



Application of Image Analysis to the monitoring of fermentation



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INTRODUCTION

The possibility of monitoring a process on-line is a goal for many industries, as it permits enhanced control. Traditionally, control of the pitching rate and the evolution of the number of yeast cells during fermentation is a time consuming task in the brewing industry, frequently involving a cautious sampling and a lot of laboratorial work.

Nowadays image analysis is commonly used in a wide range of applications due to the development of faster computers, advanced frame grabbers, and sophisticated software. Image analysis provides an alternative to the monitoring of biomass during fermentation. Further, it can be made on-line, thus eliminating

Image analysis procedure:

Image acquisition was accomplished through the visualization a Axioscop microscope (Zeiss, Oberkochen), with a 10x magnification, and digitised with the help of a CCD AVC D5 Sony camera (Sony, Tokyo) and a DT3155 Data Translation frame grabber (Data Translation, Marlboro). The images were digitised with a 768x576 pixel size and 256 grey levels by the Image Pro Plus (Media Cybernetics, Silver Spring) software package (Figure 1) which was used for counting. No special treatments were applied

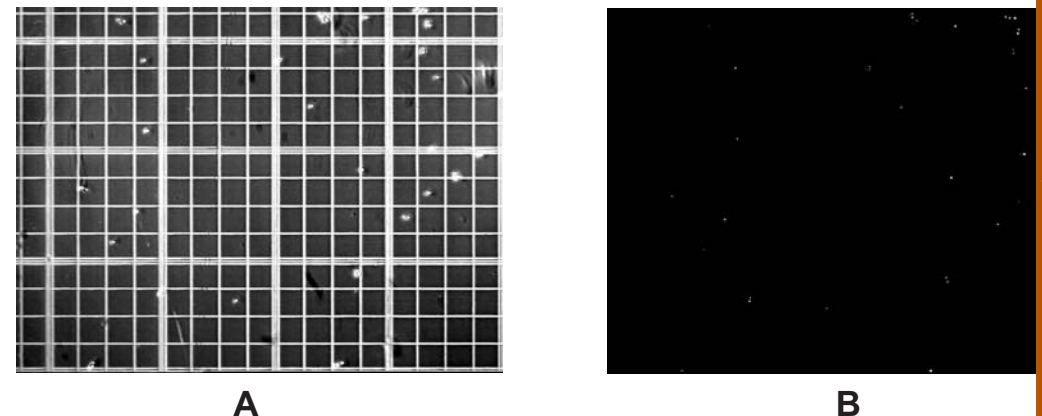


Figure 1: Digitised images of the Neubauer chamber before (A) and after (B) the elimination of the lines by decreasing the intensity of light and focusing (100x).

the risks of contamination and facilitating the work by less skilled workers.

The image processing starts with the visualisation step. Different devices can be used: for biomass characterisation optical microscopes are the most common tools. Generally an electronic eye (camera) is substituted to the human eye. The video signal is further processed into a digital one, the image, i.e. a set of picture elements arranged according to lines and columns.

In order to demonstrate the capabilities of image analysis in brewing, biomass concentration in beer fermentation, performed in EBC tubes, was monitored through an image analysis technique and validated with data obtained from hematocytometer cell count.

MATERIALS AND METHODS

Worth Primary Fermentation :

The primary fermentation of the worth was carried out in EBC tubes, with industrially prepared worth, at 10 °C. The inoculum was prepared from an industrial strain and the initial concentration in the fermenter was 2.5×10^7 cells/ml. The fermentation proceeded during 108 hours until the suspended biomass concentration dropped significantly (flocculation). Samples were taken twice daily.

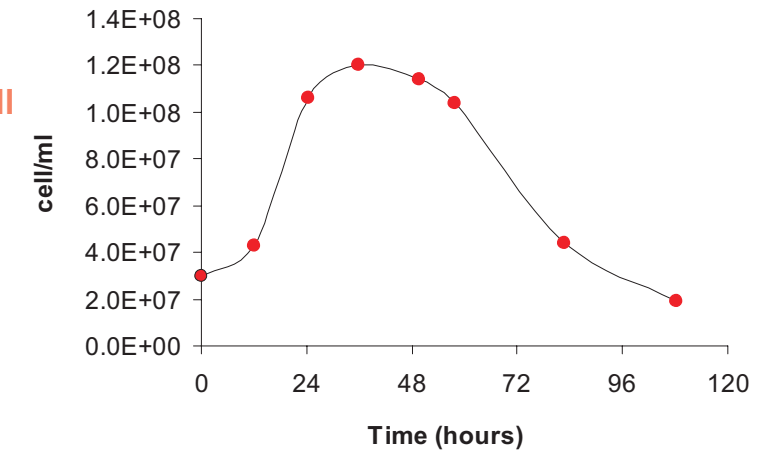
Cell count:

The samples were collected from the EBC tube and the cells in suspension were counted by two parallel procedures, using a Neubauer chamber (manual count and using an image analysis system) in order to compare the results obtained by each method.

RESULTS AND CONCLUSIONS

Figure 2 shows the evolution of a typical fermentation in a EBC tube . It is clearly seen the suspended cell concentration drop after 48 hours, due to the yeast flocculation.

Figure 2: Evolution of the cell concentration during a typical fermentation in a EBC tube.



The cell counts obtained through both methods (manual count and image analysis) for two parallel fermentations are compared in Figure 3. The correlation between the methods is clearly demonstrated.

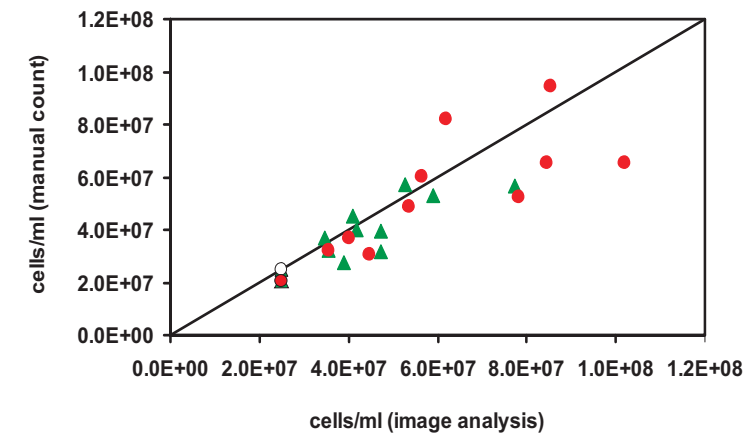


Figure 3: Correlation between the cell counts performed using image analysis and manual count. The different symbols refer to different fermentations.

The basis for an automatic image analysis cell count method is set up. Its on-line implementation is, theoretically, straightforward. However, a significant amount of work has still to be done in order to obtain an easy-to-use, fast and reliable system capable of being operated in an industrial environment.