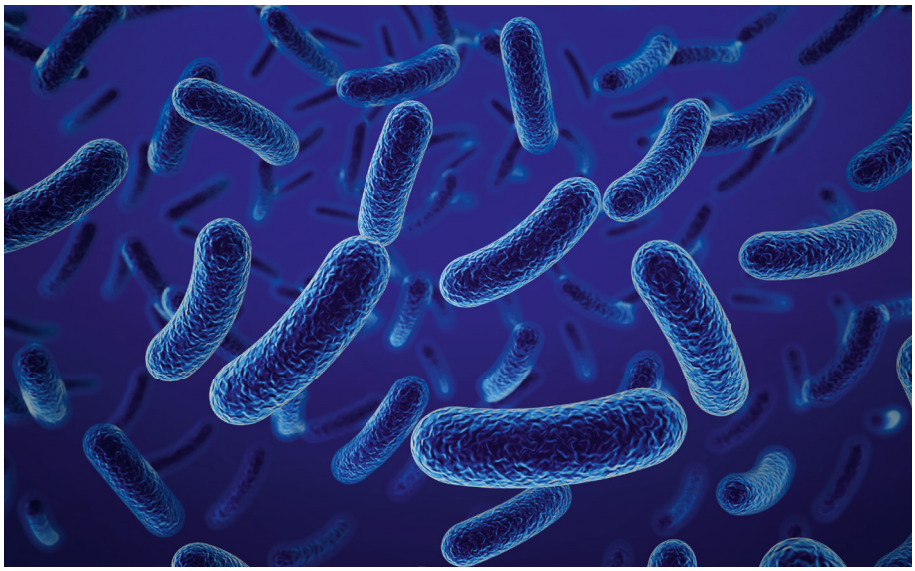
REAL-TIME MONITORING OF MICROBIAL VIABILITY



TNO innovation for life

For the industry, knowledge of microbial viability is the key to increased production efficacy, treatment procedure selection, process optimization, and high product quality. TNO has developed a user-friendly fluorescence based test for microbial live-dead assessment. This rapid and cost-efficient method was successfully tested with a diverse range of different bacteria (including spores), protozoa and fungi.

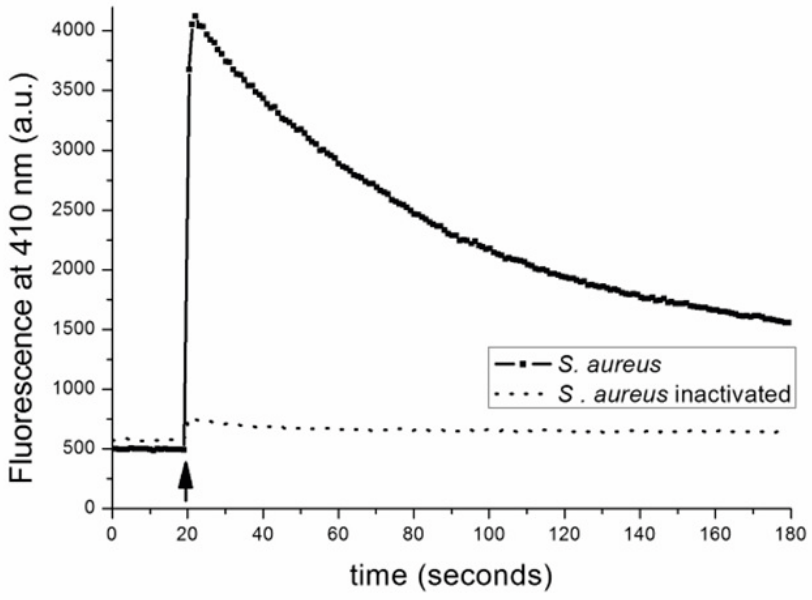
Viability is influenced by a multitude of physical and biological parameters. Knowledge of microbial viability is of pivotal importance in all industrial processes employing microbiology. The fluorescence-based test of TNO is an efficient alternative to methods like plate counting, microscopic live-dead assessment, ATP measurements, or flow cytometry, and does not require molecular expertise.

CURRENT TECHNOLOGY

Monitoring of microbial viability is typically performed using traditional cultivation on solid media and counting of resulting colonies. This approach can take several days depending on the microbial species. ATP measurements have proven to be only rough indicators of viability as the ATP content is linked to the growth phase, chemical environment, choice of medium, presence of metabolic inhibitors, etc. Alternative methods like microscopic live-dead assessment and flow cytometry using viability probes are time-consuming and require specialized and expensive equipment and trained laboratory personnel. Such slow and costly traditional methods do therefore not allow for the fast screening of viability and the high-throughput analysis of a large number of samples.

RAPID SCREENING TEST OF TNO

TNO has developed an innovative technology which allows for rapid and easy screening for viable cells. The principle is based on the fact that the inner compartment of dead cells reflects the conditions of the surrounding environment, whereas live intact cells actively maintain their distinct compart-mentalization and their characteristic internal properties.



Fluorescent signal at addition of probe and buffer (arrow), a quantitative measure for the amount of living cells in a mixture.

A fluorogenic biomarker is added to a mixture of live and dead cells. In the surrounding medium and in dead cells the fluorescence is quenched, whereas once internalized by live cells, a fluorescent signal is emitted upon light exposure. The signal is quantitatively monitored and indicates the presence of live cells. The method in addition monitors the ability of the live cells to maintain a controlled intracellular environment under biocidal conditions, which can serve as a fitness parameter.

MANY APPLICATIONS

The Real-Time Viability (RTV) technology is a strategic diagnostic tool for all applications where knowledge about microbial viability is important. The broad range of applications includes both process optimization and assessment of product quality. A large number of samples can be rapidly screened.

The technology in its current stage is especially well suited for viability assessment of food ingredients including:
- fitness of probiotics
- viability of starter cultures

Near-future applications include determination of:
- killing efficacy of antimicrobial compounds, biocides, and antibiotics
- minimal inhibitory concentrations (MIC)
- microbial contamination of surfaces, air, and water
- efficacy of biofilm removal

The method is compatible with sample filtration and is not affected by organic matter in the cell suspension. However, as the principle is based on the loss of a controlled intracellular environment, short term effects of UV killing and some antibiotics cannot be assessed.

References to RTV method and applications:

- *van Melis CC et al., (2012), Int J Food Microbiol. 160(2): 124–30.*
- *Nocker A et al., (2012), J Microbiol Methods. 90(2): 86–95.*
- *Nocker A, et al., (2011), Appl Environ Microbiol. 77(18): 6433–40.*
- *Kort R, et al., (2010) BMC Biotechnol. 18;10:45.*
- *Kort, R. et al., EP Patent 2.232.243*

ADVANTAGES

The real-time viability (RTV) method will be of exceptional benefit for all applications where information about microbial viability and fitness is needed. The test is based on the uptake of a fluorogenic biomarker and is applicable to virtually all cell types including gram-negative and -positive bacteria, bacterial spores, protozoa, fungi, and other eukaryotic cells, independent of their culturability. High-throughput is made possible by a microtiter plate format. Detection of fluorescence indicates viability. The method is fast, extremely user-friendly, cost-efficient, and versatile. It avoids long incubation times and provides answers within minutes after the sample is taken.

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TNO

TNO has over 3000 professionals who put their knowledge and experience to work in creating smart solutions to complex issues. These innovations help to sustainably strengthen industrial competitiveness and social wellbeing. We are partnered by some 3000 companies and organisations, including SMEs, in the Netherlands and around the world. On the topic of Healthy Living we initiate technological and societal innovation for healthy living and a dynamic society.

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