

## **METHODS**

### **Strain and growth conditions**

- Grown at 30°C in fermenter (similar to chemostat)
- Inoculated in a medium previously described by Sierkstra et al. (1992)
- Yeast grown at D of 0.05, 0.1, 0.15, 0.19 and 0.29 h<sup>-1</sup> with 1.5 g NH<sub>4</sub>Cl l<sup>-1</sup> and 9.5 g glucose l<sup>-1</sup>

### **Labelling of oligonucleotides and Northern blot analysis**

- Northern blot for detection of GAP1, HIS4, ILV5, GLN1, and GDH1
- ACT1 internal control for amount of RNA blotted for GAP1, HIS4, and ILV5
- H2A/H2B was used as internal control for detection of GLN1 and GDH1

### **Enzyme Assays**

- Activities of NADPH-GDH and NAD-GDH were measured under V<sub>max</sub> conditions essentially described by Boles et al. (1993)
- GS Activity was measured as described by Mitchell & Magasanik (1983).

## **RESULTS**

### **Growth conditions and cell morphology**

- Glucose concentrations higher than 8.5 g l<sup>-1</sup> no further increase in biomass was recorded
- *S. cerevisiae* was nitrogen-limited under the conditions employed.

### **Physiological Parameters**

- CO<sub>2</sub> production increased from 1.6 to 9.8 mmol g<sup>-1</sup> l<sup>-1</sup>
- O<sub>2</sub> consumption increased from 1.5 to 9.0 mmol g<sup>-1</sup> l<sup>-1</sup>
- Respiration quotient constant
- No major changes occurred in the carbon metabolism under these conditions
- Biomass remained constant
- Ammonia and glucose consumption increased linearly with D and no changes in the biomass observed

### **2-Oxoglutarate and amino acid concentrations**

- 2-oxoglutarate, glutamate, and glutamine play an essential role in interaction between carbon and nitrogen metabolism.
- 2-oxoglutarate, glutamine, and glutamate concentrations remained at about 5, 27, and 180 μmol g<sup>-1</sup>, respectively.

### **Expression of nitrogen-regulated genes**

- GDH1 encodes for NADPH-GDH
- GLN1 encodes for GS activity
- GAP1 encodes for general amino acid permease
- ILV5 encodes for α-acetolactate reductoisomerase
- HIS4 encodes for histidinol dehydrogenase
- Expression of GAP1, GLN1, and GDH1 was repressed during growth of glutamine and depressed during growth on proline and glutamate
- No change in expression of ILV5 and HIS4 genes were observed during growth on various nitrogen sources
- In nitrogen limited continuous culture: amount of GAP1 mRNA increased with D

- Increase in GAP1 correlates with increase in nitrogen and carbon fluxes, as well as decrease the extracellular ammonia concentration
- Levels of GLN1, GDH1, ILV5, and HIS4 mRNAs did not change with increasing D

### **Enzyme Activities**

- Glucose and ammonia fluxes increased linearly with D
- Proline and glutamate-grown cultures: activity of NADPH-GDH slightly higher than in glutamine-grown cultures
- Activities of both NAD-GDH and GS were higher during growth on proline and glutamate than during growth on glutamine
- Nitrogen-limited continuous culture:
- NADPH-GDH increased from 1.10 to 2.03  $\mu\text{mol min}^{-1}$
- GS increased from 0.36 to 1.02  $\mu\text{mol min}^{-1}$
- D increased from 0.05 to 0.29  $\text{h}^{-1}$
- NAD-GDH activity decreased from 0.033 to 0.012  $\mu\text{mol min}^{-1}$
- Enzymes are also regulated at a post-transcriptional level.

### **Possible Equations**

- First nutrient: Ammonia (consumed)
- Second nutrient: Glucose (consumed)
- Population: Yeast
- D: dilution rate (vol/time)