Flow Cytometric Determinations of Glycogen Content of Yeast During Fermentation

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ABSTRACT

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Using flow cytometric analysis, the rapid detection of glycogen content of yeast cells in process is possible. Glycogen is a sensitive parameter which can express the physiological state of the yeast cells. A procedure was developed to stain and measure glycogen in yeast cells using flow cytometry. Glycogen content of lager yeast cells was tested after 1 and 15 h of nutrient limitation.

Key words: Flow cytometry, glycogen, yeast.

INTRODUCTION

During fermentation yeast cells incorporate reserve substances such as glycogen and neutral lipids. In yeasts, stored glycogen consists of α-1,4 and α-1,6 glycosidic connected glucose units. Glycogen has a structure similar to amylopectin but it is not as high an energy source as neutral lipids. The complex knotted structure, makes it possible for the cell to metabolise glycogen directly and release glucose. In the adaption phase and the beginning of early exponential growth phase, glycogen is accumulated in the cell, while at times of nutrient limitation, i.e., at the end of fermentation, glycogen is metabolised. This implies that when cropped yeasts are stored at high temperatures (15–22°C for lager yeasts), under oxygen supply (i.e. for blowing out CO_2), and without a wort top-up, yeast cells start an aerobic proliferation and the yeast cells consume their accumulated glycogen content. The more glycogen consumed, the less chance for the cell to start a new round of cell cycle. Information about the glycogen synthesis of yeasts can serve as a sensitive parameter for controlling functionality of cells.

MATERIALS AND METHODS

A procedure was developed to stain glycogen in yeast cells to detect this molecule using flow cytometry[1]. The specific staining was carried out with acriflavine according to the procedure of Gharton *et al.*[2] and Meyer *et al.*[4] with minor modifications[3]. For flow cytometric analysis a PAS, Partec GmbH, Münster, was used. Acriflavine was excited at 488 nm; emission was detected at 515 nm.

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RESULTS AND DISCUSSION

Controlling glycogen synthesis of yeasts in process showed an oscillating course (Fig. 1a). These cells were stored at 5°C and subsequently inoculated into wort. After inoculation, these cropped yeast cells accumulate glycogen until the beginning of exponential proliferation. Then a reduction in glycogen takes place during heavy proliferation. At the end of fermentation, the yeast cells again accumulate glycogen. When the yeast cells were stored at a higher temperature (22°C), glycogen synthesis took a different course during fermentation (Fig. 1b). In Figs. 2 and 3, the results of glycogen synthesis are shown under nutrient limitation. After 1 h of wort nutrient limitation (Fig. 2), two cell fractions with different glycogen contents are present in the population analysed flow cytomet-

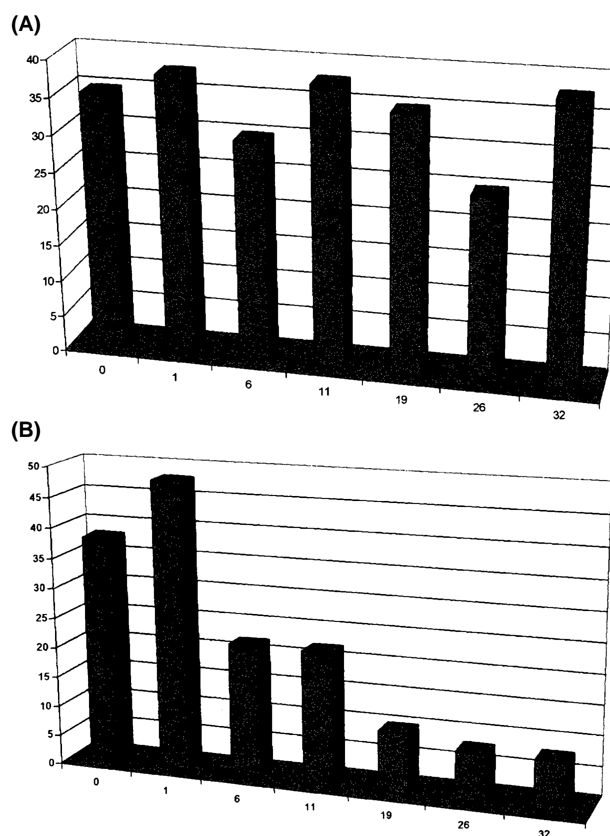

FIG. 1. (A) Glycogen synthesis during the fermentation process of a lager yeast population stored at 5°C (x axis = yeast sampling/day and y axis = fluorescence peak maximum). (B) Glycogen synthesis during the fermentation process of a lager yeast population stored at 22°C (x axis = yeast sampling/day and y axis = fluorescence peak maximum).

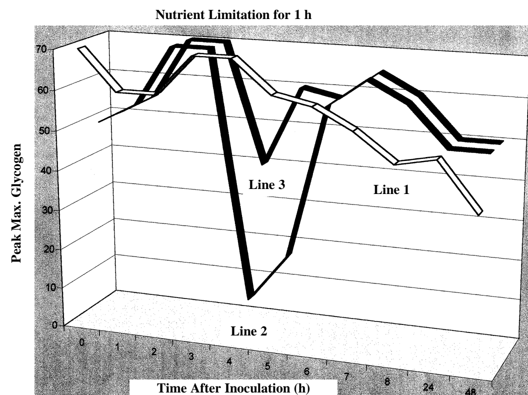

FIG. 2. Frequency distribution of two peaks after nutrient limitation of 1 h. (Line 1: Control); Peak one of flow cytometric frequency distribution—newborn, senescent and membrane damaged cells which start glycogen decomposition at a rapid rate, (Line 2); Peak two are "adult" cells with a good proliferating status (Line 3).

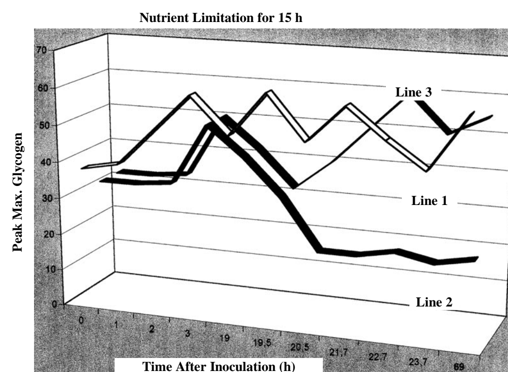

FIG. 3. Frequency distribution after nutrient limitation of 15 h. After long term nutrient limitation (peak one), cells are unable to start normal glycogen synthesis. These are dying cells. (Line 1: control cells; Line 2: newborn, senescent and membrane damaged cells; Line 3: adult cells with good proliferating status.)

rically. Under the first peak are cells, which are newborn, senescent, and membrane damaged. Under the second peak are "adult" cells with good proliferation.

The older and damaged cells metabolise glycogen more rapidly and economically. Both cell fractions recover very quickly after a top- up with wort and continue normally with glycogen synthesis. After 15 h of nutrient limitation (Fig. 3) the first peak fraction shows no recovery to normal proliferation. These cells die by autolysis, while the second peak cell fraction recovers to normal proliferation with normal glycogen synthesis after some hours.

With flow cytometric analysis, a rapid detection of the glycogen content of yeast cells in process is possible. Glycogen is a sensitive parameter, which can express the physi--ological state of the yeast cells. Currently there is a lack of functional data in this area in most brewery laboratory procedures. Results on glycogen content could fill this gap.

High glycogen content of yeast cells is associated with high vitality of the yeast population. The present results suggest that yeasts achieve high proliferation rates after a reduction in glycogen content. On the other hand, low glycogen content suggests an exhausted state for the yeast cells and such populations are not recommended for utilization for new fermentations.

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