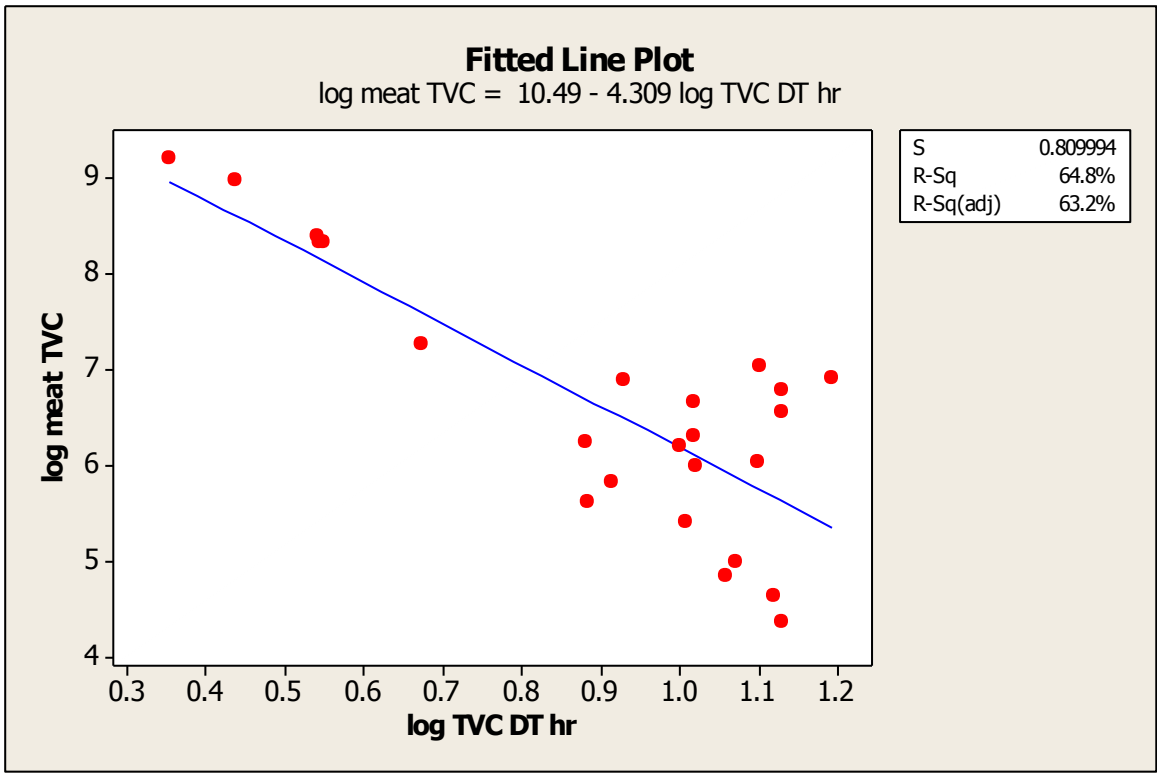

Figure 7: Log cfu/g coliforms in meat versus log DT (hr)

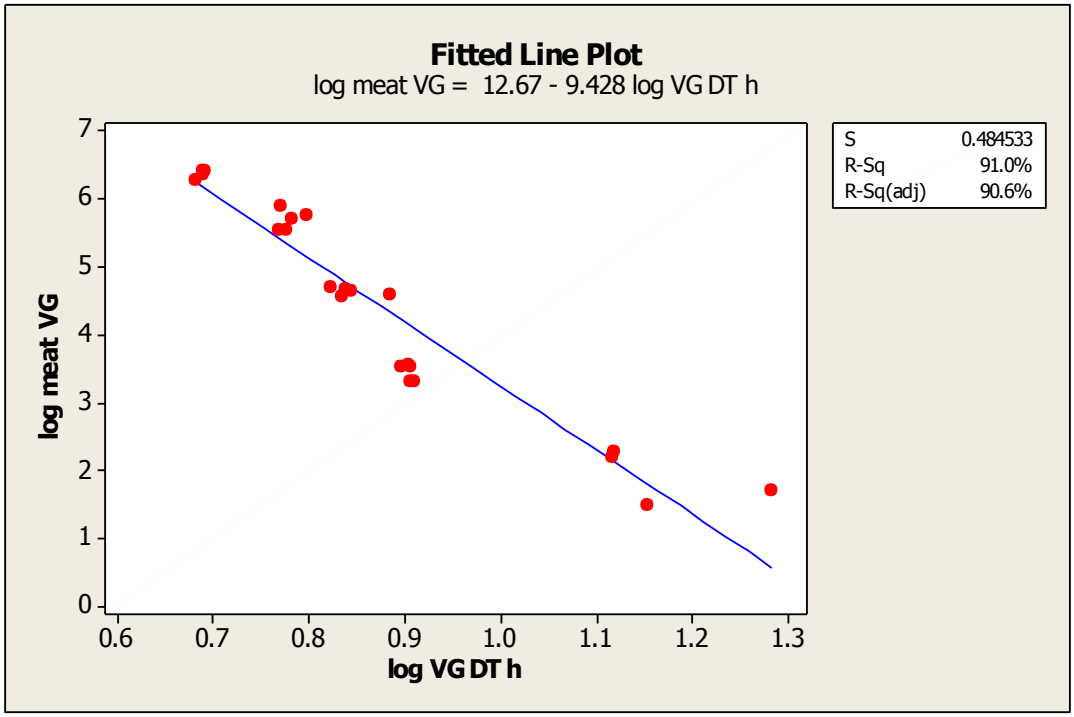

Figure 8: Log cfu/ml yeasts versus log DT (hr)