Cultivation of Sunflower suspension cultures in shaking flasks with an *online* monitoring system

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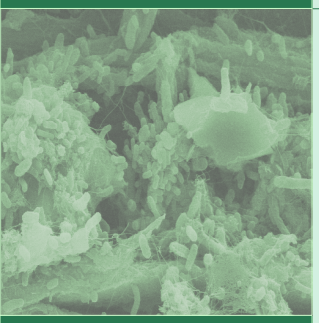


Introduction:

§ Monitoring of biological activity in shaking flasks insufficient
à time-consuming, dangerous for sterility, delay between real status & measured state

§ Accurate **monitoring** & realistic **scale up** hindered
à consequences: growth limitations, premature interruption before reaching growth maximum

§ Application of **miniaturized parallel cultivation systems with *online* sensor technology** (Fig. 1 and 7) for microorganisms
à utilization for cultivation of plant cells just marginally reviewed

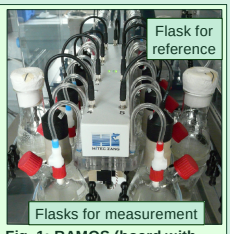


Fig. 1: RAMOS (board with flasks for reference & measurement)

Fig. 2: *Helianthus annuus* – sunflower

Aim:

§ Screening suspensions of sunflower (*H. annuus*, Fig. 2) concerning media & cell line optimization in the parallel cultivation system **RAMOS** [1] (**R**espiration **A**ctivity **MO**nitoring **S**ystem, Fig. 1 and 7)

§ Transfer of plant *in vitro* cultures into RAMOS: **handling**, setup & interpretation of data

§ Optimized synthesis of plant secondary metabolite **α-Tocopherol** (vitamin E, Fig. 5) for industrial applications e.g. in cosmetic industry & pharmacy [2, 4]

Materials and Methods:

Callus (Fig. 3): undifferentiated plant cells via impact of plant growth regulators like the Auxin 2,4-Dichlorphenoxyacetic acid (2,4-D)

Plant suspension culture: callus suspended & cultivated in liquid media (Fig. 4)

Cultivation parameter for further exp.:

§ 26°C, 110 rpm, dark, sunflower suspension cultur, inoculum 20% (v/v) [3]

§ **L**insmaier & **S**koog media à variation of 2,4-D concentration: 0,1 & 0,2 mg/L

RAMOS: measurement of difference & O_2 partial pressure in each flask [1] à RQ

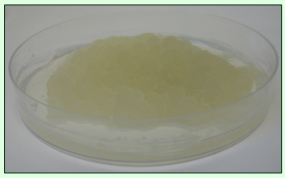


Fig. 3: Callus of *Helianthus annuus*, approx. 2 weeks old

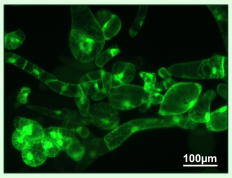


Fig. 4: Suspension of sunflower, stained with FDA (fluor. microscopy, vital cells are green)

Plant cells in contrast to microorganisms:

§ Sensitivity of plant *in vitro* cultures in terms of growth & metabolite synthesis high

§ Increase of viscosity during stationary phase heavy à risk of limitations (O_2 and other nutrients)

§ Growth rate low à high risk for contamination, long term experiments

§ Agglomeration of plant cells in suspension intense à difficult handling e.g. for reproducible inoculation & single cell analysis

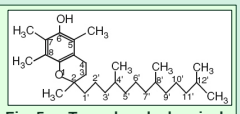


Fig. 5: α-Tocopherol, chemical structure [4]

Results:

§ Classification into growth phases (Fig. 8 above)

§ Cultivations take long time, intense dependency of cultivation from inoculum (type, concentration, amount/volume) (Fig. 8)

§ Growth dependent from 2,4-D concentration
à limitation earlier at halved Auxin concentration, restricted metabolism

§ Growth independent from working volume (tested at 30 and 50 ml in 250 ml total flask volume; not shown)

§ Growth phase: respiration quotient RQ decreasing from 1,3 to 1,2

§ Stationary phase: slow decrease of RQ to 1,1
à incipient increase of viscosity of cell suspension

§ Death of cells: abrupt increase of RQ

§ Validation of data with help of identic experiments

Oxygen transfer rate $OTR = \frac{\Delta p_{O_2} * V_g}{\Delta t * R * T * V_l}$ $[\frac{mol}{l * h}]$

Carbon dioxide transfer rate $CTR = \frac{\Delta p_{CO_2} * V_g}{\Delta t * R * T * V_l}$ $[\frac{mol}{l * h}]$

Respiration quotient $RQ = \frac{CTR}{OTR}$

Fig. 6: Equations for calculation of oxygen transfer rate OTR, carbon dioxide transfer rate CTR & respiration quotient RQ

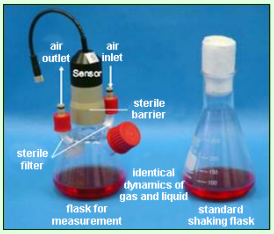
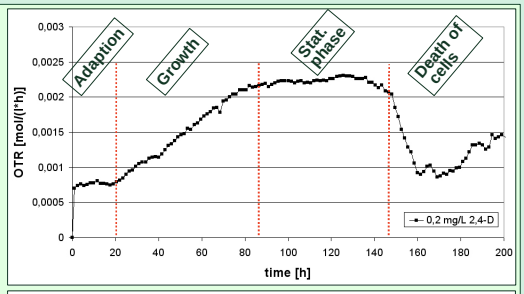


Fig. 7: RAMOS (comparison of standard flask with flask for measurement; [1])



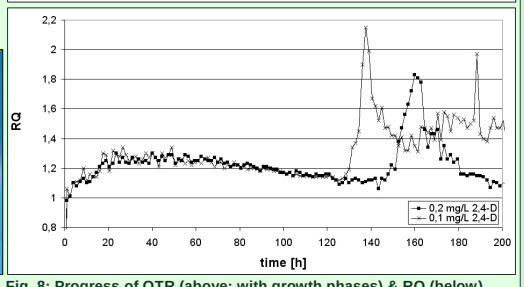


Fig. 8: Progress of OTR (above; with growth phases) & RQ (below) during cultivation of *H. annuus* suspension culture in RAMOS with 0,1 mg/L (×) and 0,2 mg/L (•) 2,4-D (26°C, 110 rpm, dark)

Conclusion RAMOS:

§ Advantages: marginal amount of work, easy handling in comparison to standard miniaturised cultivation strategies, optimisation and scale up

§ Disadvantage: complex establishment of plant suspension cultures in RAMOS and development of setup

§ Outlook: transformation experiments with callus and suspension cultures of sunflower with genetically modified *Agrobacterium tumefaciens*
à additional increase of α-Tocopherol yield

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[4] Dörmann, Peter (2007): Functional diversity of tocochromanols on plants. Planta 225: 269-276.

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