

Supporting Information for “Ultrasound Enhanced Attenuated Total Reflection Mid-Infrared Spectroscopy In-line Probe – Acquisition of Cell Spectra in a Bioreactor”

Cosima Koch,[†] Markus Brandstetter,^{†, §} Patrick Wechselberger,^{‡, ⊥} Bettina Lorantfy,^{‡, ||} Maria Reyes Plata,[†] Stefan Radel,[†] Christoph Herwig,^{‡, ⊥} Bernhard Lendl^{*†}

[†]Institute of Chemical Technologies and Analytics, Vienna University of Technology, Getreidemarkt 9/164-UPA, 1060 Vienna, Austria

[‡]Institute of Chemical Engineering, Vienna University of Technology, Gumpendorferstraße 1a, 1060 Vienna, Austria

[⊥]Christian Doppler Laboratory for Mechanistic and Physiological Methods for Improved Bioprocesses, Institute of Chemical Engineering, Vienna University of Technology, Getreidemarkt 9/166, 1060 Vienna, Austria

*corresponding author: bernhard.lendl@tuwien.ac.at

DESCRIPTION OF CONTENTS:

Experimental: Details on media preparation and cultivation conditions, page S2

Table S1: Overview of setups and conditions of the fed-batch fermentations, page S2

Figure S1: Spectra of *S. cerevisiae* acquired in-line during batch phase, page S3

EXPERIMENTAL

All reagents for yeast media were supplied by Carl Roth GmbH (Karlsruhe, Germany).

Yeast peptone glucose (YEPG) medium for preculture: 40 g L⁻¹ glucose, 5 g L⁻¹ peptone and 5 g L⁻¹ yeast extract. The YEPG medium was autoclaved at 121°C for 20min. 200 mL of YEPG medium were inoculated with 1 mL of frozen stock of *S. cerevisiae* (strain: CBS 8340) under sterile conditions and subsequently incubated for 24 h at 30°C and 220 rpm in shake flasks.

Batch medium (6 L): 20 g L⁻¹ glucose, 5 g L⁻¹ ammonium sulphate, 3 g L⁻¹ potassium hydrogen phosphate, 0.5 g L⁻¹ magnesium sulfate heptahydrate. Additionally, 2.67 mL L⁻¹ of sterile vitamin and trace element solutions, respectively, as well as 0.1 mL L⁻¹ of sterile antifoam were added after autoclavation (121°C, 20 min).

Fed-batch feed medium (4 L): 100 g L⁻¹ glucose, 3 g L⁻¹ potassium hydrogen phosphate, 0.5 g L⁻¹ magnesium sulfate heptahydrate and 2.67 mL L⁻¹ of sterile vitamin and trace element solutions, respectively, as well as 0.1 mL L⁻¹ of sterile antifoam.

During batch fermentation, conditions were as follows: 6 L min⁻¹ (1 vvm) of airflow, 800 rpm speed of agitation, temperature 30°C and the pH was automatically adjusted to 4.8 by addition of 2 M NaOH. The batch process was started by inoculation with 600 mL of preculture (10% of batch volume). The fed-batch phase was run under the same conditions as the batch phase except that the airflow was increased with culture volume to remain at 1 vvm. An overview of the employed conditions and used set-ups can be found below (Table S1:). A lower growth rate (i.e. feed rate) was set for fermentation D to increase temporal resolution and allow for a tighter time grid of off-line samples.

Table S1: Overview of setups and conditions of the four fed-batch fermentations.

ID	Fermenter	Stirring rate (rpm)	Set growth rate μ (h ⁻¹)
A	Chemap	600, 800, 1000, 1200	0.2
B	Chemap	800	0.2
C	Chemap	800	0.2
D	Techfors	800	0.15

FIGURE S1:

Inline spectra of yeast acquired during the batch phase of the fermentation.

