Classification of *Saccharomyces cerevisiae* morphology using image analysis

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Population dynamics of microbial systems can be described by several approaches and in various levels of complexity, each of them arising from specific goals and limitations. From the process-engineering viewpoint there is a need for a comprehensive mathematical model describing population dynamics in terms of measurable entities (microbes) and chemicals involved (limiting substrate, dissolved oxygen, etc.), as well as process configuration (number and type of reactors, interconnections, etc.) and process parameters (inlet flow rate and composition, reactor holdup, and more).

The description of intricate population dynamics and the inference of cell stages lead to complex models with a great number of parameters. Knowledge about whole cell cycle and morphology classification is imperative, since a considerable difference exists between the cell description employed in model formulation and the laboratory reality.

As soon as in biological systems exists a relationship between cell morphology and productivity, some authors drive efforts towards the on-line measurement of biomass component to avoid process delays or to determine cellular characteristics related to its morphology and/or physiology through image analysis.

Saccharomyces cerevisiae size and shape distribution are affected by growth rate, mutation, and environmental conditions (composition, temperature, pressure, presence of oxidant agents, etc.). Although its shape usually assumes an ellipsoid contour, it is modified along the cell cycle by bud formation and growing attached to the mother.

This work deals with *S. cerevisiae* classification based on morphology analysis. Image acquisition was conducted in an optical microscope (with 400x magnification) coupled with a black and white camera and linked to a microcomputer by a frame grabber. Traditional tools generally used for image enhancing were employed. Feature extraction and objects separation were necessary to classify "mothers" and "daughters" and to determine its frequency in the analyzed samples.

Cells were automatically divided in five different classes with respect to bud size compared to whole object area. A discrimination considering bud area as the minimum area determined after employing watershed algorithm for its separation was performed through image analysis employing Matlab v.6.1 (The Mathworks Inc.). This methodology was validated with distinct samples and employed along *Sacharomyces cerevisiae* growth in different operational conditions.

Bud Classification

The proposed methodology for *S. cerevisiae* morphology analysis was applied to a batch growth. The results present a daughter detachment before it reaches same length of the respective mother and demonstrate to be a potencial tool for cell cycle studies.

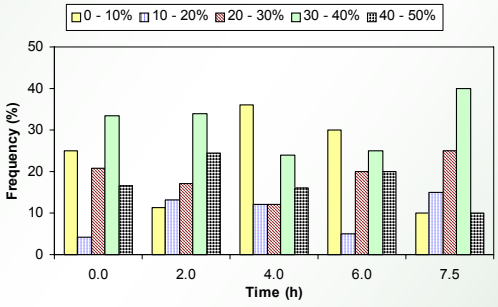
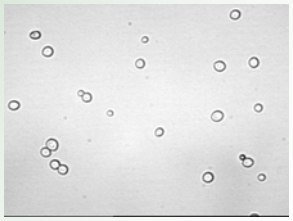


Image Analysis Procedure


Original Image

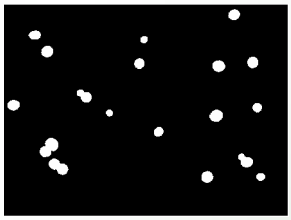


Image after binarization and morphological operations

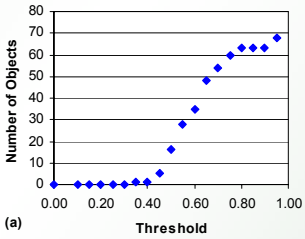

Cropped Image

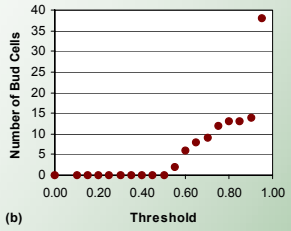

Watershed Algorithm

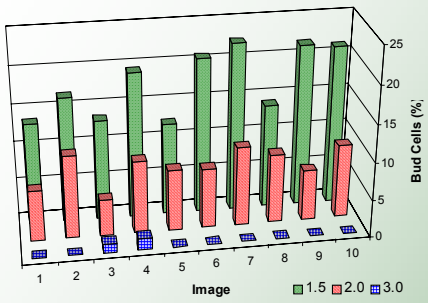
Feature Extraction

Threshold Analysis

Exists a threshold range where these properties remain constant (between 0.8 and 0.9), i.e. the results obtained in this range are relatively independent of the threshold chosen







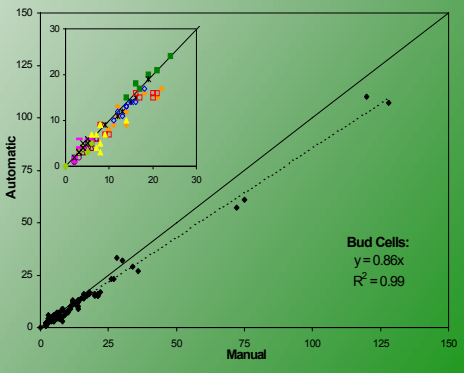
Elongation

An elongation factor (Fmax/Fmin) of 3.0 is totally inappropriate to this yeast strain under the experimental conditions employed not detecting doublets

Data Consistency

To check the consistency of the results obtained through automatically calculated properties, a manual determination of total and bud cells was performed and the compared data are presented.

A good correlation was reached for total objects number with an average error inferior to 5%. For bud cells, a constant deviation with respect to the number of objects analysed is observed when comparing automatic to manual determinations (correction factor of about 14%). These results were extracted among 100 pictures corresponding to 2000 objects.



Acknowledgments

CNPq Conselho Nacional de Desenvolvimento Científico e Tecnológico

FCT Fundação para a Ciência e a Tecnologia
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CAPES