Comparative Studies on the Proliferation of Sunflower Suspension Cells in Disposable Bioreactors

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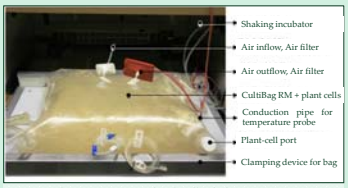
Introduction

The cultivation of plant cells continuously became more important in the last years due to a high amount of different substances which could be used by the pharmaceutical and cosmetic industry. However, during cultivation of plant suspension foam generation and shear stress occurs which may have negative effect on cell growth. In addition, cell growth, aggregation and the release of exopolysaccarides lead to a continuous change of the rheological behaviour, which requires constant process adjustment.

Besides STR's (stirred tank reactor), disposable (single-use) systems such as the Biostat CultiBag RM, which is well established in cultivation volumes of 2 to 20 L, are used for plant cell cultivation. By inducing an orbitally shaking motion or a wave-mixed motion, mixing is achieved and oxygen is transferred from the aerated reactor chamber into the culture medium.



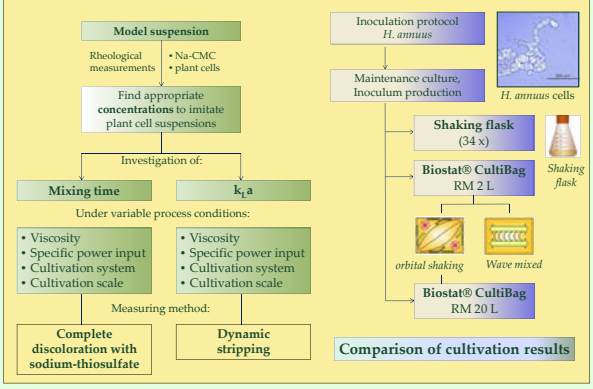
Biostat CultiBag RM 20 L with orbitally shaking motion to cultivate *Helianthus annuus* inside the shaking inkubator (Infors HT)

Objective

The cultivation of *Helianthus annuus* suspension cultures in single-use bioreactors of different culture volume should be adapted and established by determining process parameters. A comparison with the cultivation attempts conducted by the TU Dresden (Geipel, Werner) should point out the advantages and disadvantages of the culture systems concerning growth rate and foam generation.

Methods

Mixing time and **k_La-value** related to increasing viscosity during plant cell cultivation were determined by a **model suspension**. The **cultivation conditions** should be **kept constant** by adjusting the specific power input at constant k_La-value.



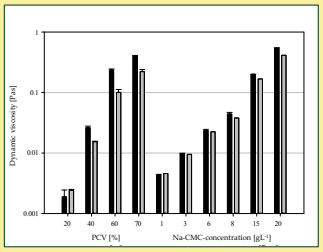
Results (1)

Rheological measurements:

- **Na-Carboxymethylcellulose** as a model suspension in concentrations ranging from 1 gL^{-1} to 20 gL^{-1}
- **BY2-supension-cells** (*N. tabacum*) with cell density ranging from 20 % to 70 % Packed Cell Volume (PCV)

Viscosity:

- Increasing dynamic viscosity with increasing cell number
- Na-CMC with concentrations ranging from 1 gL^{-1} to 20 gL^{-1} are useful to analyse cell densities ranging from 30 % to 70 % PCV



Dynamic viscosity [1] in different concentrated (s) plant cell suspensions and Na-CMC at shear rates ranging from 1 s^{-1} ■ to 10 s^{-1} ■

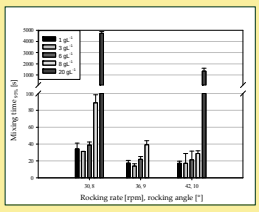
Results (2)

Mixing time

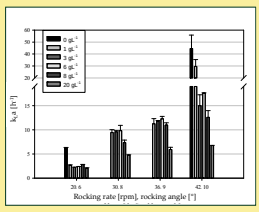
- **Increasing mixing time** with decreasing power input and increasing viscosity
- Significant influence of the medium viscosity in the bag reactor with Na-CMC conc. > 8 gL^{-1}
- Formation of **segregation gaps**

Oxygen transfer

- **Increasing k_La** with increasing power input and decreasing viscosity
- Significant influence of the medium viscosity in the bag reactor with Na-CMC conc. > 8 gL^{-1}
- Formation of **segregation gaps**



Mixing time with increasing viscosity (Na-CMC)[1] using the example of the wave mixed CultiBag RM 2L

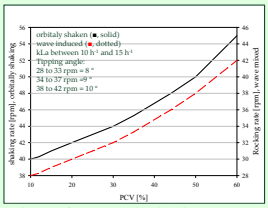


k_La with increasing viscosity (Na-CMC)[1] using the example of the wave mixed CultiBag RM 2L

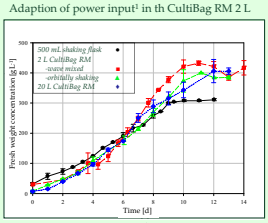
Results (3)

Adjustment of cultivation terms

- **Specific growth rate among** 0.25 d^{-1} und 0.3 d^{-1}
- **Maximum fresh weight** between 310 gL^{-1} (shaking flask) and 430 gL^{-1} (wave mixed CultiBag RM)
- Minimal **foam generation** due to surface aeration
- **Loss of volume** in the aerated bag reactor
- Comparable results for the fresh weight concentration in disposable bioreactors
- Accelerated rate of growth in the bag reactor (2 to 20 L) in comparison to the 4 l STR



Adaption of power input[1] in th CultiBag RM 2 L



Fresh weight concentration[1] during the cultivation of (n=2) *H. annuus* in Linsmeier Skoog media.

Results of the cultivation

Characteristic Variable	*Shaking flask (ZHAW*	*Shaking flask (TUD, Geipel)[2]*	*Biostat CultiBag RM*		
			2 l wave	*2 l orbital*	*20 l orbital*
$x_{DW\text{-}min}$ [gL^{-1}]	2,235 ± 0,601	1,815 ± 0,3	1,930 ± 0,000	1,390	0,290
$x_{DW\text{-}max}$ [gL^{-1}]	15,08 ± 1,471	14.581 ± 0,5	15,76 ± 0,419	13,91 ± 0,602	13,67 ± 0,981
$\mu_{max\text{-}DW}$ [d^{-1}]	0,294 ± 0,036	0,288 ± 0,0621	0,252 ± 0,007	0,262 ± 0,003	0,231 ± 0,052
$Y_{x/s}$ [g_{xDW}/g_s]	0,558 ± 0,024	-	0,789 ± 0,075	0,699 ± 0,077	0,511 ± 0,056

Characteristic variabless for the cultivation of *H. annuus* in different cultivation systems.
DW = calculated from dry weight concentration

Summary

- 1 gL^{-1} to 20 gL^{-1} Na-CMC as model solution for plant cell suspensions to determine mixing times and k_La-values for increasing viscosity.
- Adjustment of power input to the cell density at low fluctuating k_La leads to comparable maximum specific growth rate of approximately 0,3 d^{-1} for the cultivation of *H. annuus* in disposable bioreactors
- Cultivation of *H. annuus* in disposable bioreactors possible without process disturbing foam formation

Outlook

- Determination of more values for mixing time and k_La, especially in CultiBag RM 2 L and 20 L
- Adaption of specific power input to the viscosity of media
- Comparison of cultivation results between STR and disposable bioreactors in larger scales
- Investigations concerning shear stress in the CultiBag RM 2 L and 20 L

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Literature

1) Greulich, J. not published diploma thesis: "Vergleichende Untersuchungen zur Massenvermehrung von Sonnenblumenzellen in Suspensionskultur in Standard- und Einwegbioreaktoren"
2) Geipel, K.; not published measuring values for the cultivation of *H. annuus* in glas-shaking-flasks; ILBT-TUD, 2012

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