Automatic Determination of Yeast Cells Viability by Image Analysis

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There are numerous methods to determine cell viability. Such methods usually measure the loss of replication ability, cell damage or the reduction in metabolic activity [1]. Since conventional methods are time absorbing in detection and enumeration, have poor sensitivity and specificity and the reproducibility is relatively low, rapid methods are needed. The direct epifluorescence technique can be used to give rapid estimates and is often used for monitoring the microbiological quality of milk and other food-stuffs [2]. An advantage of this method over other rapid methods is that morphological characteristics of microorganisms can be ascertained [3]. However, large number of cells are necessary to obtain statiscally significant data, which makes this task laborious and time consuming. Image analysis and other technological improvements have extended the applications of epifluorescent microscopy, hence avoiding operator fatigue.

Epifluorescence microscopy with acridine orange as dying agent, is used for determination of viable and non-viable yeast cells. This fluorescent dye is a hydrophobic weak base which is known to bind to negatively charged, particularly phosphate groups. It attaches to adjacent phosphate groups in single-stranded nucleic acids (RNA) and fluoresces red/orange due to dye-dye interactions. Molecules of acridine orange will also intercale singly into double-stranded nucleic acids (DNA), and fluoresce green [4]. The cells are viewed thereafter in an ultra violet microscope and the images can be rapidly photographed. Subsequently these photos are digitised and the images can be enhanced by the Corel Photopaint™ 6.0 program (Corel Corp.) with the possibility of multiplying, adding and subtracting of the channel (RGB) images which can increase the contrast between viable (red/orange) and non-viable (green) cells. Image analysis can be performed in the Global Lab Image 3.21 (Data Translation, Inc.) where many parameters can be calculated, yet only area calculation and particles (cells) numbers are useful to this work.

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