Universidade do Minho

Use of Image Analysis Technique for the Identification and Pursuit of Ethanol Processes

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Introduction

- The yeast *Saccharomyces cerevisiae* is one of the most important microorganisms employed in the ethanol production.
- From the process-engineering viewpoint there is a need for a comprehensive mathematical model describing microbial population dynamics in terms of
  - measurable entities (microbes)
  - chemicals involved (limiting substrate, dissolved oxygen, etc.)
  - process configuration (number and type of reactors, interconnections, etc.)
  - process parameters (inlet flow rate and composition, reactor holdup, and more).
- 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.

- *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 through image analysis.
- The projected area of cells and number of bud cells were used to evaluate morphological changes.
- Information on cell size distribution and buds formation along the cell cycle is reported.

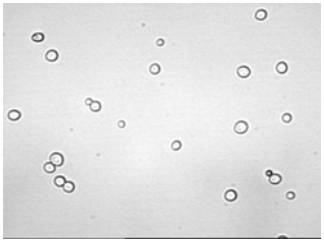
Image Analysis

- Image Analysis allows for:
  - Enhancement of pictures
  - Automatic identification and isolation of particles
  - Fast means of getting morphologic information, thus saving tremendous effort and time

- 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.
- 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 5 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*.
- This methodology was validated with distinct samples and employed along *S. cerevisiae* growth in different operational conditions (pressure, gas composition)

Image Analysis Procedure



Original Image

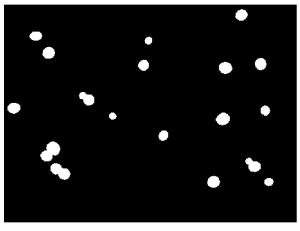


Image after binarization and morphological operations

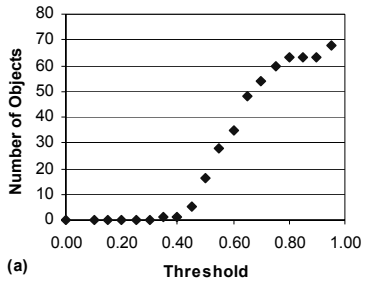
Cropped Image

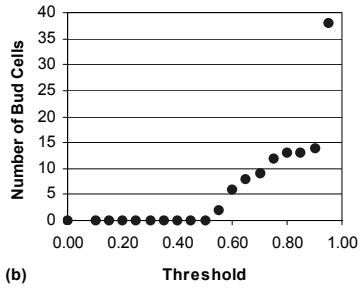
Watershed Algorithm

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

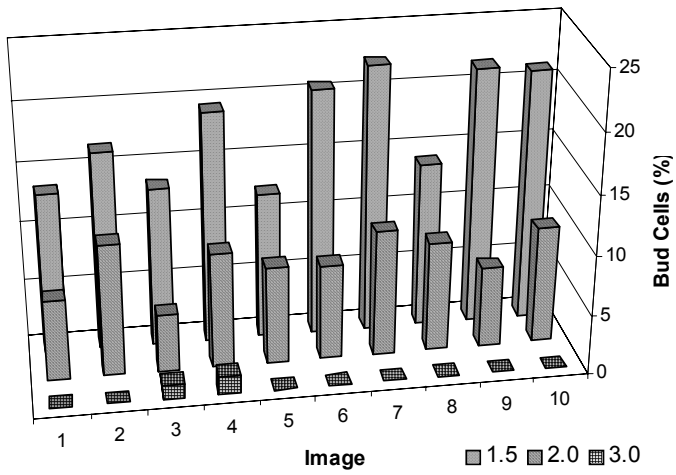




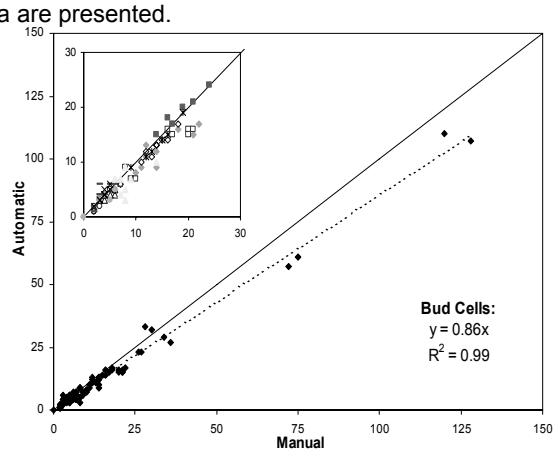
Elongation

- Assuming that the cell projection onto the image is an ellipse, a parameter called "elongation" (major axis length / minor axis length) was computed to discriminate non-bud from bud cells.
- Based on the effect of different elongation values studied (1.5, 2.0 and 3.0), a value of 1.5 for elongation factor was selected for all the analysis to discriminate bud from single cells.
- A typical elongation distribution for cells of *S. cerevisiae* is presented in the next slide.

Elongation (F_{max}/F_{min})



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 analyzed is observed when comparing automatic to manual determinations (correction factor of about 14%).
- These results were extracted among 100 pictures corresponding to 2000 objects.

Conclusions

- An automated image analysis procedure was developed to allow for the discrimination between buds from single cells, as well as to determine cell size distribution in each experimental condition.
- The main advantage is related to the large number of objects analysed leading to a more representative data analysis.
- The measurement of bud cells is a better method to identify cell activity inhibition than cell size determination.
- The methodology herein proposed can be very useful in the physiological state assessment and in the cell division analysis of yeast cell cultures used in ethanol production.

More Information may be browsed throughout the Bioprocess System Engineering group's web page at:

www.deb.uminho.pt/BioPSEg