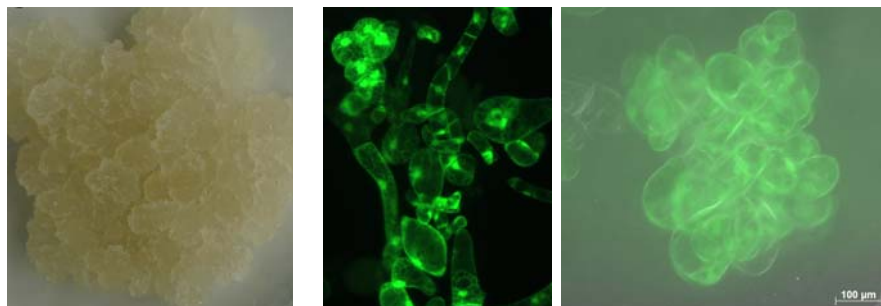


Bioreactor cultivation of Sunflower cells

Callus and suspension cultures for the biotechnological production of α -tocopherol



Callus of *Helianthus annuus*

Suspension of *H. annuus*, stained with FDA (fluor. diacetat, green: viable cells; fluorescence microscope)

The Sunflower as vitamin supplier:

For engineering, construction and optimisation of processes for the biotechnological in vitro production of secondary plant metabolites the **Annual Sunflower** (*Helianthus annuus* L.) was chosen as model system [1].

At the Chair of Bioprocess Engineering an untransformed **callus** cell line (totipotent cells) is already cultured successful for some years. Out of that callus a well and stable growing **callus suspension** culture was established [2].

To enhance the yield of the secondary metabolite **α -tocopherol** (vitamin E) transformation experiments with *Agrobacterium tumefaciens* are in progress. **Analysis** of the secondary plant compound is carried out using GC/MS with δ -tocopherol as internal standard. Previously, the protocol (extraction, derivatization, measurement) and the calibration of target product and internal standard were implemented.

- [1] Pavlov, Werner, Ilieva, Bley (2005): Characteristics of *Helianthus annuus* Plant Cell Culture as a Producer of Immunologically Active Exopolysaccharides, Eng. Life Sci. 5, No. 3.
[2] Haas, Weber, Ludwig-Müller, Deponte, Bley, Georgiev (2008): Flow Cytometry and Phytochemical Analysis of a Sunflower Cell Suspension Culture in a 5-L Bioreactor, Z. Naturforsch. 63c.

Plant cells in shaken bioreactors:

The **Respiration Activity MO**nitoring **S**ystem (RAMOS[®], HiTec Zang GmbH [3]) is used for a fast screening of in vitro plant cell suspensions and optimisation of the culture conditions.

This system enables the growth of plant cells in shaking flasks with online monitoring. With the help of a sensor in each bottleneck, total pressure and oxygen partial pressure in the flasks can be measured non-invasive. Furthermore, an internal calculation provides growth relevant data like **oxygen transfer rate** OTR or respiratory quotient RQ. Thus, predictions concerning cell growth, kinetics and phases are possible.

The heterotrophic growing plant cells metabolize sucrose and need additional **growth regulators** like the auxin 2,4-Dichlorophenoxyacetic acid to remain in the undifferentiated callus stage. So far, in the system tests with untransformed cell cultures of *H. annuus* have been examined e.g. by variation of substrate concentration.



RAMOS[®] including 8 shaking flasks for measurement, each with suspension of *H. annuus*

flask for measurement (left), standard flask (right), after [3]

- [3] Zang, Andrea (2010): HiTec Zang GmbH. Website <http://www.hitec-zang.de/>