

Scale-up Production of Pharmaceutical Proteins in Plant Cell Suspensions with Orbitally Shaken Disposable Bioreactors

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Introduction

Plant cells are well suited for the production of pharmaceutical and industrial proteins either as whole-plant systems or cell suspension cultures. The latter have the advantage of being cultivated in containment under defined conditions that allow rigid process control.

Cylindrical orbitally shaken single-use bioreactors could be favourable types of cultivation vessels for plant suspension cells because they combine reduced cell stress and contamination risks, that are characteristics of surface aerated reactors, with the flexibility and cost effectiveness of disposables.

We used a monoclonal *Nicotiana tabacum* cv BY-2 plant suspension cell line that secretes the human vitronectin-specific IgG₁ antibody to the medium to investigate the suitability and scalability of orbitally shaken disposable bioreactors for cultivation.

Materials and Methods

Online measurement of oxygen consumption

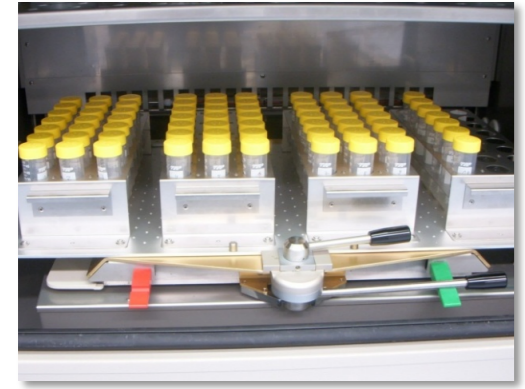


RAMOS based measurement of OTR
See also poster:
Characterization of orbitally shaken single-use bioreactors for plant cell cultivation



Non-invasive oxygen sensors from PreSens for the detection of DOT

Orbitally shaken vessels for BY-2 cultivation



Spintubes, 240 rpm
V_R = 50 mL, V_L = 10 mL



Shake flasks, 180 rpm
V_R = 250 mL, V_L = 50 mL

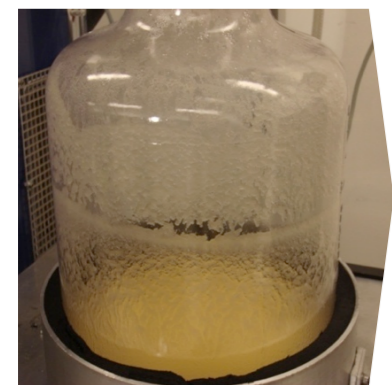


Nalgene carboy, 180 rpm
V_R = 20 L, V_L = 5 L



SB200-X, 80 rpm
V_R = 350 L, V_L = 100 L

Scale-up production & DSP



4 L pre-culture in 20 L vessel



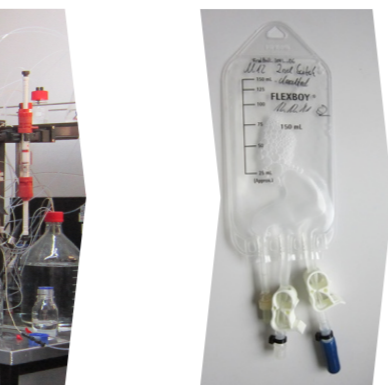
100 L culture in SB200-X



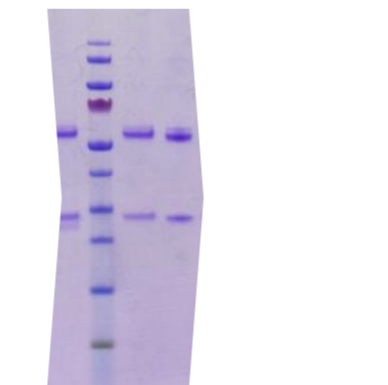
Harvest



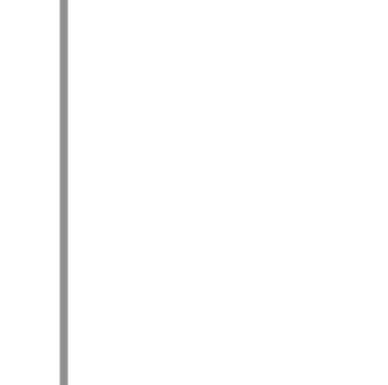
EBA



Protein A chromatography



Final bulk



Analytics

Results

1) Online Measurement of Oxygen Transfer Rate (OTR) and Dissolved Oxygen Tension (DOT)

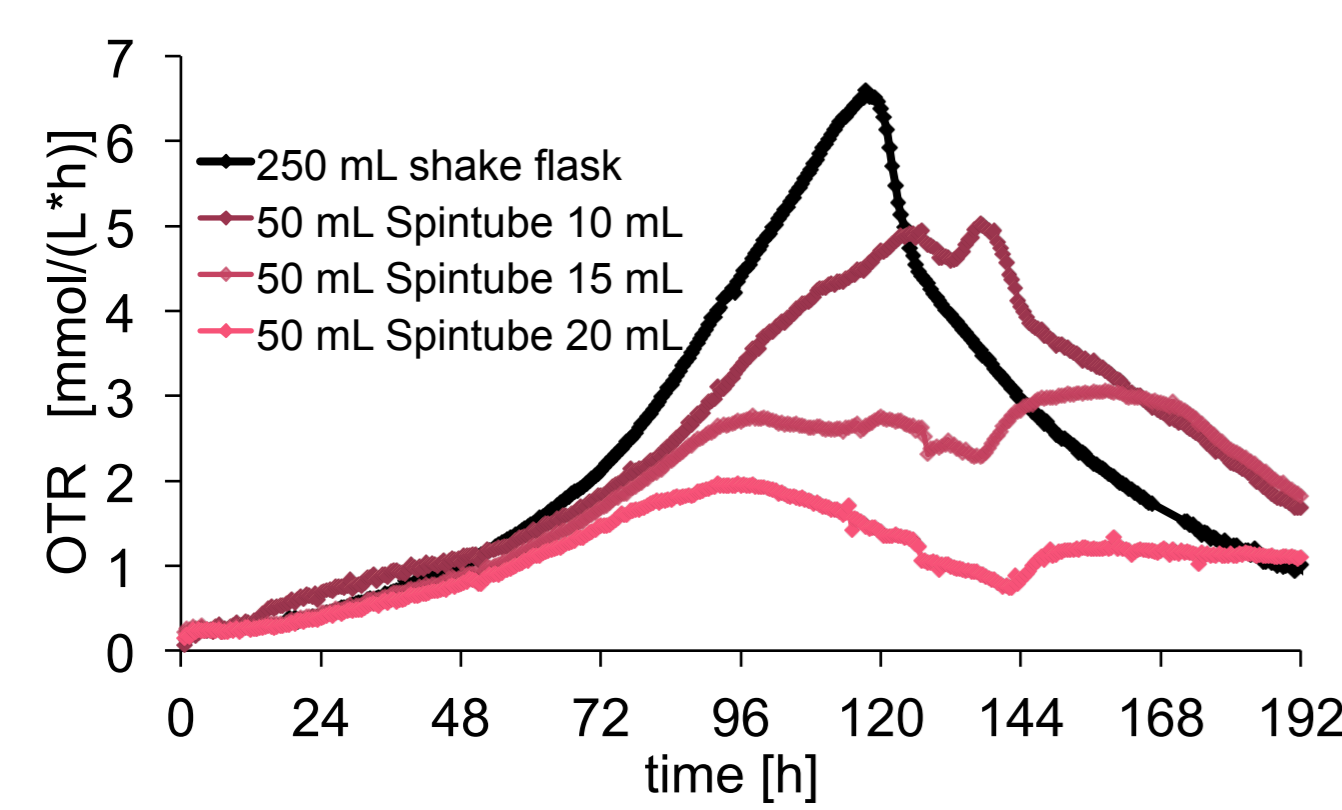


Figure 1. OTRs of BY-2 cells in 50 mL spintube bioreactors with different filling volumes

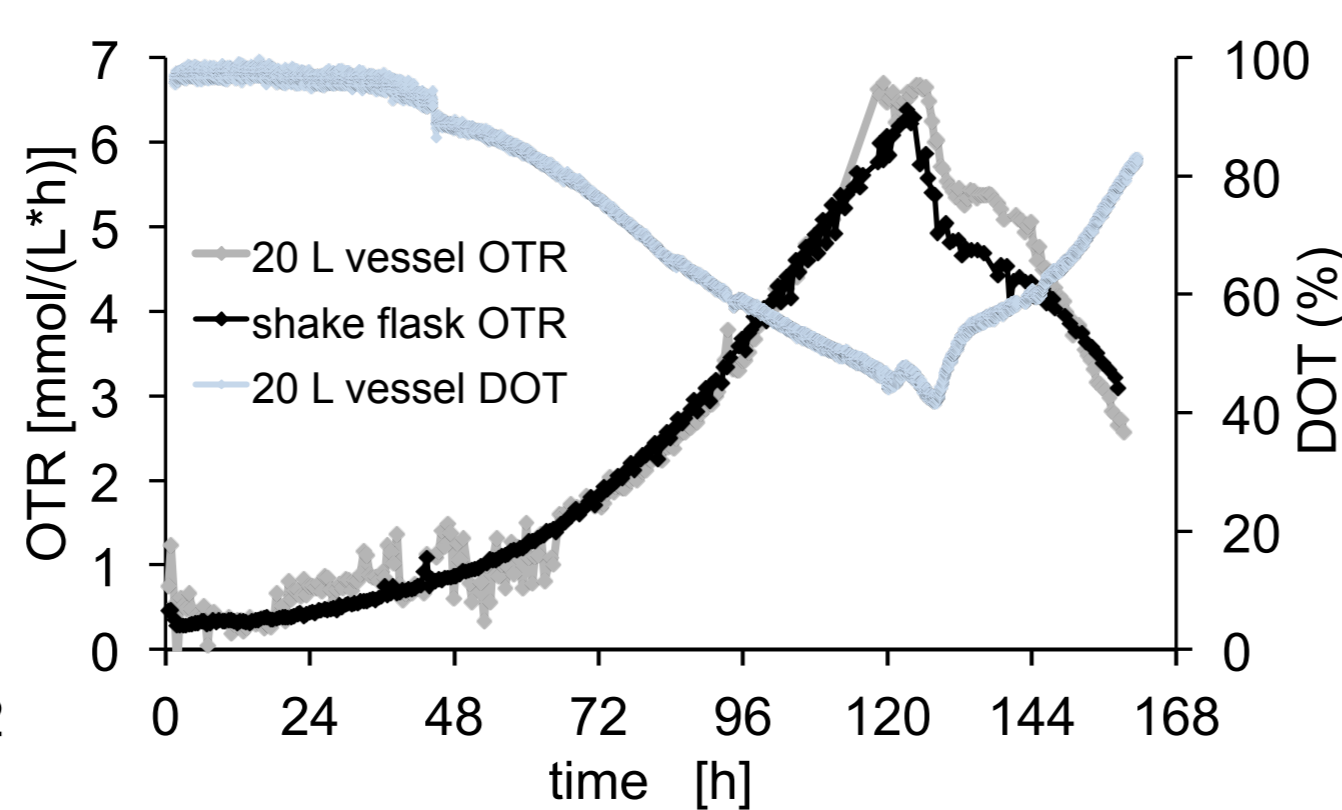


Figure 2. Simultaneous measurement of OTR and DOT for BY-2 cells cultivated in 20 L Nalgene vessel

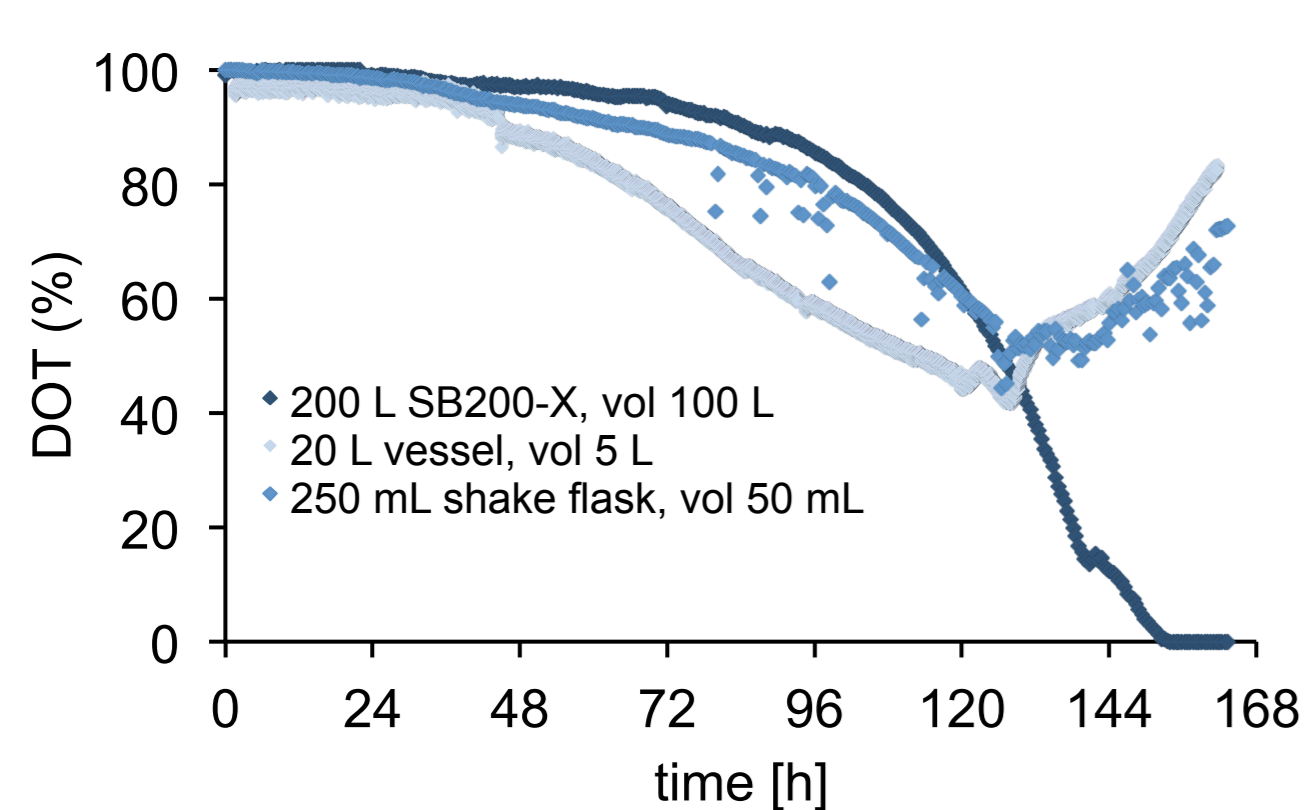


Figure 3. DOTs of BY-2 cells grown in different cultivation vessels with working volumes of 50 mL – 100 L

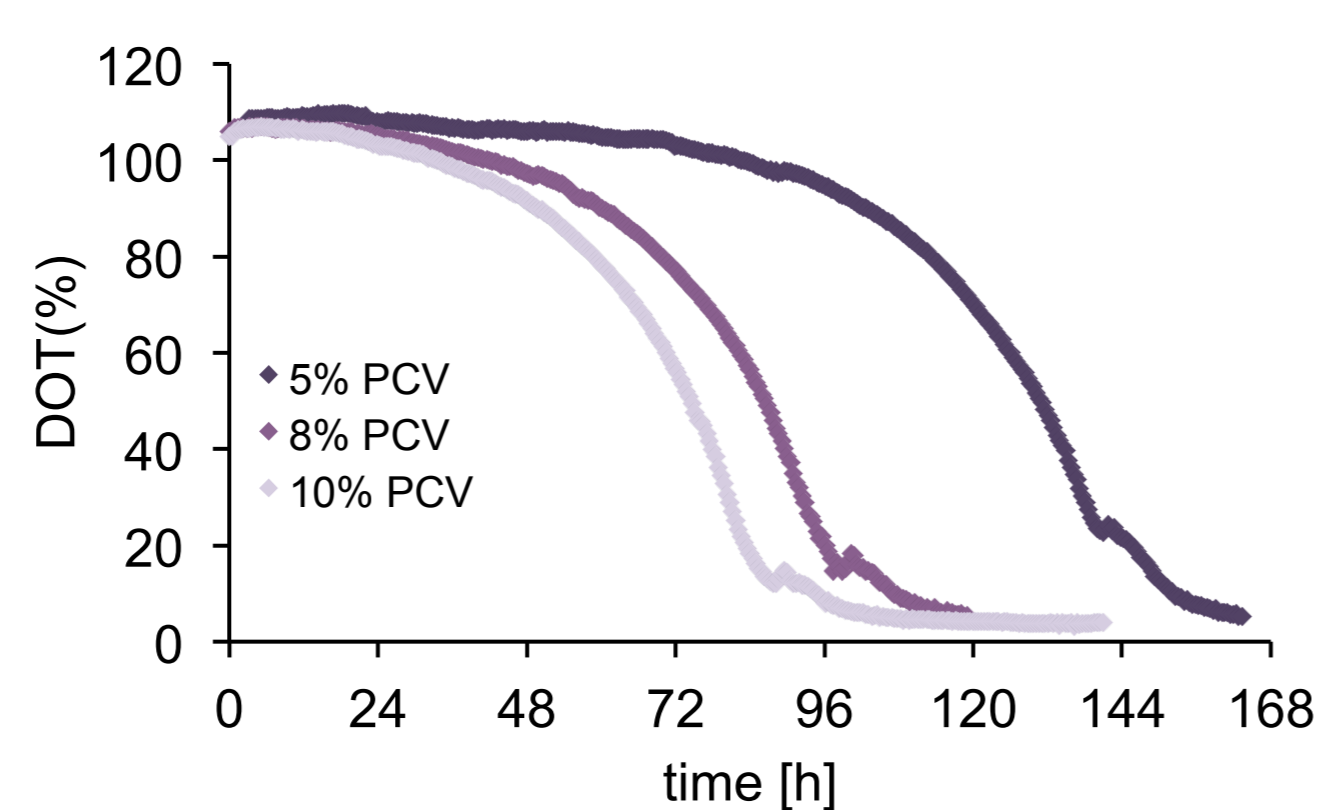


Figure 4. DOTs of BY-2 cells grown in SB200-X (100 L working volume) with varying inoculation densities

2) Scale-up Cultivation & DSP

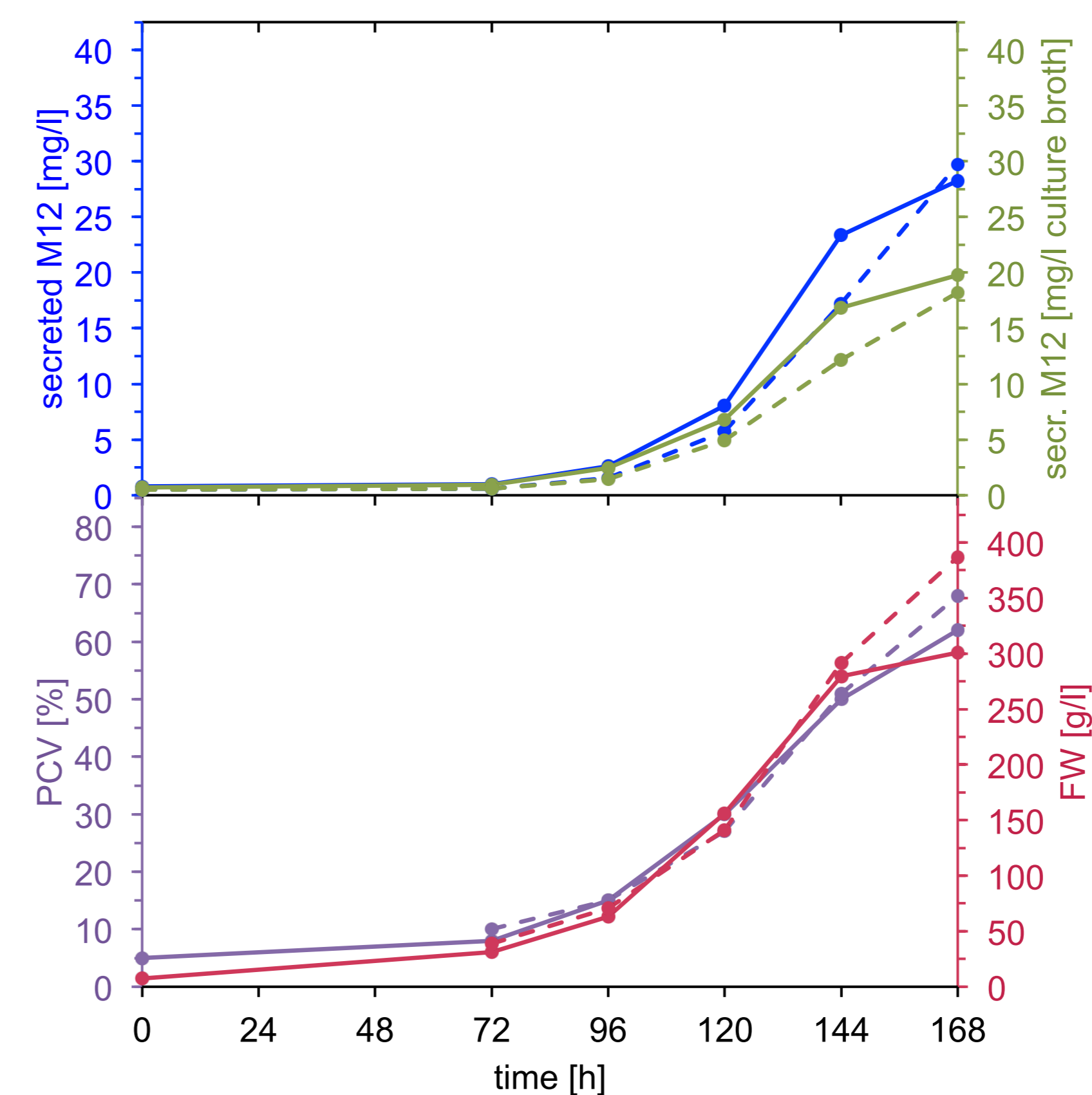
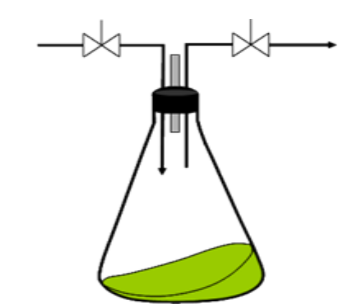


Figure 5. Growth parameters and M12 antibody yields of parallel BY-2 cultivations in shake flask and SB200-X



500 mL shake flask: 150 mL working volume, results displayed in solid lines



200 L SB200-X: 100 L working volume, results displayed in dashed lines

- Three repeated batch fermentations in SB200-X resulted in the production of 5.7 g M12 antibody with an overall process time of 18 days.
- The secreted M12 antibody was purified from the spent medium with a two-step process comprising ion exchange chromatography and protein A chromatography. A product recovery of 90% was achieved.

3) Biochemical Characterization of the Product

- The characteristics of the purified M12 antibody from BY-2 were compared with those of its mammalian (CHO DG44) produced equivalent.

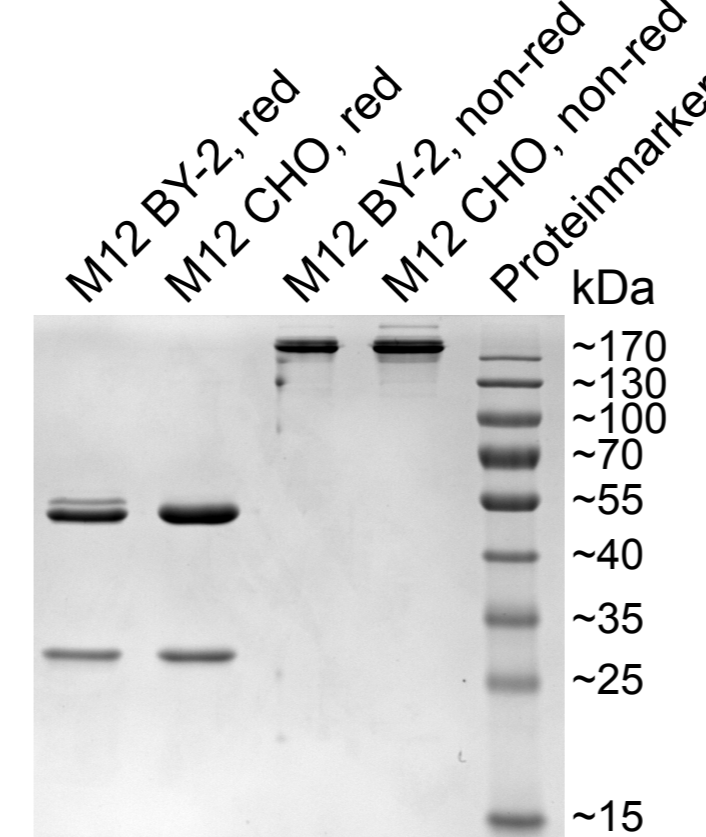


Figure 6. Coomassie stained SDS PAGE

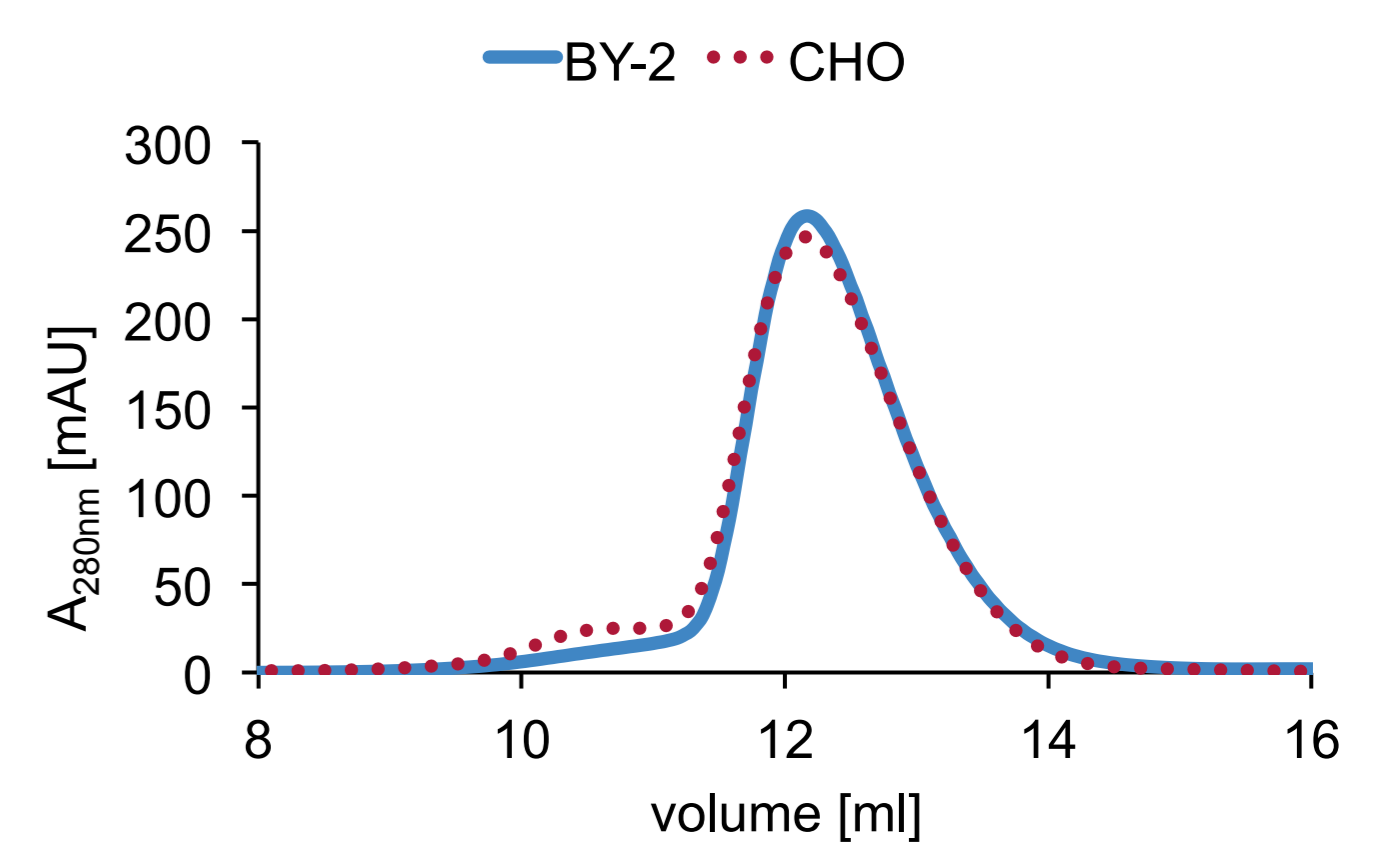


Figure 7. Analytical gel filtration

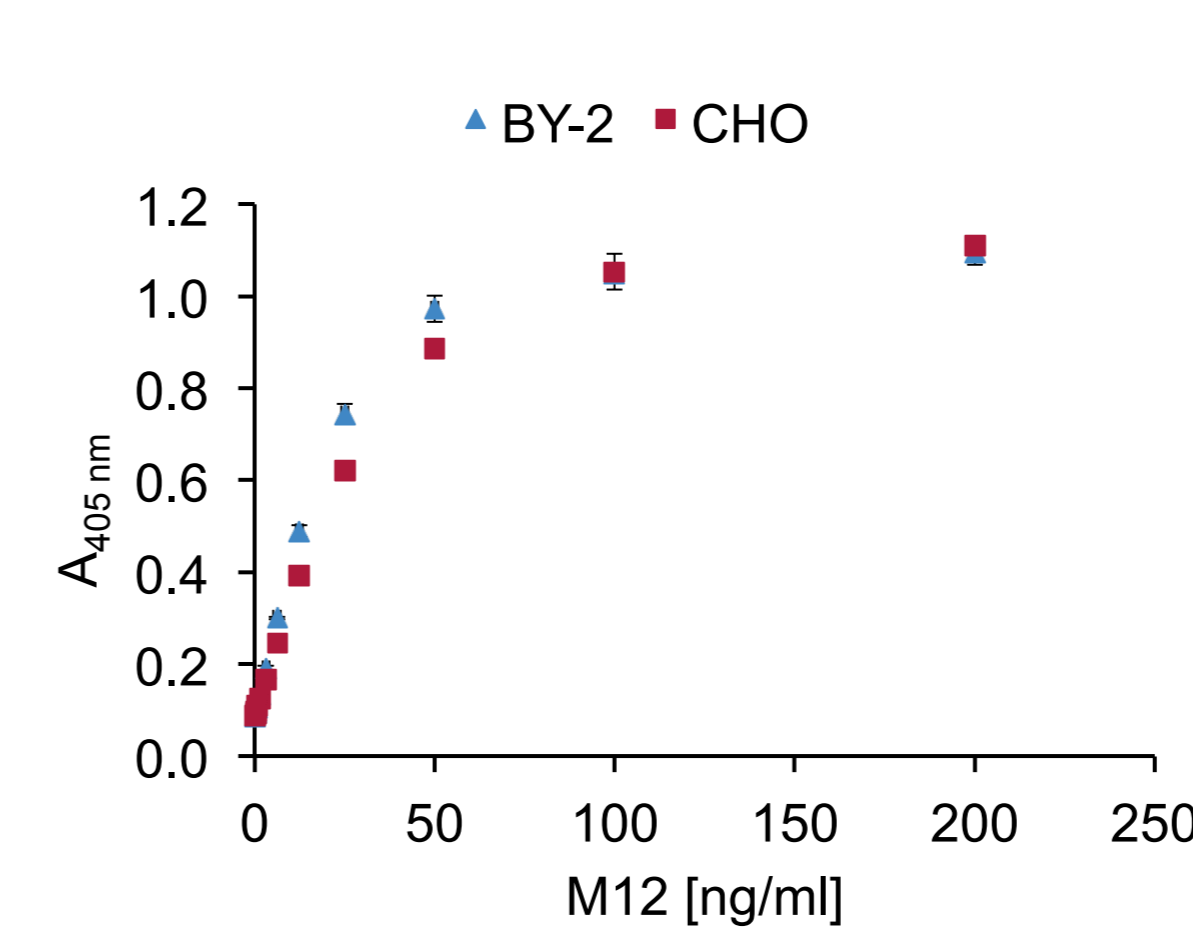


Figure 8. Vitronectin binding assay

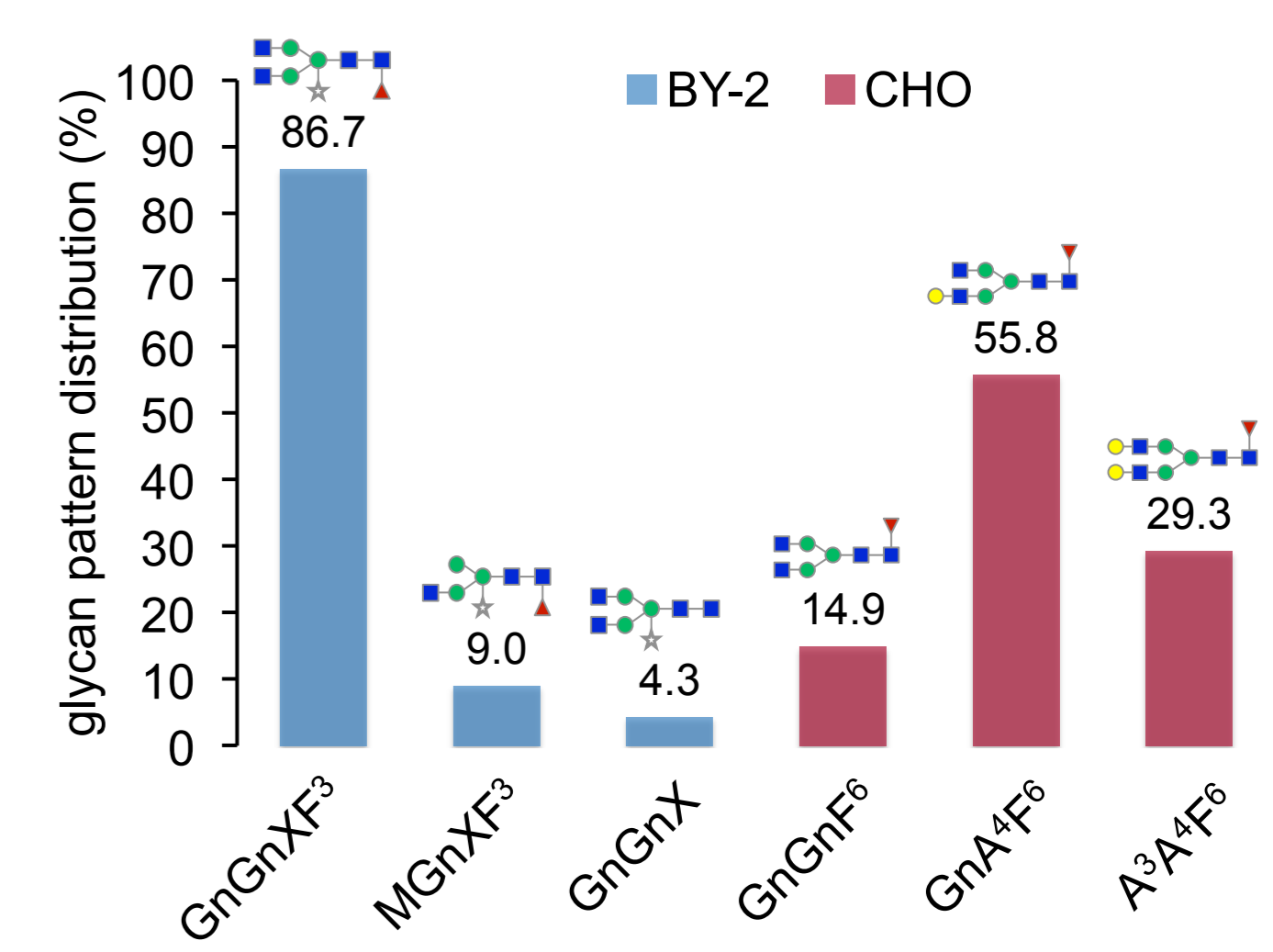


Figure 9. N-glycan profiles

Conclusions

- Optical detection of dissolved oxygen via PreSens technology is a suitable means for online monitoring the growth/oxygen consumption of plant suspension cells.
- Orbitally shaken disposable bioreactors are well suited for the cultivation of BY-2 suspension cells. A 20-fold process scale-up in culture volume did not adversely affect the productivity of the plant cells.
- Successful scale-up production and efficient DSP of the M12 from BY-2 was demonstrated.
- Direct comparison of the plant cell produced antibody and the CHO produced equivalent revealed comparable biochemical properties. The N-glycan profile of the M12 from BY-2 was more homogeneous than its mammalian equivalent.