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# A fluorescence-based method for the assessment of polyhydroxyalkanoates (PHA) production

**Anne Elain**
Université de Bretagne-Sud, France

Polyhydroxyalkanoates (PHAs) are polyesters acting as energy and nutrition reserves for many prokaryotes. The substrate, cultivation strategy and production strain, will determine the chemical nature and the physical characteristics (i.e., molecular weight, tensile strength, melting temperature, degradability timelines, etc.) of these polyesters. This opens the door for substituting petrol-based thermoplasts, elastomers or latexes, in multiple industrial and biomedical applications. For instance, in the biomedical field, the great adaptability of PHA, in association with their biocompatibility and their bio-absorption capabilities, make them attractive for use as in vivo implants, regeneration devices or drug delivery systems (3, 4). Until now, the major challenge is to reduce the cost of the biosynthesis process to permit the development of a reliable and sustainable PHA production chain. One aspect of major economic importance is to carefully optimize the operating conditions in order to maximize biomass growth and polymer yield. Hence, a rapid and reliable method of screening and monitoring process performances (i.e., cell growth and PHA contents), is needed. Although, GC-MS analysis provides the most accurate results relative to PHA quantification and monomer composition, it involves extraction and derivatization steps which are complex and time-consuming when applied to a large number of samples. In this study, we developed a method that used the lipophilic fluorescent probe Nile Red (1 mg L−1 solution in DMSO) directly into the culture broth to stain the PHA inclusions inside bacterial cells followed by detection of the emitted fluorescence by both microscopic and spectrometric techniques. Epifluorescence microscopy provides a rapid tool to distinguish producing from non-producing bacterial species and the relative fluorescence intensity (FI) determined at the maximum of emission spectra in the wavelength region of 560-710 nm, allows a fast assessment of the cultivation condition and physical process that may enhance PHA production yield. The method was found effective to select bacterial strains efficient for PHA synthesis among a marine collection. Subsequently, the NR assay was used to determine the C0/N0 ratio of the producing media that may improve the polymer yield as well as to follow the time course of fermentation. The coupling of fluorescent dye staining to epifluorescence microscopy is thought to open up new possibilities for high-throughput screening applications and identification of novel PHA producers.

## Biography

Anne Elain received her PhD degree in Process Engineering from Rennes I University, France, in 1999. She then joined the Biomaterials and Nanotechnologies Lab (now IRDL) of Bretagne Sud University, France. Currently, she leads the Department of Biochemical Engineering. Her general research interest areas are applied microbiology and fermentation technology for the production of high value-added products and process optimizing (yield, sustainability, economic cost, etc.).

anne.elain@univ-ubs.fr

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