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Review

Application of flow cytometry to wine microorganisms

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ABSTRACT

Flow cytometry (FCM) is a powerful technique allowing detection and enumeration of microbial populations in food and during food process. Thanks to the fluorescent dyes used and specific probes, FCM provides information about cell physiological state and allows enumeration of a microorganism in a mixed culture. Thus, this technique is increasingly used to quantify pathogen, spoilage microorganisms and microorganisms of interest. Since one decade, FCM applications to the wine field increase greatly to determine population and physiological state of microorganisms performing alcoholic and malolactic fermentations. Wine spoilage microorganisms were also studied. In this review we briefly describe FCM principles. Next, a deep revision concerning enumeration of wine microorganisms by FCM is presented including the fluorescent dyes used and techniques allowing a yeast and bacteria species specific enumeration. Then, the last chapter is dedicated to fluorescent dyes which are used to date in fluorescent microscopy but applicable in FCM. This chapter also describes other interesting "future" techniques which could be applied to study the wine microorganisms. Thus, this review seeks to highlight the main advantages of the flow cytometry applied to wine microbiology.

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1. Flow cytometry: an overview and principles

Flow cytometry (FCM) is defined as the study of isolated cells in a liquid flow. It is an individual, quantitative and qualitative method used to characterize particles in liquid suspension. Flow cytometry is a powerful technique used to analyze multiple parameters of individual cells within heterogeneous populations. Several reviews and books have been dedicated to the principle of flow cytometry and given numerous details (Díaz et al., 2010; Longobardi-Givan, 2001; O'Neill et al., 2013). In the sake of brevity, a flow cytometer is composed of three main parts: fluidic, optical, and, electronic systems (Fig. 1).

1.1. Fluidic system

The fluidic component is composed of a flow chamber that separates and aligns particles (Fig. 1). Most flow cytometers accomplish this by injecting the sample stream containing the cells into a flowing stream of sheath fluid or saline solution. The sheath liquid undergoes gradual acceleration which stretches the liquid sample and thus aligns the cells in the jet center (Díaz et al., 2010). Then the cells pass through the light source with the refraction or scattering of light at all angles. The light sources may be a laser, arc lamp or light emitting diode (LED). Most flow cytometers use a laser as light source. A laser provides a bright and consistent light source with a narrow, specific and well-defined wavelength. Many different types of lasers are currently available and this number is increasing. Some commonly used lasers include argon, krypton, helium-neon, cadmium helium lasers and Yag lasers (Shapiro, 2003).

1.2. Optical system

The light emitted from the cells after they are irradiated in the flow chamber is directed to a detector array by a complex system of mirrors and filters which compose the optical system (Fig. 1). Forward scatter (FSC), or low-angle light scatter, is the amount of light scattered in the forward direction when the laser light strikes the cell (Díaz et al., 2010). The magnitude of forward scatter is roughly proportional to the size of the cell and this data can be used to quantify this parameter. Moreover, a cell traveling through the laser beam will scatter light at all angles. Light scattering at larger angles, for example to the side, is caused by granularity and structural complexity inside the cell (Díaz et al., 2010). This side-scattered (SSC) light is focused through a lens system and collected by a separate detector, usually located 90° from the laser beam's path. The characteristics of front and side light scattering can be used to identify cell types that differ by their size and granularity. A second focusing lens is used to direct the light rays to a set of filters that separate the various wavelengths present. High-pass filters allow light only above a certain wave frequency while low pass filters allow light only below a certain frequency. Together, these two filters form a band pass filter. In a population of labelled cells, cells cross the path of the laser and generate a fluorescence signal. The fluorescent light is then directed to the appropriate detector.

1.3. Electronic system

Light which passes through an optical system will be converted into electronic signals generated by photodiodes and photomultiplier tubes (PMT) and collected to enable data acquisition and analysis (Fig. 1). This is accomplished through photodiodes (frontal dispersion; FSC) and PMT for fluorescent rays and signals associated with side scatter light (SSC). Some modern flow cytometers can have up to 12 PMT, though this number has not increased with the development of new technologies.

1.4. Flow cytometry for enological studies

Most flow cytometry protocols applied in enology that will be described in details in the following sections concern detection and enumeration of wine microorganisms, defined respectively as presence/absence of microorganisms and determination of the population level. Using different dyes coupled to flow cytometry, physiological analysis of wine yeast and bacteria could be performed. Viability could be assessed which corresponds to living cells which are the sole able to perform alcoholic fermentation. However, viable cells could have a weak activity which will impact the alcoholic fermentation rate, for this reason it is interesting to measure the yeast vitality which reflects yeast activity. Finally, the presence of Viable But not Culturable (VBNC) cells could also be assessed. This specific physiological state is characterized by the inability of the cells to grow in a culture media, even though they are still viable and maintain a detectable metabolic activity. Such physiological state is important to monitor, since in wine conditions it could lead to future wine spoilage as described below.

Thanks to these different physiological characteristics, a precise monitoring of the alcoholic fermentation and the malolactic fermentation can be performed. The first fermentation is conducted by yeasts which convert sugars such as glucose, fructose into cellular energy, producing ethanol and carbon dioxide. The second fermentation is achieved by bacteria. This biological process converts malic acid to lactic acid and carbon dioxide into cells, leading to an increase of the wine pH value.

2. Enumeration of wine microorganisms by FCM

Wine is a complex matrix containing many compounds and microorganisms such as yeasts, lactic acid bacteria (LAB) and sometimes acetic acid bacteria (AAB). The yeast responsible for alcoholic fermentation (AF) is mainly *Saccharomyces cerevisiae*. Non-*Saccharomyces* yeasts are also present on grape berries, in grape must and during AF. Moreover, yeasts such as *Brettanomyces bruxellensis* and *Candida vini*, considered as spoilage yeasts, may be