



## Speedy Breedy - Lab Memo 36

### Measurement of Bacterial contamination and effect of Biocides in Functional Fluids

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#### ***Objective***

The objective is to establish Speedy Breedy as an on-site, rapid technique to quantify microbial contamination in functional fluids, assess the efficacy of biocide systems, and propose a suitable protocol to monitor the rate of degradation.

The aim is to set up a surveillance system to mitigate the risk of contamination in cutting oils and other functional fluids, reducing chemicals usage, equipment failures, poor quality manufacturing, and health issues for operators.

#### ***Principle & background***

Functional Fluids are used for many different purposes including coolants, lubricants and cutting oils. Bacterial contamination of Functional Fluids leads to degradation so most contain a biocide to stop bacterial growth however biocides have a limited operation life and bacteria frequently overcome the biocide, degrading the fluid, making it ineffective, and creating a health hazard for the operator.

Unfortunately, there is no simple, rapid method that can be used on the factory floor to determine when the fluid needs to be changed, or biocide added. This leads to waste if the fluid is changed too frequently and loss of function if it is left too late.

Speedy Breedy is a portable device that can be used on the factory floor and determines contamination by measuring sensitive pressure changes within a closed vessel due to microbial respiration, and assigns a Time to Detection which is indicative of the level of contamination.

#### ***Experiment to show detection of contamination in cutting fluid***

Based on previous work that indicated Speedy Breedy could successfully grow and detect both *E. coli* and *Pseudomonas* in new Hocut 3280 (an industrial cutting oil) containing no biocide, an experiment was performed to determine whether Speedy Breedy can differentiate between new and contaminated cutting oils that do contain biocide, therefore mimicking the working environment:

#### ***Method***

One sterile Speedy Breedy vessel pre-filled with TSB nutrient capsules was filled with 25 ml clean sterile water and 25 ml of new Hocut 3280 (containing biocide).

A second identical vessel was filled with 25 ml clean sterile water and 25 ml of old Hocut 3280 (containing biocide) that had been used in a metal cutting machine for some time and believed to be contaminated with bacteria. An odour was noted. The vessels were inserted into Speedy Breedy (old Hocut in the right chamber, new Hocut in the left chamber) and a test run performed using a standard 36 Deg C protocol.

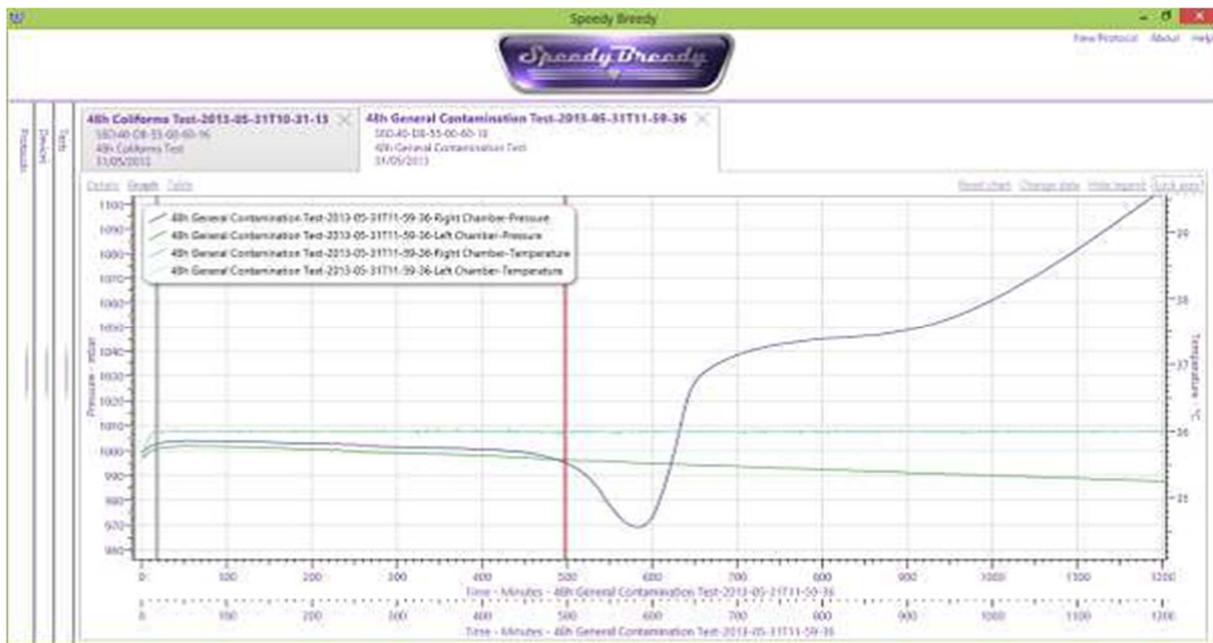


## Results

Graph 1. (below): An event was recorded in the right chamber (blue line) at 500 minutes as indicated by the vertical red line event mark on the graph above.

Speedy Breedy communicated a microbial contamination event for old Hocut. No event was recorded in the left chamber (green line -new Hocut).

When the vessels were removed, the “contaminated” vessel containing old Hocut showed significant turbidity and the liquid was much lighter in colour than the second vessel containing new Hocut where no event was detected.



## Conclusion

Speedy Breedy is a simple to use instrument that can differentiate between samples of cutting oil where the biocide is working effectively to oil containing biocide that has been degraded after use.

Having established that bacteria will not grow in the oil when the biocide is active but do when the biocide is degraded, an obvious protocol would be to tested the cutting oil over a period of time to determine when contamination becomes a problem and fluid either needs to be changed or additional biocide introduced.

This would form a routine monitoring system that could reduce costs, improve quality and Reduce health issues in the factory.

An independent industry expert has been consulted and agrees the logic of the proposed testing regime. It is the remit of the biocide supplier to provide efficacy data against different species.

***Proposed regime for routine testing of cutting oils and other functional fluids for microbial contamination and biocide efficacy.***

Speedy Breedy can be used to monitor functional fluids over a period of time in order to determine:

- The inherent background level of contamination if present.

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- The effectiveness of any biocides used.
- The change in bacterial numbers with time in order to show whether this is getting worse and
- Remedial action needs to be taken.

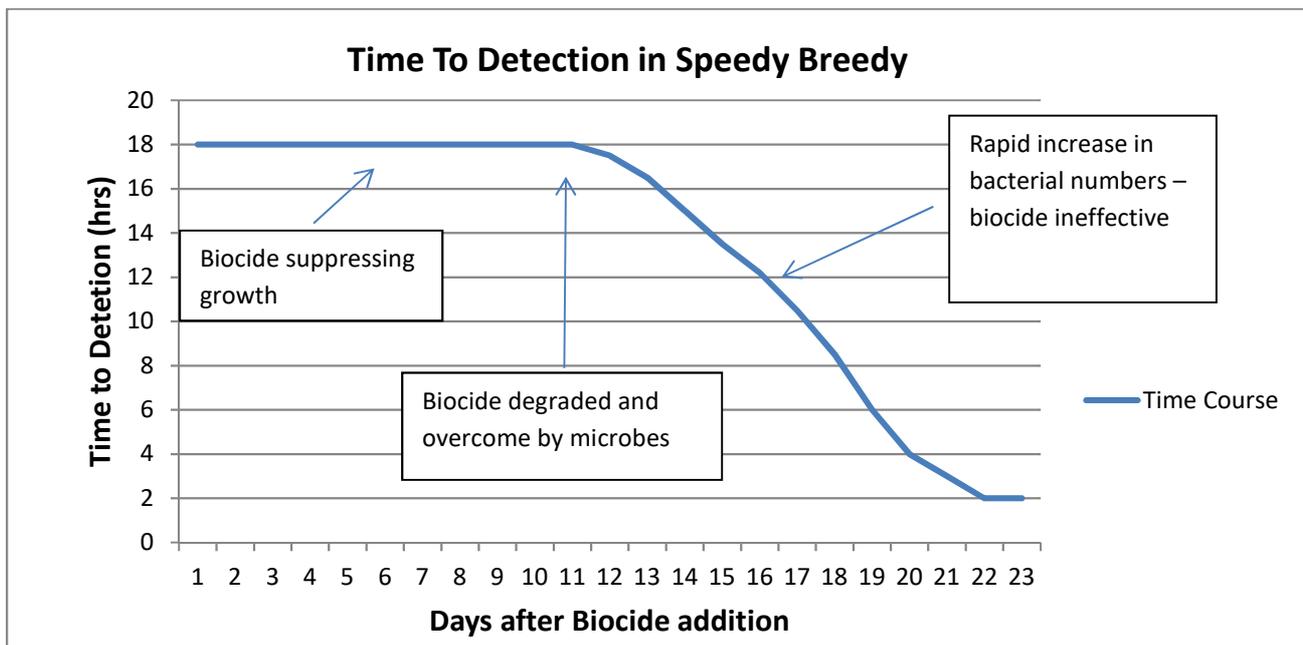
By testing samples each day (or similar) and plotting results it will be possible to determine whether there is a net increase or decrease in numbers of contaminating bacteria, indicating the effectiveness of the biocides used.

### ***Hypothesis***

The hypothesis is that when biocide is added to the functional fluid it will suppress the growth of microbial contaminants for an unknown period of time but will eventually degrade and become ineffective against the bacteria. This is typical of biocide action in many industries. When samples are taken regularly in a test sequence and Time To Detection (TTD) in Speedy Breedy is plotted against the time from addition of new fluid or biocide, Speedy Breedy will effectively determine when the biocide is inactive because the microbes will grow more quickly.

The period that the biocide will be active cannot be predicted because each biocide and concentration will work differently however after several test sequences a body of knowledge will be built up that will aid in determining the frequency of testing required to identify when biocide is degraded.

### **Results are indicated in the graph below:**



### **Explanation:**

In this test sequence the functional fluid has been sampled on a daily basis, tested in Speedy Breedy, the TTD logged and then plotted for each day after addition of biocide. An arbitrary cut-off TTD at 18 hours was set up but this may be longer – a 24 hour protocol might work better taking into account shift patterns and so forth. Any sample taking longer than this to detect means that the bacteria are effectively suppressed by the biocide.



At the start of the sequence when the biocide is new and fully active, bacterial suppression will be high and a TTD of greater than 18 hours is likely. As seen in the example.

In the example given, at Day 11 the TTD falls below the 18 hour threshold and on subsequent days the TTD becomes increasingly shorter indicating that the bacterial load is rising rapidly because the biocide is degrading and is no longer effective. The fast and easy assessment of contamination using Speedy Breedy therefore supports a better understanding of the effectiveness and longevity of the biocide and aids in managing replacement and addition of biocide. This can result in considerable savings in chemicals usage and gives an indication whether problematic organisms are under control. It will also aid in assessing the correct biocides and concentrations for that particular process.

### Protocol:

TSB culture vessels are used in Speedy Breedy to detect Total Viable Count (TVC) of the broad spectrum of organisms in the functional fluid, with a culture temperature of 36 Deg C and a mixing speed of 90 rpm Bactest can provide a TVC protocol for use in Speedy Breedy in this application.

- 25 ml samples of the functional fluid are taken daily using a clean sample vessel (50 ml disposable syringes are good for this purpose), mixed with 25 ml of clean water and added to a Speedy Breedy TSB vessel.
- Samples are run in Speedy Breedy using the TVC protocol provided.
- Time To Detection is monitored for each day in the test sequence and plotted as indicated on the graph above.
- The efficacy of the biocide is determined from the graph and new biocide added as required.
- Further testing is continued to show effectiveness of the new biocide addition.

### Discussion

It was found that many functional fluids including cutting oils mix very well with water when used at a 1:1 ratio and that Speedy Breedy's culture medium capsules readily dissolved in the mixture.

This document is for general guidance only and sets out a proposed start point for experiments to establish Speedy Breedy as a microbial monitoring system for functional fluids. It provides no guarantee of success as each process is different and it is the responsibility of the user to establish efficacy of procedures and protocols used in their process.