## **Strain and Culture Conditions**

The haploid yeast *Saccharomyces cerevisiae* CEN.PK113-7D, obtained from the *Centraalbureau van Schimmelcultures* (Fungal Biodiversity Center, Utrecht, The Netherlands), was used in this study. The cultivation was performed using a low-salt Verduyn minimal medium [1] with a glucose concentration of 7.5 g/L. 1L-Erlenmeyer flasks containing 100 mL medium were inoculated with cells from a cryovial (glycerol, -80oC) and the inoculation cultures were subsequently grown for 10 h at 200 rpm and 30 °C. The inoculation culture was used to inoculate a 7 L bioreactor (Applikon Biotechnology B.V., Delft, The Netherlands) containing a working volume of 4 L, controlled by a Biostat B Plus controller (Sartorius AG, Göttingen, Germany). The reactor was aerated with pressurized air at 1 L/min (0.25 vvm) using a Smart series mass flow controller 5850S (Brooks Instrument, PA, USA). The reactor was operated at 0.3 bar overpressure, at 30 oC, with a stirrer speed of 600 rpm. The pH of the broth was maintained at 5.0 by automated addition of either 4M KOH or 2M H2SO4. Once the batch phase was completed (indicated by a fast decrease in CO2 signal and a sharp increase in dissolved oxygen (DO)), the chemostat phase (steady-state) was started at a dilution rate of 0.1 h-1 for 50 h. DO was not controlled but was well above > 60% during the whole chemostat phase. After about 5 residence times, sampling for proteomics was performed.

## **Dynamic Feast Famine Setup**

After five residence times (50 h) of continuous feeding, the feeding was changed to block-wise, leading to a feast/famine regime [2]. Cycles of 400 s were applied by a feeding medium for 20 s, followed by a period of 380 s of no feeding. The medium pump was controlled using an automatic timer (PTC-1A, Programmable timing controller, Omega Engineering Inc., Stamford, CT, USA). During the 20-second feeding period, 43 ± 1 mL of fresh medium were added. The same volume was subsequently withdrawn during about 260s at a flow rate of 0.166 ± 0.001 mL s−1 maintaining the broth volume nearly constant at 4 L. After about 5 residence times, sampling for proteomics was performed.

## **Sample acquisition and analysis**

### Extracellular metabolites

For the analysis of extracellular metabolites, 1.5 ml broth was taken using a syringe containing ~26 g pre-cooled (-20 oC) stainless steel beads, which was subsequently filtered as described by Mashego et al. [3]. Extracellular acetate, ethanol, glucose and glycerol concentration were measured using HPLC or enzymatic assay, as described by Canelas et al. [4]. Biomass concentrations (cell dry weight) were determined using a gravimetrical method using the method described by Suarez-Mendez et al. [2]. The CO2 and O2 fractions in the off-gas were determined using a combined infrared/paramagnetic NGA2000 analyzer (Rosemount Analytics, CA, USA).

### Intracellular metabolites

Samples for the measurement of intracellular were taken by rapidly withdrawing 1 ml of broth and quenching it in 5 ml cold (-40 oC) methanol, as described by Lange et al. and Canelas et al. [1,5]. Taken samples were weighted, and subsequently poured into a filtration setup (using a Supor-200 cellulose membrane, 0.2 µm, 47 mm, Pall Corporation), which already contained 15 ml pre-cooled (-40 oC) methanol. After this, vacuum was applied, followed by the addition of 15 ml cold (-40 oC) methanol to wash the biomass [6]. The filter with the washed biomass was subsequently transferred to a 50 ml falcon tube containing 30 ml of a 75% (v/v) ethanol solution, preheated to 75 oC. To this, 100 µl 13C yeast cell extract was added as internal standard [7]. The tube was then shaken and put into a water bath at 95 oC for 3 minutes to extract the intracellular metabolites. After extraction, the tubes were immediately cooled in an ice bath, and the filter was removed. The cell extract was subsequently stored at -80 oC and later concentrated through complete evaporation of the aqueous ethanol solution and resuspended into 500 µl milliQ water, as described by Mashego et al. [8]. The resuspended samples were centrifuged at 15000 g for 5 minutes at 1 oC, and the supernatant was transferred to a new tube, which was subsequently centrifuged again to remove all solid components in the sample. The obtained supernatant was then transferred into a screw-capped vial and stored at -80 oC. Samples were analysed by GC-MS [7–9] and LC-MS [10].

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