EFFECT OF AERATION DURING PROPAGATION ON THE BEHAVIOUR OF YEASTS DURING FERMENTATION OF SYNTHETIC MEDIA

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Abstract

Aerobically propagated yeast show a higher specific growth rate than anaerobically propagated yeast, when glucose is the substrate during fermentation of synthetic media. Total glucose consumption is observed within l0hrs with aerobic yeasts. With anaerobic yeasts, a concentration of approximately 2.5g of substrate remains in the culture medium after a period of l0hrs fermentation. Diauxie is less pronounced with anaerobically propagated yeasts. With maltose as substrate, results are similar to results obtained with glucose as substrate. There are however noticeable differences in the specific growth rates. Specific growth rate with glucose as substrate is higher than fermentation intensity with maltose as substrate. Growth during subsequent respiration of ethanol is faster with maltose as substrate than with glucose as substrate. These results would suggest a minor repression of respiration by the catabolism of glucose when compared to the catabolism of maltose. Maximum production of secondary metabolites is higher with aerobic yeasts than with anaerobic yeasts.

Keywords: Aerobically propagated yeasts, anaerobically propagated yeasts, diauxie, ethanol, fermentation intensity, repression of respiration, specific growth rate.

Introduction

The propagation and maintenance of yeasts adds an appreciable level of control to the brewing process. The development of pure yeast strains and their importance in the brewing process has been going on for over a century, and is still an area of active research. In 1883, Emil Christian Hansen described the first techniques for successfully isolating single yeast cells and propagating them to a larger scale. The purpose of a yeast starter, or pitching yeast, is to produce sufficient quantity if yeasts for subsequent fermentations. Propagation conditions should be such that a maximum amount of yeast is produced which provide optimal fermentation performance once pitched. However after several fermentation cycles, there is need to revert to freshly propagated yeasts, since too frequent number of successive harvest could lead to a modification of yeasts. The influence of the method of pitching yeasts propagation on the behaviour of yeast during fermentation has previously been studied. These results have shown that the methods of propagation of pitching yeasts has a considerable effect on fermentation with regards to beer flavor, lipid composition, sterol synthesis, production of fatty acids and production of secondary metabolites.

Studies by Amata were carried out under non-identical culture conditions. The first fermentation is carried out with aerated wort and pitching yeasts from classic or anaerobic propagations. The objective of this work is to study the influence of pitching yeast propagation on the fermentation of synthetic media under similar fermentation conditions. Limited sugar concentrations in relation to nitrogen sources will give room for an adaptation of yeasts to the respiratory stage of fermentation. In these studies the following metabolic parameters will be considered: specific rate of fermentation, specific growth rate, production of some secondary metabolites and the on-set of diauxie.

Materials and Methods

Experimental: The yeast strain used, Saccharomyces uvarum K19 is from the collection of "Laboratoire de Micro biologic Industrielle dc L'ENSAIA-INPL, Nancy, France". Propagation of the yeast cells was carried out in Pasteur flasks of carrying capacities. This method used is a modification of the method described and as reported.

Fermentation: Fermentation was carried out at room temperature in 1-litre flat bottom flasks, with constant agitation at 250rpm. Initial cellular concentration at time of pitching was adjusted to 0.65x10 cells/ml. Initial glucose and maltose concentrations were fixed at Sgl"[1]. Culture media

contained fixed nitrogen sources as described, and the pH of the medium was adjusted to pH 4.5. Samples were collected on an hourly basis.

Analysis: Cellular concentration in suspension was determined by standard turbidimetric methods. relating optical density measurements on a calorimeter to dry weight of cells. The optical density measurements were carried out at wavelengths of 660nm. Ethanol, acetic acid and acetaldehyde concentrations were measured by gas chromatography. The characteristics of the apparatus used are as described. Glucose in the fermentation medium was measured using the method proposed, while maltose was measured using methods proposed.

Results and Discussion

Specific Growth Rate and Substrate Consumption

Figs Ib and 2b represented growth rates on glucose with yeasts obtained respectively from aerobic and anaerobic propagations. With glucose as substrate medium, cellular growth takes place without an initial lag phase and at a constant rate. The specific growth rate is higher with yeasts obtained from aerobic propagation (figlb0 when compared to the specific growth rate with yeast from anaerobic propagation (fig2b). The specific growth rates are in the order of u, = 0.32IV^{1} and (.1=0.26'" respectively. The yields biomass are however relatively

Glucose

identical for two systems of propagation, in the order of 0.15g.g-l glucose. It is however observed that glucose is completely metabolized during growth at rates u, = 0.32h" with yeast from aerobic propagation (figla) whereas glucose is more slowly metabolized with yeast from anaerobic propagation (fig2a), the growth rate dropping even when the concentration of glucose in the medium still remains (approximately 2.5gl"). The yields ethanol during this phase of fermentation is relatively identical (0.34g,g' and 0.32g.g"^{1}) respectively for both aerobic and anaerobic propagations. With aerobic pitching yeasts, ethanol consumption starts after glucose has been completely metabolized (figla). The specific growth rate on ethanol is in the range of u, = 0.05h (fig la). With anaerobic pitching yeast the growth rate in ethanol is relatively similar (. μ = 0.05'", fig 2b) and growth on ethanol starts even when glucose is not totally metabolized (fig2a), this presupposes a repression of glucose metabolism by the respiratory enzymes involved in the metabolism of ethanol. This situation results in a reduced diauxie with anaerobic pitching yeasts, when compared to aerobic pitching yeasts.

With maltose as substrate, growth rate is initiated without a lag phase and at a constant rate. The specific growth rate being higher with aerobic pitching yeasts when compared with that of anaerobic pitch yeasts (fig 3b and fig 4b respectively). The specific growth rates are μ = Q.32h'" and μ= 0.19h"^{1} respectively for aerobic and anaerobic pitching yeasts.

During the initial growth phase, the yields biomass are reasonably identical in the

Maltose

the order of 0.17g.g maltose. It can be observed that maltose is completely catabolised during growth at μ = 0.23'".

With aerobic pitching yeasts (fig3a). With anaerobic pitching yeast, after the growth phase at H = 0.19^{1} a concentration of 2.5g.g of maltose still remains in the medium. The yields ethanol during this phase of fermentation are reasonably identical, in the order Biomass

of 0.38g.g. Consumption of ethanol commences after complete consumption of maltose (fig3a) with aerobic pitching yeasts and the specific growth rate on ethanol is. With anaerobic pitching yeasts, ethanol consumption starts even when maltose is not completely consumed (fig 4a). Te specific growth rate during this respiratory phase is. The diauxie is less pronounced with anaerobic pitching yeasts when compared with aerobic pitching yeasts. Of the two methods of propagation, growth with glucose as substrate is faster than growth during fermentation with maltose as substrate. Growth during subsequent respiration of ethanol is faster with repression of ethanol respiration by glucose metabolism when compared to maltose metabolism, (figs Ib, 2b, 3b and 4b respectively).

Production of Secondary Metabolites

The production curves of acetate concentration are relatively identical (figs la, 2a, 3a and 4a respectively), rapid increase in production at the beginning of fermentation, followed by a reconsommation at the end of the fermentation phase. Production of acetate by aerobic pitching yeasts is higher than with anaerobic pitching yeasts (Amata, et al. 1990). Maximum production of acetate with glucose as substrate is higher than with maltose as substrate. In the case of acetaldehyde production, a higher increase in concentration is observed principally during the fermentation phase with aerobic pitching yeast when compared to anaerobic pitching yeasts. Acetaldehyde is slowly consumed during the respiratory phase.

Conclusion

Results so far have shown that pitching yeast aeration has an appreciable influence on the adaptation of yeast to the respiratory stages of growth during fermentation of synthetic media. The specific growth rate during fermentation is also appreciably influenced by aeration during propagation, these rates being higher with glucose as substrate. However aeration during propagation does not affect rates during the respiratory phase (during ethanol consumption). These results would suggest that aeration during propagation is beneficial to subsequent fermentation.

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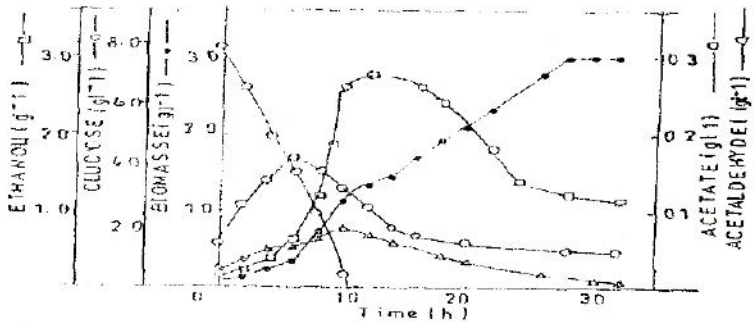


Fig la Growth kinetics on glucose medium with yeasts from aerobic propagation

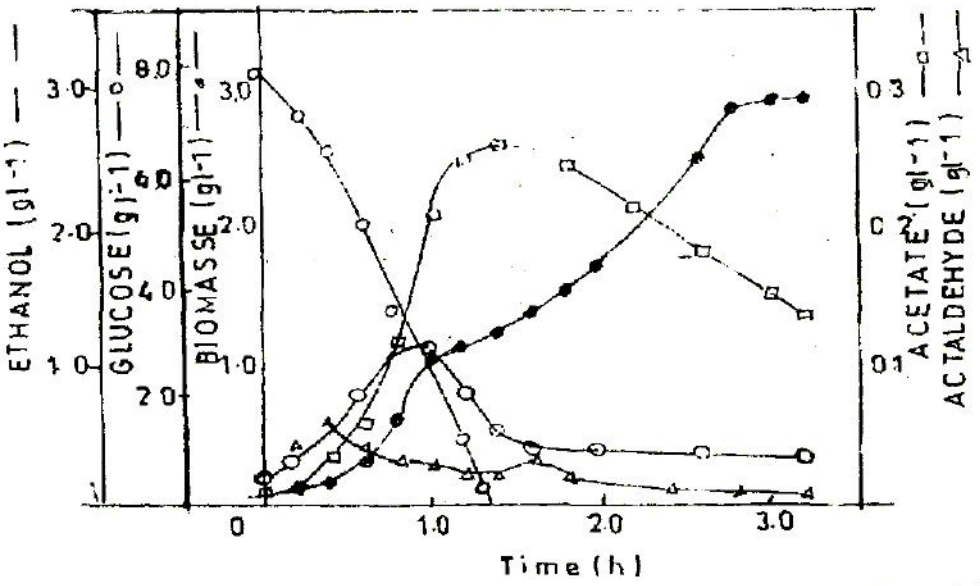


Fig 2a Growth kinetics on glucose medium with yeasts from anaerobic propagation

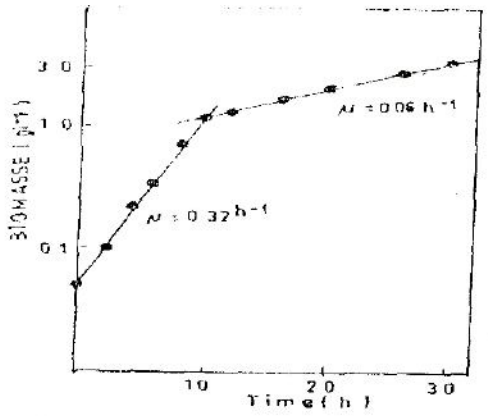


Fig 1b Specific Growth Rate : aerobic pitching yeasts

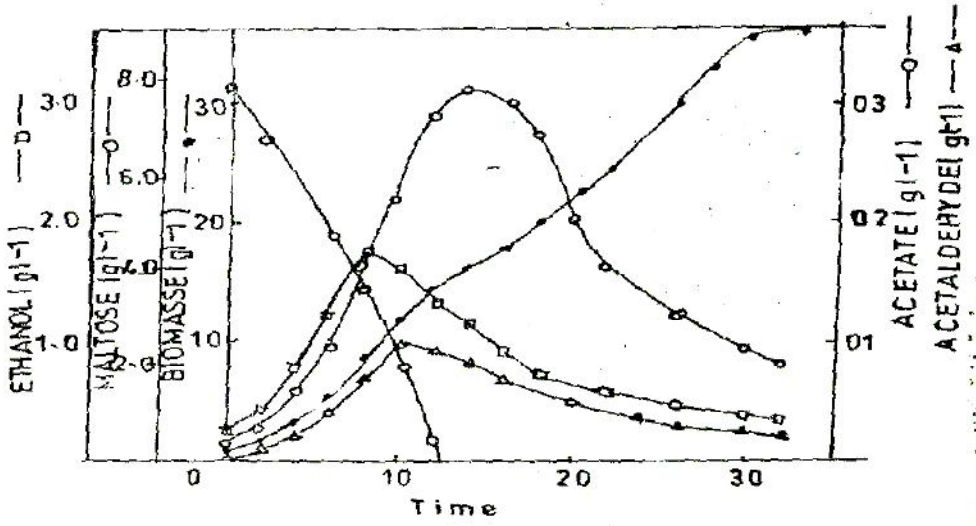


Fig 3a Growth kinetic on maltose medium with yeasts from aerobic propagation

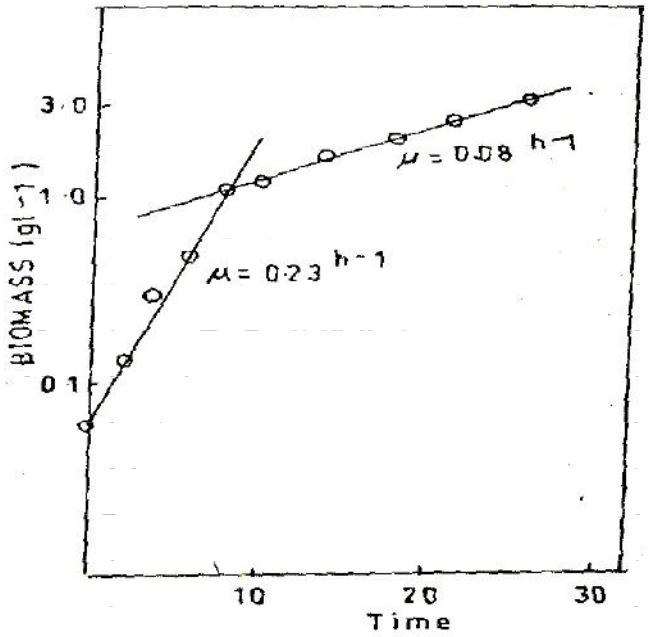


Fig 3b Specific Growth Rate : aerobic pitching yeasts

... anaerobic pitching yeasts

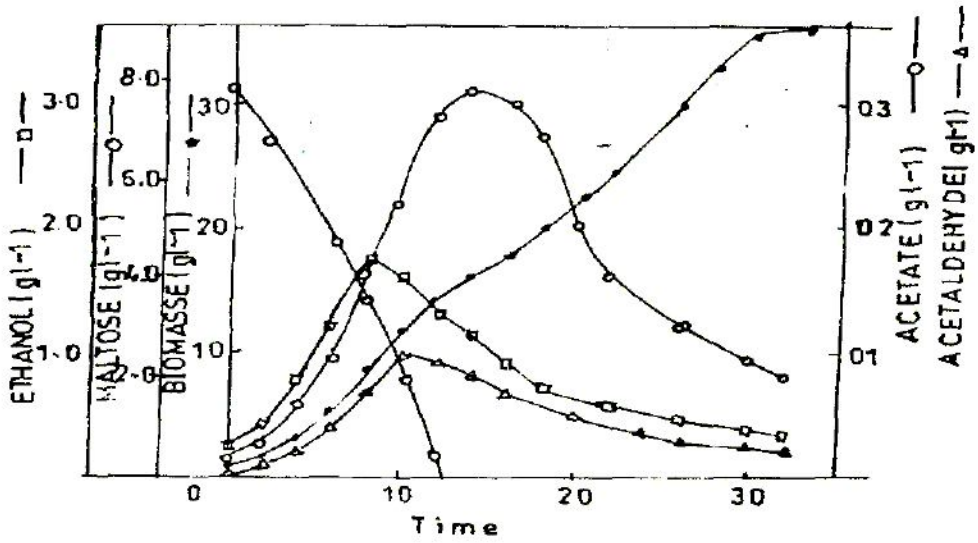
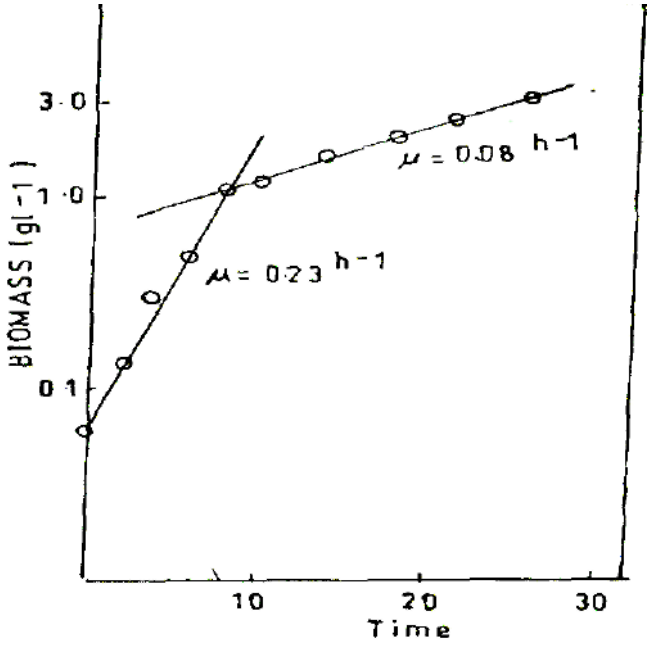


Fig 3a Growth kinetic on maltose medium with yeasts from aerobic propagation



F*g 3b Specific Growth Rate - aerobic pitching yeasts