Development and characterisation of the orbitally shaken pilot scale single-use bioreactor SB10-X and growth characterisations of *Sf*9 cells

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INTRODUCTION

The demand for more flexible and faster protein production processes with greater profitability has driven the implementation of single-use bioreactor technology, based on lower capital costs (approximately 48 %) and reduction in installation/ start-up time [1,2].

Therefore the orbitally shaken single-use bioreactor SB10-X has been developed and characterised. The determined scale-up factor kLa was used to perform a scale-up with 3 orbitally shaken bioreactors (culture volumes: 60 mL, 1.5 L and 10 L). *Sf*9 insect cells haven been used as model organism for the growth characterisations in the bioreactors.

MATERIALS AND METHODS

Volumetric mass transfer coefficient k_La

Dynamic gassing out method. Aeration with air. DO measured with noninvasive oxygen sensors (Presens). The k_La value in the 250 mL shake flask was previously determined (data not shown).

Mixing times

Global discoluration method using iodometry: thiosulfate redox reaction with iodine and a starch indicator.

Volumetric electric power input

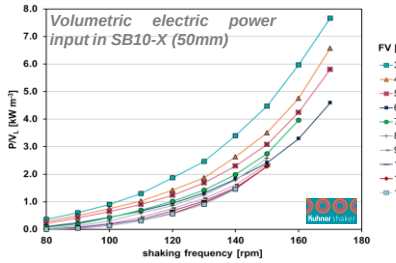
The power needed for shaking the water subtracted with the power of the corresponding amount of ice (resp. solid) divided by the infilled volume.

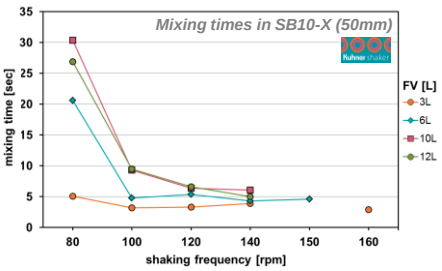
Growth characterisation

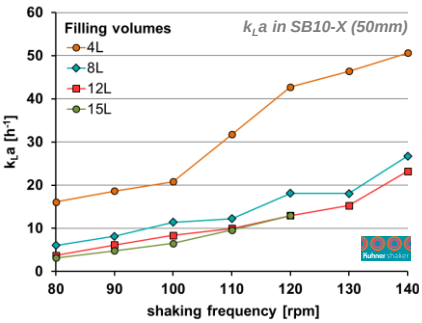
Parameter	Settings
Cell line	Spodoptera frugiperda (*Sf*9) by Invitrogen™
Medium	Serum free *Sf*-900™ III (Invitrogen™)
Bioreactors	250 mL and 3 L shake flask (Corning) and SB10-X (Adolf Kühner AG), working volumes: 60 mL, 1.5 L and 10 L.
Temperature	27 °C
Orbital shaking	250 mL: 100 rpm, 25 mm shaking diameter 3 L: 120 rpm, 50 mm shaking diameter 10 L: 100 – 110 rpm, 50 mm shaking diameter
Aeration	250 mL: indirect head space gassing with air 3 L: indirect head space gassing with air 10 L: 0.1 vvm with air
DO	Maintained above 50 – 80%
pH	No pH regulation
Scale-up	Scale-up factor k_La= 10 - 14 h^{-1}
Cell counting device	Cedex HiRes (Roche)
Analyser	BioProfile 100 Plus (Novo Biomedical)
Cultivation time	9 days

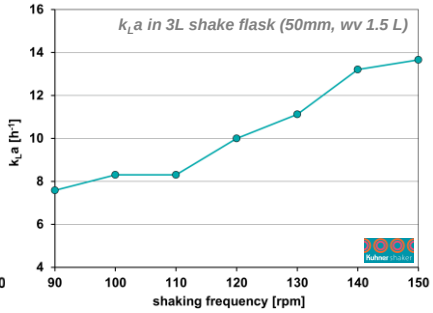
RESULTS AND DISCUSSION

Bioreactor characterisation

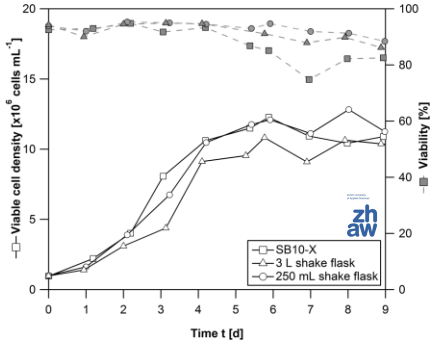


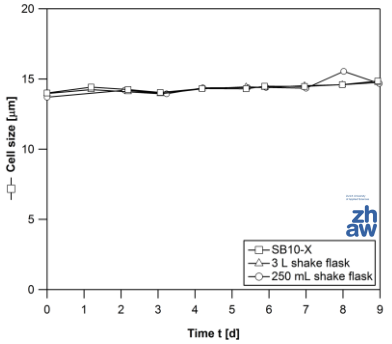






*Sf*9 growth characterisation





	250 mL shake flask	3 L shake flask	SB10-X
VCDmax [x10^6 cells mL^{-1}]	12.82	10.82	12.27
Growth rate µ [h^{-1}]	0.024	0.025	0.029
Doubling time [h]	28.3	29.0	23.6

- Cells entered the exponential growth phase after day 1 and grew uniformly until day 4
- Subsequently, cells reached the stationary and death phase with decreased viabilities (<90%)
- One exception is the viability seen for the SB10-X on day 7 revealing a non-typical viability decrease below 80% → temporarily oxygen overflow in the reactor leading to minor cell death
- VCDmax were comparable in all three shaken bioreactors

DISCUSSION

- The determined scale-up parameters show that the SB10-X is a suitable bioreactor for the cultivation of shear sensitive cells such as *Sf*9 cells
- Growth characterisations were successfully demonstrated
- VCDmax, growth rates and doubling times are comparable to literature data reported for wave-mixed and stirred cultivation systems
- Using the k_La value ensures successful scale-up from 60 mL to 10 L working volume in orbitally shaken single-use bioreactors with *Sf*9 suspension cells

LITERATURE

[1] Eibl, R., Steiger, N., Wellnitz, S., Vicente, T., et al., Fast single-use VLP vaccine productions based on insect cells and the baculovirus expression vector system: influenza as case study, in: Eibl, D., Eibl, R. (Eds.), Disposable Bioreactors II, Springer, Heidelberg 2014, pp. 99-125.
[2] Lopes, A. G., Single-use in the biopharmaceutical industry: A review of current technology impact, challenges and limitations. Food Bioprod. Process. 2015, 93, 98-114.

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