Fast Monitoring of Growth of Industrial Yeast Cultures by Synergy H1

Gary Prescott[1], and Anita Van Landschoot[2]

[1]BioTek Instruments, Inc., Luzern, Switzerland • [2]University College Ghent and Ghent University, Ghent, Belgium

Overview

Strains of the yeast *Saccharomyces* serve critical roles in the production of many beverages, but also in the production of ethanol for biofuel. The ability to obtain the desired product reliably and repeatedly in the same amounts requires careful monitoring of not only the input materials, but also the growth of the yeast strain during the propagation or the hydratation/revitalization in the case dried yeast is used for the fermentation process. Likewise this often requires monitoring the growth of these strains under various conditions to optimize the industrial conditions. Here we describe the use of the Synergy™ H1 Hybrid Multi-Mode Microplate Reader to provide temperature control, suspension agitation, and monitor cellular yeast growth using light scatter in 96-well microplates at 600 nm. Measurements were made every 2 minutes and data are collected using Gen5™ Data Analysis Software (BioTek Instruments, Inc).

Introduction

Yeast are single celled eukaryotic fungi organisms that reproduce asexually by budding or division (Figure 1). While yeast can vary in size, they typically measure 3-8 µm in diameter. *Saccharomyces cerevisiae* is the most commonly used strain in scientific research, baking and fermentation and has become synonymous with the term yeast.

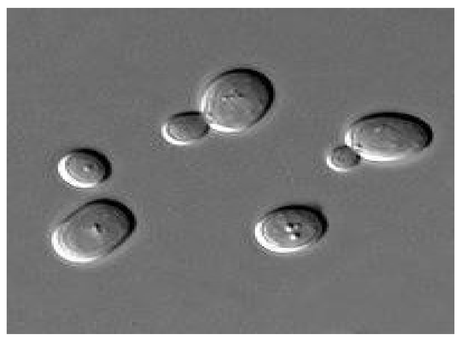

Figure 1 – Cells of *Saccharomyces cerevisiae*

Yeast has been used for thousands of years to ferment alcohol. The paintings discovered in the pharaoh's tombs depicting Egyptians using yeast for fermentation of beverages and to leaven bread have documented the usage of yeast for at least 5000 years [1]. It wasn't until the 18th century that the biological sciences had advanced enough to identify yeast organisms and to deduce that sugars were fermented into ethanol with a carbon dioxide being emitted as a by-product. Since that time bakers, scientists and yeast manufactures have been working to identify strains of yeast that meet specific needs.

Yeast Growth Phases

When cultured for the fermentation of beer, yeast cells in culture follow a very predictable pattern of growth that can easily be divided into four stages: (1) lag phase; (2) log phase; (3) deceleration phase; and (4) stationary phase. During the lag phase, no growth occurs as newly pitched yeast mature and acclimate to the environment. This is followed by the log phase, where cells are rapidly growing and dividing. Nutrients are in excess relative to cell number and waste is being sufficiently diluted as to be insignificant. The growth rate in this phase will follow first order kinetics. As cell numbers increase cell growth begins to slow as a number of parameters (e. g. substrate and waste), each with saturation effects, become significant. Eventually the yeast cells reach the stationary phase, where no growth occurs due to high metabolite concentration or complete substrate consumption (Figure 2).

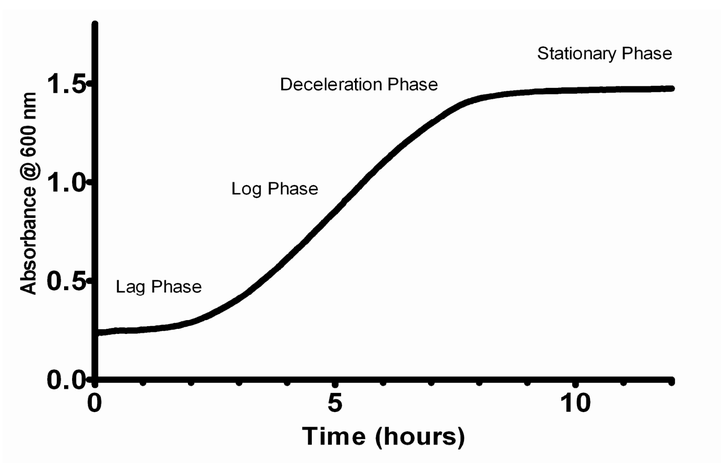

Figure 2 - Typical Yeast Growth Curve. *Saccharomyces cerevisiae grown in YPD media at 30°C for 12 hours with data measurements every 2 minutes.*

The clonal nature of budding yeast cell division allows for the screening of large numbers of organisms for mutants that exhibit desired characteristics. Individual mutants replicate and divide asexually from a single progenitor. When selecting yeast strains for desired growth characteristics one method of doing so is to monitor growth under selective conditions. We have used the Synergy H1 Multimode reader to maintain temperature, provide continuous shaking, as well as perform absorbance measurements to monitor yeast cell growth under a variety of different conditions.

Materials and Methods

Assay components: YPD media were prepared as directed and sterilized by autoclaving. Different strains of yeasts were from Wyeast Laboratories (Odell, OR).

The industrial yeast strains used to study the effect of temperature, pH and density of industrial media (wort or must of white grapes) on yeast growth/biomass production were:
- Ale yeast A of a Belgian brewery,
- Dry brewing yeasts: Saflager W-34/70 and Safbrew T-58
- Dry bakers/distillers yeast: Fermipan Red
- Dry wine yeast: Springer Oenologie

Assay Plates: 96-well, flat bottom, clear, non-treated plates from Corning Life Sciences were used in all experiments.

The test experiments with YPD media followed the same general format. Overnight stock cultures (50 mL) were grown in 250 mL Erlenmeyer flasks at 30°C with orbital shaking at 125 RPM. Prior to growth experiments 150 µL of each overnight stock culture was diluted to 7.5 mL with fresh 1X YPD media. The diluted cells were then plated as needed into the assay plates.

Measurements were made every 2 minutes with continuous orbital shaking mode employed. Shaking speed was set to slow and frequency set to 559 (1 mm amplitude). Temperature control was enabled and unless stated otherwise was at 30°C. Cell growth was assessed by light scatter measurements made at 600 nm [2]. The absorbance of each well was read kinetically using a Synergy H1 hybrid multimode microplate reader (BioTek Instruments, Winooski, VT). It was controlled and data collected using Gen5 Data Analysis software (BioTek Instruments, Winooski, VT).

BioTek Instrumentation

Figure 3 - Synergy™ H1 Hybrid Multi-Mode Microplate Reader. This reader combines a filter-based and monochromator-based detection system. It provide temperature control, suspension agitation, and monitoring of cellular yeast growth using light scatter (absorbance) in 96-well microplates at 600 nm.

Growth Uniformity

Repeatable yeast cell growth across the entire plate is necessary for comparison studies. When a single strain control yeast culture is plated in all the wells of a 96-well plate the growth patterns in all of the wells are recorded (Figure 4A). When individual wells were examined in closer detail, very little differences between the wells can be discerned. This is true for not only the initial and final absorbance, but also the intervening data points as well (Figure 4B).

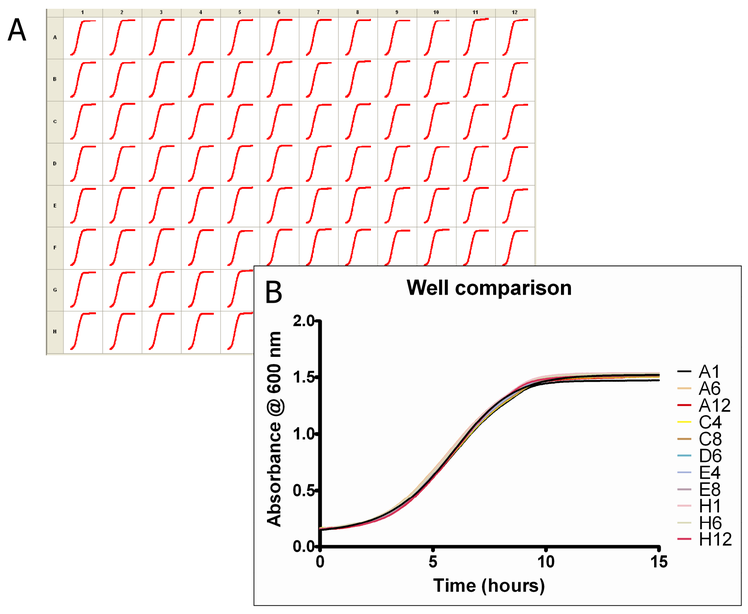

Figure 4 – A. Kinetic absorbance patterns of control yeast cultures as viewed in Gen5 Data Analysis software; **B.** Comparison of yeast growth in select wells.

Industrial Ale Yeast

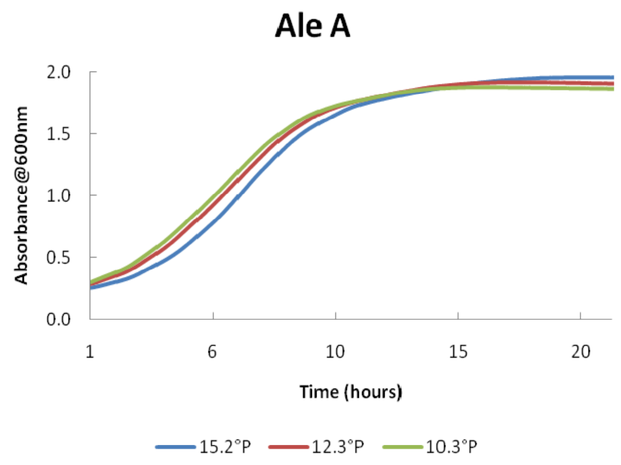

Figure 5 – The effect of pH (4.8, 5.0, 5.2, 5.5) and density (10.3, 12.3 and 15.2 °P) of wort on growth of industrial brewing yeast ale A at room temperature (~ 24°C). The wells were inoculated with 1.1 x 10^7 cells/mL. After 24 hours the cell number in the wells was 1.1-2.2 x 10^9 cells/mL. Every growth curve represents the growth curves for the four pH's in the wort with the corresponding density.

Dry Brewing Yeasts

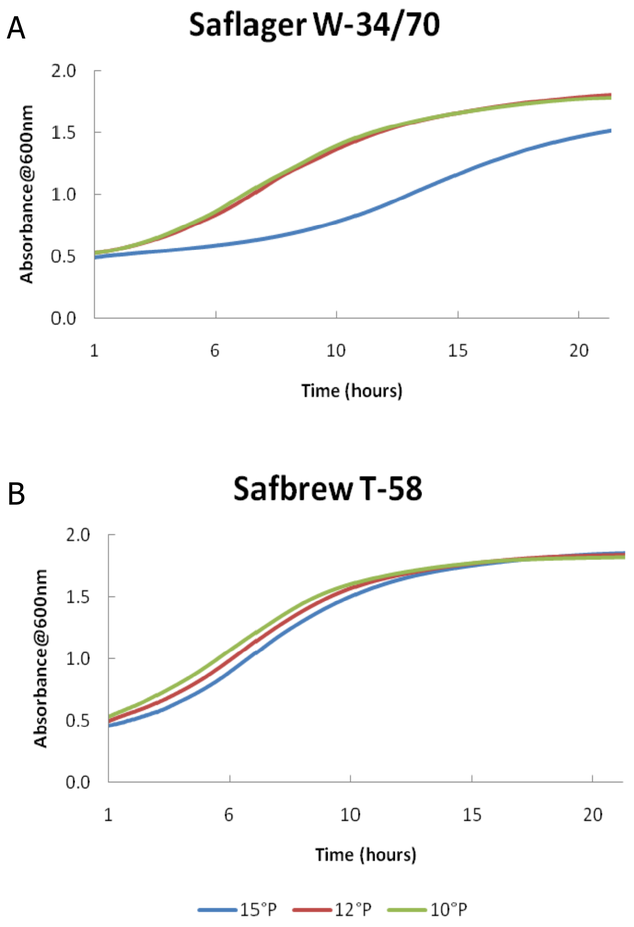

Figure 6 – The effect of pH (4.8, 5.0, 5.2, 5.5) and density (10.3, 12.3 and 15.2 °P) of wort on growth of dry brewing yeasts at room temperature (~ 24°C). **A.** Saflager W-34/70; **B.** Safbrew T-58. The wells were inoculated with 1.8 x 10^7 cells/mL (Saflager W-34/70) or 1.9 x 10^7 cells/mL (Safbrew T-58). After 24 hours the cell number in the wells was 4-5 x 10^8 cells/mL for Safbrew T-58. Every growth curve represents the growth curves for the four pH's in the wort with the corresponding density.

Dry Bakers/Distillers Yeast

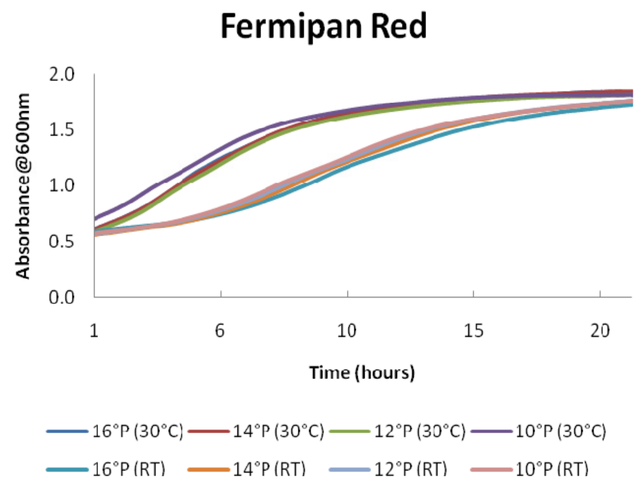

Figure 7 – The effect of pH (4.8, 5.0, 5.2, 5.4) and density (10.3, 12.4, 14.5 and 16,5 °P) of wort on growth of dry yeast Fermipan Red at room temperature (RT; ~ 24°C) and at 30°C. The wells were inoculated with 2.5 x 10^7 cells/mL (RT) or 2.4 x 10^7 cells/mL (30°C). After 24 hours the cell number in the wells was 2.4-4.7 x 10^8 cells/mL. Every growth curve represents the growth curves for the four pH's in the wort with the corresponding density.

Dry Wine Yeast

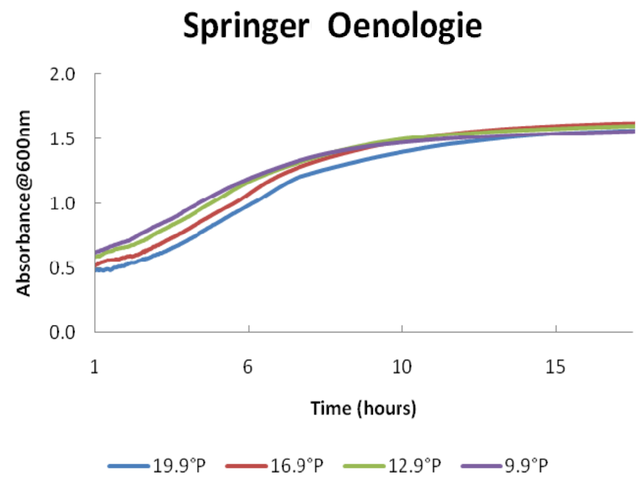

Figure 8 – The effect of density (9.9, 12.9, 16.9 and 19.9 °P) of must of white grapes on growth of dry wine yeast Springer Oenologie at 30°C. The wells were inoculated with 3.6 x 10^7 cells/mL. The pH of the must was 3.2. After 24 hours the cell number in the wells was 0.8-1.1 x 10^9 cells/mL.

Growth Results

Medium: density - pH	Yeast	Temperature	Time ODmax/2	ODmax
Wort: 15.2°P - pH: 4.8, 5.0, 5.2, 5.5	Ale A	RT	6h55-7h05	1.68-1.73
Wort: 12.3°P - pH: 4.8, 5.0, 5.2, 5.5	Ale A	RT	6h11-6h29	1.61-1.64
Wort: 10.3°P - pH: 4.8, 5.0, 5.2, 5.5	Ale A	RT	5h57-6h21	1.60-1.88
Wort: 15.2°P - pH: 4.8, 5.0, 5.2, 5.5	Saflager W-34/70	RT	13h25-13h51	1.16-1.22
Wort: 12.3°P - pH: 4.8, 5.0, 5.2, 5.5	Saflager W-34/70	RT	7h13-7h43	1.35-1.40
Wort: 10.3°P - pH: 4.8, 5.0, 5.2, 5.5	Saflager W-34/70	RT	6h35-7h15	1.31-1.35
Wort: 15.2°P - pH: 4.8, 5.0, 5.2, 5.5	Safbrew T-58	RT	7h33-7h47	1.44-1.47
Wort: 12.3°P - pH: 4.8, 5.0, 5.2, 5.5	Safbrew T-58	RT	6h41-7h19	1.39-1.44
Wort: 10.3°P - pH: 4.8, 5.0, 5.2, 5.5	Safbrew T-58	RT	6h19-6h45	1.36-1.37
Wort: 16.5°P - pH: 4.8, 5.0, 5.2, 5.4	Fermipan Red	RT	10h11-10h35	1.22-1.24
Wort: 14.5°P - pH: 4.8, 5.0, 5.2, 5.4	Fermipan Red	RT	9h45- 9h59	1.30-1.33
Wort: 12.4°P - pH: 4.8, 5.0, 5.2, 5.4	Fermipan Red	RT	9h31- 9h45	1.31-1.34
Wort: 10.3°P - pH: 4.8, 5.0, 5.2, 5.4	Fermipan Red	RT	9h15- 9h33	1.29-1.33
Wort: 16.5°P - pH: 4.8, 5.0, 5.2, 5.4	Fermipan Red	30°C	5h25-5h47	1.25-1.35
Wort: 14.5°P - pH: 4.8, 5.0, 5.2, 5.4	Fermipan Red	30°C	5h25-6h01	1.32-1.34
Wort: 12.4°P - pH: 4.8, 5.0, 5.2, 5.4	Fermipan Red	30°C	5h45-6h15	1.32-1.34
Wort: 10.3°P - pH: 4.8, 5.0, 5.2, 5.4	Fermipan Red	30°C	4h45-5h09	1.21-1.30
Must: 19.9°P	Springer Oenologie	30°C	6h45	1.14
Must: 16.9°P	Springer Oenologie	30°C	6h09	1.16
Must: 12.9°P	Springer Oenologie	30°C	5h27	1.12
Must: 9.9°P	Springer Oenologie	30°C	5h09	1.09

Table 1 – Experimental conditions and growth results of 5 industrial yeasts
RT: room temperature, ~24°C; ODmax: absorbance after 24 hours - absorbance at the start; Time ODmax/2: time to reach half of ODmax (time of lag phase thus included).

Conclusions

Lowering the pH of the wort to an industrial acceptable value of 4.8 has no effect on yeast growth for the different densities of the wort. Lowering the density of the wort or must to ~10°P has a positive effect on the yeast growth. This proves that a ~10°P medium is more than sufficient for yeast growth of four to seven times.

In the case it is industrial relevant, a higher yeast growth temperature can be used. This is illustrated with the yeast Fermipan Red: at 30°C the same final cell number is obtained in half of the time compared to at room temperature.

Finally, the Synergy H1 is a useful high throughput system to screen for optimal growth/revitalization conditions for industrial yeasts.

Acknowledgements

We would like to thank Dr. Paul Held for providing the introduction and growth uniformity results. Also, a warm thanks to Sylvie Vandoorne for her assistance in generating the kinetic curves of all industrial yeast strains. Finally, a special thank to Ann De Winne from Beun de Ronde Serlabo for inviting us to participate to this event.

[1] Jean-Luc Legras, Didier Merdinoglu, Jean-Marie Cornuet and Francis Karst. (2007.) "Bread, beer and wine: *Saccharomyces cerevisiae* diversity reflects human history". Molecular Ecology 16 (10): 2091–2102.

[2] Stubbings, W.J., Bostock, J.M., Ingham, E., and Chopra, I., (2004) "Assessment of a microplate method for determining the post-antibiotic effect in *Staphylococcus aureus* and *Escherichia coli*". J Antimicrobial Chemotherapy 54 (1): 139-143.

This poster was presented on May 5th, 2011 at Knowledge for Growth 2011 in Ghent, Belgium.