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Clostridia in milk: hydrogen detection through Raman gas spectroscopy

Clostridium are bacteria known to produce gases, especially carbon dioxide and hydrogen. Raman spectroscopy to detect them because it allows the simultaneous measurement of different chemical species including carbon dioxide and hydrogen, the latter of which is impossible to measure using absorption spectroscopy in an optical path of the order of a centimeter. The setup consists of a laser source, whose beam is focused in the headspace of the vial under examination. Orthogonally, the scattered Raman light is collected by a custom made spectrometer coupled to a CMOS industrial camera. The setup is shown in figure 1.

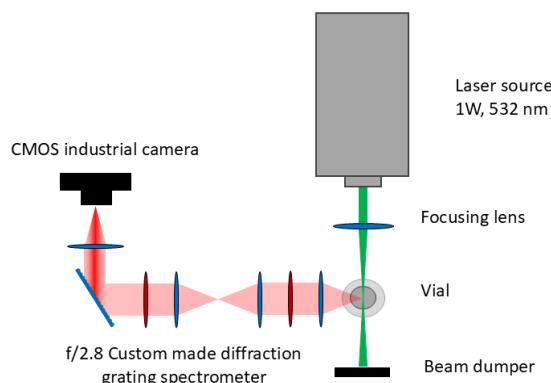


Figure 1: Experimental setup.

The procedure is therefore similar to that applied in the classical most probable number (MPN) method but the positivity of a vial is determined on the basis of the hydrogen concentration present in the headspace of vials containing potentially infected milk. The system has been optimized to achieve a sensitivity of 29 spores per liter, which is of particular relevance given that nowadays there is no well-defined threshold to reject milk infected by clostridia but only an indicative value and would therefore be a valid tool to determine it. To limit the effect of fluorescence in the measurements, both hardware solutions, such as the use of two long-pass filters, and software solutions, such as the development of algorithms for the creation of spectra not saturated by fluorescence, were adopted.

References

- [1] Barbiero, D., Melison, F., Cocola, L., Fedel, M., Andrichetto, C., Dea, P. D., & Poletto, L. (2024). Raman Spectroscopy Applied to Early Detection of Clostridium Infection in Milk. In *Applied Spectroscopy* (Vol. 78, Issue 12, pp. 1256–1262). SAGE Publications. <https://doi.org/10.1177/00037028241252693>