Universitat Autònoma de Barcelona

Design of a fluorometric fast method for microbiological quantitation in food adjuvant products.

Lluís Montserrat Sanz, June 19th 2019

Study goals:
- Have conclusive evidence to decide the microbiological quality of a product.
- Results must be obtained in 24h or less.
- The method must be design to be used in a spectrofluorometer.

Why choose ATP quantitation fluorescence?
- Fluorescence allows to detect specifically a certain fluorophore, with great precision at a low concentrations.
- Fluorometric assays are fast and fluorometric kits are globally available.
- ATP is a high-energy molecule described as the energy currency in all living systems, and its chemical energy drives most of cellular processes[1], which can be used as a biological indicator.

Method design:

Bacillus spp. based products:
- Dilution (between 10 – 1000 UFC/mL final concentration)
- Intracellular ATP extraction: Mix 1mL of diluted product with 1mL H_2O_2 10%, 30 min in constant mixing

Enzyme mix products:

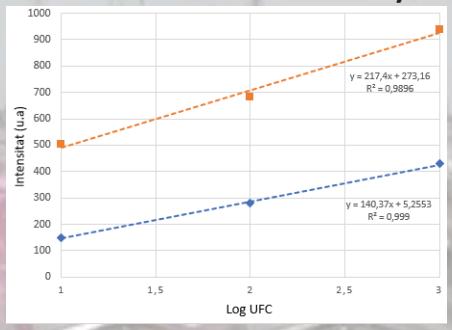
Then:
- Mix 100 µL of product with 50 µL ATP Assay Buffer
- Add 155'4 µL Reaction Mix
- Incubate 30 min without light exposure
- Reading of the sample

Table 1.Reference methods average time:

Analysis	Total time:
TVC (total viable count)	1 day
Coliforms	1 day
Fungi and yeast	5 days

Figure 1.Agilent Cary Eclipse Spectrofluorometer[2]

Figure 2. Relation between log UFC and fluorescence intensity:



Conclusions:
- Is possible to relate log UFC with fluorometric intensity.
- It could be possible to count spore concentration in spore products.
- Is possible to detect biological activity in enzyme mix products.
- This method only works on TVC; fungi and yeast filter tests are not conclusive and require further analysis.

1: MITCHELL P. Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. Nature. 1961. Volume 191. p. 144–8.
2: Agilent cary eclipse spectrofluorometer. Seen at: https://www.agilent.com/en/products/fluorescence/fluorescence-systems/cary-eclipse-fluorescence-spectrophotometer