Speedy Breedy- Lab Memo 15

Coliform detection

Author: Martina Palmer Date: Aug 2013

Principle and Background:

Quickly determining the presence or absence of Coliforms or E.Coli in water or other samples is an important ability for anyone working with potable water or other items for human consumption.

Fermenting lactose, the resulting acid production and therefore the colour change from purple to yellow in MacConkey's broth at 36°C is a long established indication for the presence of Coliforms or E.Coli. , ,

A bacterial respirometer (Speedy Breedy) was used to conduct this experiment. It is a novel technology utilising changes in gas composition within a sealed vessel to determine bacterial growth. However due to the nature of the machine, the inside of the chamber and therefore the medium cannot be visually monitored and it being a new technology there is no data available to indicate when the colour change and therefore a presumptive identification of Coliforms or E.Coli actually occurs.

Experiment

Each of the vessels was filled deionised water run through a sterile filter. 3 vessels were inoculated with lenticule discs containing either 37 CFUs and 3 vessels were inoculated with lenticule discs containing 4.04x104 CFUs.

The vessels were then inserted into 3 Speedy Breedys and note was taken of their positions. The protocol "48hr Coliform test" was run on each of the Speedy Breedys.

The vessels were removed from the Speedy Breedy at three distinct points: At event detection, at the lowest pressure point and when the pressure had returned to the same level it was at before the event. The colour was recorded immediately after the vessel was removed from the instrument.

Results

Vessel	Starting inoculum	Time to detection	Removed after	Colour	Cloudiness
1	$4.04x10^4$	412	412	Purple	No
2	$4.04x10^4$	412	456	Purple	Yes
3	$4.04x10^4$	412	527	Yellow	Yes
4	37	619	619	Purple	No
5	37	619	757	Purple	Yes
6	37	619	811	Yellow	Yes

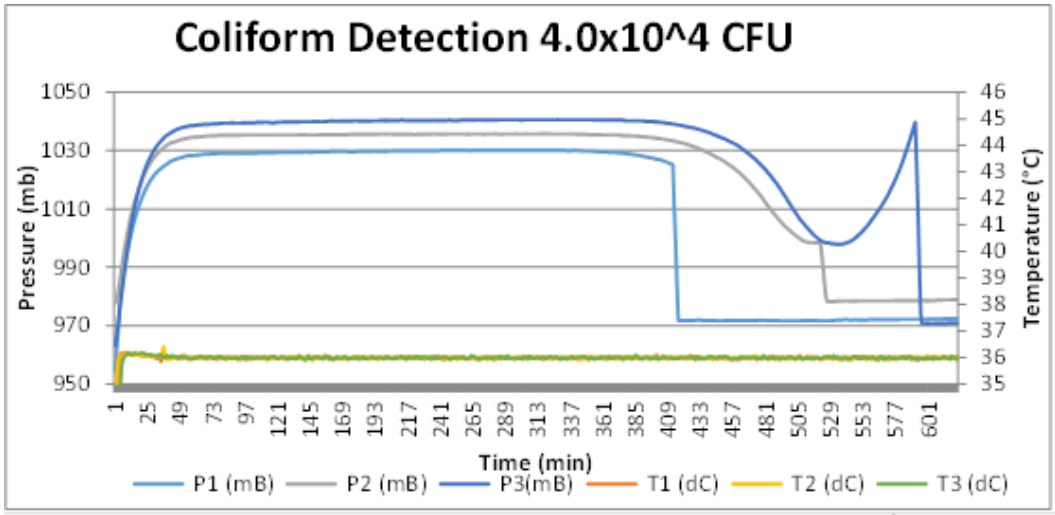
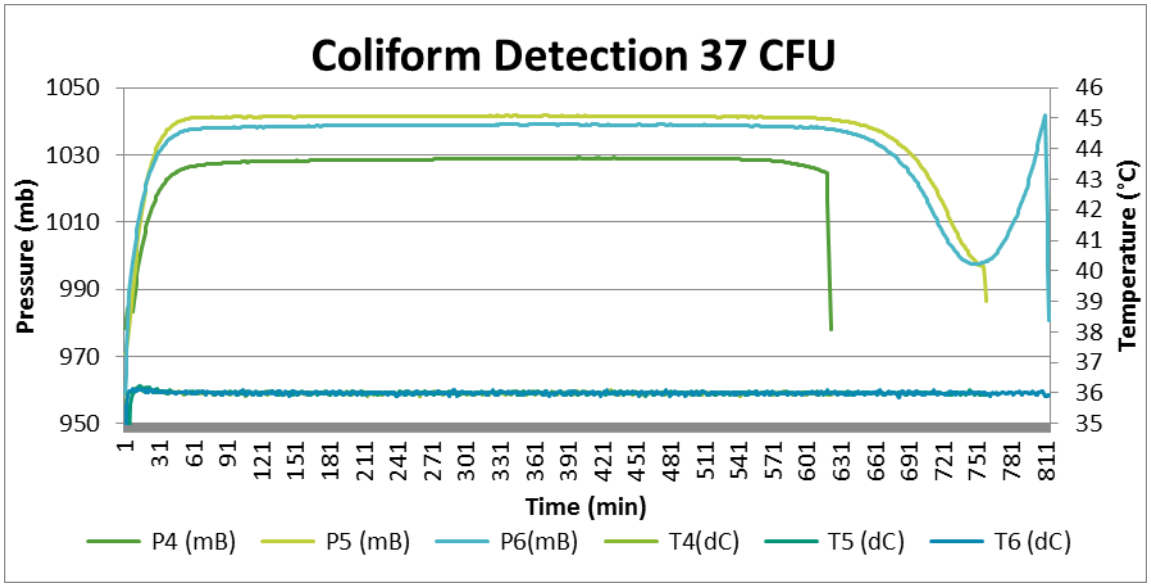


Figure 1 Graph showing the different points of vessel removal with a starting inoculum of 4.0x10⁴ Cfu.



Speedy Breedy- Lab Memo 15

Discussion

The colour change is facilitated through fermentation of lactose, a process which requires anaerobic conditions.

The depletion of oxygen is most likely indicated by the drop in pressure, followed by gas production from the fermentation. Therefore it seems logical that the first indication of a colour change follows a period of fermentation with gas production, as indicated by the rising pressure in series 3 and 6.

It is an important finding that due to the sensitivity of Speedy Breedy at the point of event detection there was no visible colour change and little to no cloudiness within the vessel. The protocols to detect coliforms and E.Coli must take this into account and cannot be allowed to stop at the point of event detection, but must be permitted to run for at least 2 hours in order to prevent false negative results from being reported.

The results show that at the point of event detection contamination has been identified, however if a presumptive identification of Coliforms is required, the vessel should remain in Speedy Breedy for approximately 2.5 hours after an event has occurred or until a return to initial pressure has been achieved as seen on the graph.

This data shows that the colour change does not occur at the point of critical mass, but after fermentation starts to occur, which needs to be taken into account when designing a protocol for the detection of E.Coli or Coliforms.

Conclusion

Using bacterial respirometers in microbiology is a new concept, however this experiment shows that Coliforms or E.Coli can confidently be identified using this novel technology after between 8:45 and 13:30 hours, depending on the level of contamination, more than 10:30 hours faster than traditional techniques.

It is important that the point of colour change be taken into account when protocols are designed and experiments conducted to avoid misinterpretation of samples.

Departments of the Environment, Health, Social Security and Public Health Laboratory Service (1982) The Bacteriological Examination of Drinking Water Supplies. Report No. 71. HMSO London.
Childs Eileen and Allen L. A. (1953) J. Hyg. Camb. 51(4). 468-477.
World Health Organization (1963) `*International Standards for Drinking Water' 2nd ed., WHO, Geneva*.