A high throughput Nile red fluorescence method for rapid quantification of intracellular bacterial polyhydroxyalkanoates

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Abstract

A rapid quantitative measurement of accumulated polyhydroxyalkanoate (PHA) is essential for rapid monitoring of PHA production by microorganisms. In the present study, a 96-well microplate was used as a high throughput means to measure the fluorescence intensity of the Nile red stained cells containing PHA. The linear correlation obtained between intracellular PHA concentration and the fluorescence intensity represents the potential of the Nile red method employment to determine PHA concentration. The optimal ranges of excitation and emission wavelengths were determined using bacterial cells containing different types of PHAs, of different co-monomers and compositions. Interestingly, in spite of different co-monomers compositions in each PHA, all tested PHAs fluoresced maximally at excitation wavelength between 520 and 550 nm, and emission wavelength between 590 and 630 nm. The developed staining method also had successfully demonstrated a good correlation between the amount of accumulated PHA based on the fluorescence intensity measurements and that from chromatographic analysis to evaluate poly(3-hydroxybutyrate) [P(3HB)], poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) [P(3HB-*co*-4HB)], poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) [P(3HB-*co*-3HV)] and poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate-*co*-4-hydroxybutyrate) [P(3HB-*co*-3HV-*co*-4HB)], using the same calibration curve, despite of different co-monomers that the PHA consist. Strongly supported by these experimental results, it can therefore be concluded that the developed staining method can be efficiently applied for rapid monitoring of PHA production.

Keywords: polyhydroxyalkanoate; nile red staining; excitation and emission wavelength; fluorescence intensity

Citation:

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