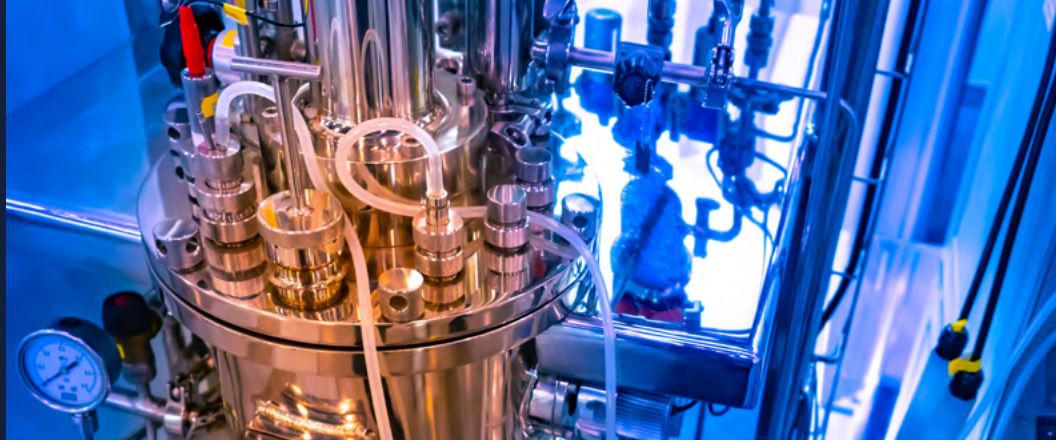
Rheolution
TEST SMARTER



Application Note | TURBIDI.T™

in collaboration with

BORDEAUX INP Enstbb

Monitoring yeast metabolism with TURBIDI.T™

SUMMARY

— TURBIDI.T™ (Rheolution, QC, Canada) has been used to characterize *S. cerevisiae* metabolic states and their evolution over time when different rates of glucose and oxygen are applied.

— Turbidity emerges as an innovative approach to provide real-time monitoring of yeast metabolism evolution.

— *S. cerevisiae* showed different turbidity profiles that reflected the shifts over different metabolic states.

— Utilizing TURBIDI.T™ for precision turbidity measurements offers a user-friendly, non-destructive and rapid approach to characterize liquid culture evolution overtime.

INTRODUCTION

Monitoring the metabolism of microbes in a bioproduction process holds pivotal significance for process optimization, product quality, and scale-up processes [1]. By quantifying microbial metabolic rates, adjustments to crucial parameters can be made to enhance production efficiency and yield. This application note explores the use of TURBIDI.T™ (Rheolution, QC, Canada) technology to monitor different yeast metabolisms through turbidity measurements.

While turbidity has not been widely used for monitoring yeast metabolism, it holds substantial potential as a valuable tool. Turbidity, which measures cloudiness in a liquid sample due to light scattering by particles in solution, can indirectly indicate microbial growth and metabolic activity. Changes in turbidity reflect alterations in cell density (see our last application note on the Quantification of cell concentration), biomass accumulation, and other physiological changes that occur during various metabolic shifts. Harnessing the power of turbidity to monitor yeast metabolism presents an innovative approach that could provide real-time insights into yeast metabolism evolution.

Saccharomyces cerevisiae is a microorganism of significant interest in bioproduction, including biofuels, pharmaceuticals, and food additives. The efficiency and yield of these processes depend on the yeast's metabolic performance, which is influenced by oxygen and nutrient conditions. *S. cerevisiae* can transition between oxidative, respiro-fermentative, and fermentative metabolism, each representing a unique strategy for energy production and utilization (**Figure 1**).

PARTICLE CONCENTRATION

CELL GROWTH

TIME MEASUREMENTS

TESTING CONFIGURATION

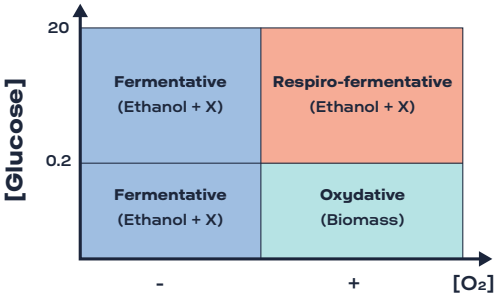


Figure 1. Schematic representation of *S. cerevisiae* metabolic states in response to oxygen and glucose concentration in the environment.

— Oxidative metabolism occurs under aerobic conditions, where ample supply of oxygen enables *S. cerevisiae* to fully oxidize glucose to produce energy in the form of ATP, which induces cell proliferation (biomass).

— Fermentative metabolism is activated under anaerobic conditions or limited oxygen, leading to the production of ethanol and less ATP. This allows the yeast to generate energy even in oxygen-deprived environments.

— Respiro-fermentative metabolism (a.k.a. Intermediate metabolism), observed in intermediate oxygen conditions, combines elements of both oxidative and fermentative pathways, enabling *S. cerevisiae* to adjust its energy production strategy based on oxygen and nutrient availability.

This study leverages the turbidity measurement capabilities of the TURBIDI.T™ instrument to monitor the dynamic evolution of *S. cerevisiae* cell cultures under specific glucose and oxygen conditions. Using a Chemostat process, which maintains a constant culture volume, we investigated how turbidity profiles change over time in response to varying nutrient and oxygen availability, reflecting the yeast's shifting metabolic states.

MATERIALS AND METHODS

1) *Saccharomyces cerevisiae* culture in a bioreactor

S. cerevisiae cultures were grown in three 2L bioreactors (BIOSTAT B; Sartorius Stedim Biotech, Göttingen, Germany) as outlined in our previous application note (**How to monitor yeast growth in a bioreactor with TURBIDI.T™?**). Each bioreactor contained a sterile synthetic culture medium, underwent autoclave sterilization, and had pH and pO_2 probes calibrated as per the BIOSTAT B software guidelines.

2) Inoculation and Monitoring

Inoculation involved introducing a 100 mL *S. cerevisiae* pre-culture prepared overnight to achieve an initial turbidity of 200 FTU. We monitored yeast growth using methods detailed in our previous application note (**How to monitor yeast growth in a bioreactor with TURBIDI.T™?**). Similarly, the evolution of cell concentration was monitored by TURBIDI.T™, referencing insights from our most recent application note (**How to quantify cell concentration with TURBIDI.T™?**). Once a steady-state condition was achieved (here noted as day 0), we initiated the Chemostat procedure.

3) Chemostat Procedure and Metabolic Configurations

The Chemostat procedure maintained continuous culture within the bioreactors. This involved a constant supply of fresh growth medium and simultaneous removal of culture fluid to maintain a steady-state equilibrium. The growth medium was a synthetic medium with inorganic salts, vitamins, oligo-elements and 30 g/L of glucose. Glucose and oxygen supply rates were configured using the BIOSTAT software. After 24 hours (1 day), the supply of glucose was changed, to observe the effect on cell culture development:

— Oxidative Metabolism: day 1 supply was 0.07 h-1 (g/L per hour) and day 2 supply was increased to 0.1 h-1. Oxygen levels were kept between 20-30% saturation.

— Respiro-Fermentative/Intermediate Metabolism: day 1 supply was 0.15 h-1 and day 2 supply was increased to 0.2 h-1. Oxygen levels were kept between 10-20% saturation.

— Fermentative Metabolism: day 1 supply was 0.2 h-1 and day 2 supply was decreased to 0.28 h-1. Oxygen levels were kept below 5% saturation.

4) Monitoring yeast metabolism in real-time with Turbidity

Turbidity measurements were conducted using the TURBIDI.T™ system. The instrument underwent preliminary calibration using formazin standards spanning from 1 to 4000 FTU. We utilized Emitt.635 cartridges at 635 nm for light emission and Receiv.ViS cartridges ranging from 400 nm to 1000 nm for light reception. Sample aliquots were collected over 2 days after yeast inoculation.

Turbidity measurements were executed in triplicate (n=3) with a 10mL sample volume per measurement. The TURBIDI.T™ system provided data, displayed through the Soft Matter Analytics App™, represented as the average ± standard deviation.

RESULTS AND DISCUSSION

The results provide insights into how *S. cerevisiae* cultures adapt to different metabolic conditions. In **Figure 2**, we illustrate the changes in culture turbidity over a 2-day period, together with the cell density at each steady state:

Oxidative Metabolism (Fig. 2A): Initially, the yeast culture had a turbidity of 2534.3 ± 30.92 FTUs (relative turbidity of 1, day 0) and a cell density of 1.80E+07 cells/mL (relative cell density of 1, day 0). Activating the Chemostat process increased turbidity by 1.75-fold (2876 ± 36.9 FTUs) and cell density by 27.3-fold (4.92E+08 cells/mL). This indicates the activation of oxidative metabolism, which thrives on sufficient glucose and oxygen, promoting yeast proliferation. By day 2, both turbidity and cell density continued to rise, resulting in a 2.36-fold increase in turbidity (7174.7 ± 160.9 FTUs) and a significant 70.2-fold rise in cell density (1.26E+09 cells/mL).

Respiro-Fermentative/Intermediate Metabolism (Fig. 2B): Yeast cultures started with a stable turbidity of 2352 ± 14.5 FTUs (relative turbidity of 1, day 0) and a cell density of 1.42E+07 cells/mL (relative cell density of 1, day 0). After one day of Chemostat operation, turbidity decreased slightly, stabilizing at 0.95-fold on day 1 (1926 FTUs). However, cell density increased by 2.675-fold (3.90E+07 cells/mL), suggesting that, although glucose and oxygen were limited compared to the oxidative state, they still supported cell proliferation. On day 2, turbidity decreased further to 0.8-fold (1840 ± 5.2 FTUs), and cell density slightly decreased as well (2.4-fold; 3.39E+07 cells/mL) likely due to limited oxygen availability.

Fermentative Metabolism (Fig. 2C): Yeast cultures began with a stable turbidity of 1863 ± 3.8 FTUs (relative turbidity of 1, day 0) and a cell density of 1.85E+07 cells/mL (relative cell density of 1, day 0). After implementing the Chemostat, turbidity decreased to 0.82-fold (1523 ± 12.6 FTUs).

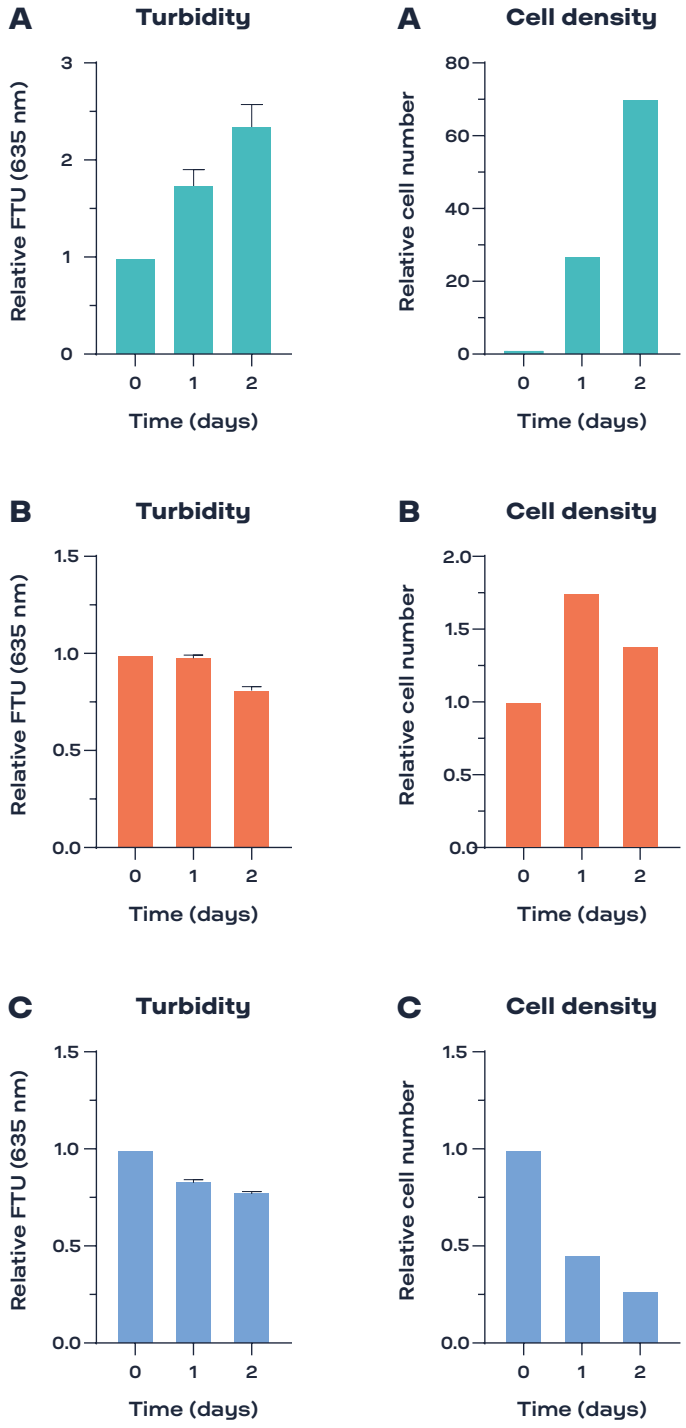


Figure 2. Real-time evolution of the culture turbidity and cell density of *S. cerevisiae* adopting (A) oxidative metabolism, (B) respiro-fermentative/intermediate metabolism or (C) fermentative metabolism, over a 2 day Chemostat process.

As oxygen became scarce, signaling a shift towards fermentative metabolism, ethanol production increased at the expense of ATP generation [2], leading to a decrease in cell proliferation, as indicated by the decline in cell density (as we see the 0.5-fold decrease; 8.48E+06 cells/mL). Limited oxygen and abundant glucose favored fermentative metabolism. On day 2, turbidity and cell density continued to decline, reaching 0.79-fold (1465 ± 12.2 FTUs) turbidity and 0.27-fold (5.08E+06 cells/mL) cell density.

The outcomes of this study underscore the potential of turbidity as an invaluable tool for tracking the dynamic metabolic responses of S. cerevisiae in bioproduction processes. As we exposed the yeast to varying glucose inputs and oxygen levels, distinct turbidity profiles emerged, mirroring the yeast's shifting metabolic states.

CONCLUSIONS & PERSPECTIVES

— The TURBIDI.T™ instrument offers a straightforward and user-friendly method to measure turbidity in yeast cell cultures within a liquid medium.

— This study reveals distinct turbidity profiles that correspond to the dynamic metabolic states of *S. cerevisiae*.

— Under aerobic conditions, we observed a significant increase in turbidity, which is correlated with an increase in cell density, contributing to the heightened turbidity levels. Intermediate oxygen conditions manifested in hybrid turbidity profiles and promoted 1.5-fold yeast proliferation in two days. Finally, limited oxygen availability led to a shift towards fermentative metabolism, consequently decreasing turbidity and cell density of the yeast culture.

— Turbidity emerged as a non-invasive and real-time method for tracking microbial metabolic responses, offering invaluable insights into optimizing bioproduction processes and enhancing product yields.

— The TURBIDI.T™ instrument's rapid data acquisition capabilities, combined with the user-friendly Soft Matter Analytics™ App, streamline the process of quantifying cell dynamics via turbidity measurements. This efficiency translates to significant time and resource savings in laboratory settings.

REFERENCES

[1] Mitra, S., & Murthy, G. S. (2022). Bioreactor control systems in the biopharmaceutical industry: a critical perspective. Systems Microbiology and Biomanufacturing, 1-22.

[2] Frick, O., & Wittmann, C. (2005). Characterization of the metabolic shift between oxidative and fermentative growth in Saccharomyces cerevisiae by comparative 13C flux analysis. Microbial cell factories, 4(1), 1-16.