Critical assessment of the Time-To-Detection method's performance to estimate microbial growth Parameters

Baka M.[1,2], Stamati I.[1,2], Noriega E.[1,2], Logist F.[1,2], Van Impe J.[1,2]
1. KU Leuven/BioTeC, Leuven, Belgium
2. Flemish Cluster Predictive Microbiology in Foods (www.cpmf2.be)

OBJECTIVE(S)
Viable plate counting is the most accurate and widely used method for microbial growth parameters' estimation (mu_{max}, lag phase); however, it is laborious and time consuming. Therefore, it is of high importance to develop techniques equally reliable, able to minimise the experimental load. Time-To-Detection (TTD) method, based on turbidity measurements, was developed to fulfill these requirements (Cuppers et al. 1993). This method is widely applied, but its performance is contradictory in literature. A sensitivity analysis of the factors influencing the method is necessary to define the boundaries of reliable performance.

METHOD(S)
Three *Listeria monocytogenes* strains (LMG 23775, LMG 23905 and LMG 21263) were selected from the BCCM/LMG bacteria collection of Gent University (Belgium). A full factorial experimental design with different temperatures (4, 8, 12°C), pH values (6.0, 6.4, 6.8), NaCl concentrations (1.9, 2.6, 3.3%) and atmospheres (aerobic and vacuum) was implemented in Brain Heart Infusion broth (BHI), based on intrinsic and extrinsic conditions found in real food products. Optical density (OD) measurements (595 nm) were taken at regular time intervals with VersaMax™ Absorbance Microplate Reader. Microbial growth parameters were estimated by using the TTD method and a detailed analysis of the different variables affecting its performance was performed. Serial decimal dilutions (N_0 values) from 10^5 to 10^0 cfu/mL were used. A threshold value of optical density was set (0.2) and the time required for each of the inoculum levels to reach this threshold is plotted as a function of $\ln(N_{turb}/N_0)$, forming a linear regression. N_{turb} is the cell population when OD is 0.2. The inverse of the slope of the regression line equals mu_{max} and the intercept with the y-axis equals the lag.

RESULTS
Experimental results obtained illustrate that the TTD method struggles to describe the occurring phenomena and accurately estimate the growth parameters under realistic food-related scenarios. The relationship between TTD and $\ln(N_{turb}/N_0)$ deviates from the typical linear regression when N_0 decreases and the stress increases, resulting in negative lag estimation and underestimation of mu_{max}. Similar behavior is observed in literature (Robinson et al. 2001), while in Mytilinaios et al. (2012) TTD's performance is successful under optimal conditions and low N_0. The method depends on the variables N_0, N_{turb} and TTD, which are all subject to uncertainty and variability. The selected N_0 range (and the dilution error) has an effect on the output of the method, as previously described. N_{turb} depends on the accuracy of the calibration curve relating OD and viable counts, and the OD threshold selected as the detection limit. For this reason, the Total Least Squares regression technique was applied in order to account for errors of both dependent and independent variables of the calibration curve. By changing the range of OD included in the calibration curve the output was evaluated. Finally, the TTD is estimated with different available models (i.e., interpolation function, Baranyi (1994) or Richards (1959)); the output of the method is significantly influenced by the model selected.

CONCLUSIONS AND IMPACT OF THE STUDY
The TTD method has limitations to precisely estimate the growth parameters of *L. monocytogenes* under stressing conditions and low inoculum levels as occurring in real food products. The results ofthis study have significant implications for estimating parameters relevant in food safety assurance systems.

REFERENCES

Baranyi J. et al. 1994. International Journal of Food Microbiology 23, 277-294.
Cuppers H. G. A. M. et al. 1993. Journal of Industrial Microbiology and Biotechnology 12, 168-171.
Mytilinaios I. et al. 2012. International Journal of Food Microbiology 154, 169-176.
Richards, F. J. 1959. Journal of Experimental Botany 10, 290-300.
Robinson T. P. et al. 2001. International Journal of Food Microbiology 70, 163-173.