C18. **Importance of flow cytometry to characterize the cellular state of microorganisms of industrial interest**

Marielle Bouix
Agroparistech – INRA, Massy, Franta

Flow cytometry represents a remarkable investigative tool for microbiologists. Indeed, progress in resolution of cytometers one hand, and the emergence of new fluorescent markers on the other hand, make now possible to analyze microorganisms and viruses. Flow cytometry may be used in many areas of microbiology. The speed of analysis and results, and its statistical value, make it an ideal tool in the diagnostic field: for exemple, microbial contaminants in food can be made in maximum 24 hours for total germs, and 48h or 72h for specific germs like *Salmonella* against respectively 72 hours and 5-6 days by the conventional culture methods. This shortening response times is critical for companies from the food industries, allowing them to release the products earlier, thus reducing storage times.

Flow cytometry is also used in the field of environmental microbiology, the study of micro-algae or viruses water, or research of *Legionella* in cooling towers, as well as medical microbiology and basic microbiology with the study of cell cycle and molecular biology with the use of fluorescent proteins as a marker gene (GFP, RFP, YFP .....).

In my laboratory, we study the microorganisms used as tools for the food ferments or microorganisms of interest in white biotechnology (production of biomolecules for chemistry or health). Flow cytometry allows in these cases to assess the physiological state of the cells.

- First by a global viability parameter that can be compared to cultivability on petri dish.
- But also by analysis of cytomic parameters such membrane integrity, membrane potential, membrane fluidity, intracellular pH, and level of oxidative stress.
- It is also possible to evaluate the expression of antigens at the cell surface in accordance with the progress of culture.
- We have also developed a measure of cell activity or vitality.

We characterize the cellular state of the microorganisms used in industrial processes to understand the mechanisms of cell damage caused by the culture conditions, and also in the case of ferments, by the processes of stabilization. Understanding these mechanisms can propose some change to the culture conditions and stabilization to minimize cell damage. Thus it is possible to improve the cell state for the maintenance of activity during fermentation, or harvest the cells in a cellular state that will enable better conservation and good ability to recovery growth after stabilization. It is this use of flow cytometry for the characterization of cellular state that is developed by using some examples on yeasts and bacteria.

Evaluation of cellular state by flow cytometry during fermentation

In winemaking, a first fermentation is performed by yeast: it must transform the entire grape sugar into ethanol (except sweet wines). In the great majority of cases, musts are now inoculated with selected yeasts. To facilitate their establishment and organoleptic expression, the level of contamination by wild yeasts should not be greater than 10^5 cells/mL. Flow cytometry is used to control the level of contamination before inoculating selected yeasts. In some cases, the fermentation proves slow, or does not end, leaving a residual abnormal sugar in wine. Flow cytometry is used to assess cell state of yeast during the fermentation, to measure cell viability, membrane potential and vitality, and so provide fermentation stops. These measures may also be performed on the yeast fermentation for the production of industrial ethanol. The vitality measurement is also used for lactic acid bacteria.

After the alcoholic fermentation, the malolactic fermentation is often required. This bacterial fermentation is not easy to master, and *Oenococcus oeni* bacteria grow slowly in the petri dishes in the laboratory. Cytometry will allow to detect and enumerate quickly these bacteria and to assess their cellular state to predict the start of malolactic fermentation in wine. We have shown that the *O. oeni* cellular state and its activity depend on his intracellular pH. The measurement of intracellular pH will therefore help to master this fermentation.

Using flow cytometry to predict the behavior of microbial starters after stabilization

The first example concerns a yeast cheese ripening. This yeast is produced and stored in liquid form at 4°C until use. Enumeration by culture petri dish is insufficient to predict the behavior of yeast in cheese. On the contrary, we have shown that the membrane potential is relevant for predicting recovery of activity of this yeast.

The second example concerns the interest of measuring membrane fluidity. We have shown that the cell membrane fluidity at harvest affect bacterial cells survival after lyophilization and storage. Membrane fluidity is strongly related to the composition of membrane fatty acids. The membrane fluidity was measured by fluorescence anisotropy, after adapting the flow cytometer for this measurement.

Then, flow cytometry reveals a very interesting and efficient tool for microbial studies and its use will grow in the years to come.