

# Development and characterisation of the orbitally shaken pilot scale single-use bioreactor SB10-X and growth characterisations of Sf9 cells



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## INTRODUCTION

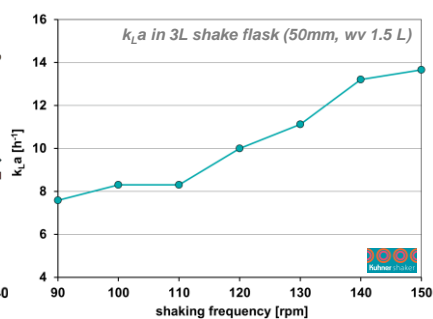
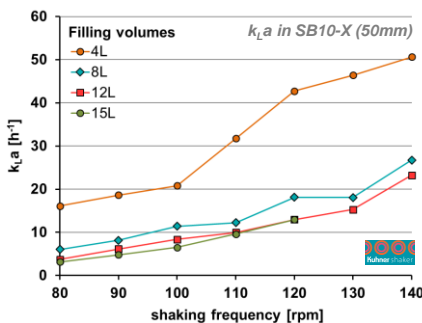
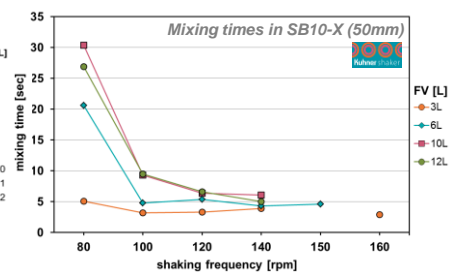
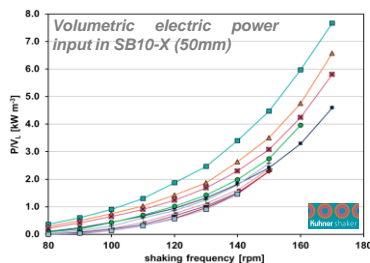
The demand for more flexible and faster protein production processes with greater profitability has driven the implementation of single-use bioreactor technology, based on lower capital costs (approximately 48 %) and reduction in installation/ start-up time [1,2].

Therefore the orbitally shaken single-use bioreactor SB10-X has been developed and characterised. The determined scale-up factor  $k_La$  was used to perform a scale-up with 3 orbitally shaken bioreactors (culture volumes: 60 mL, 1.5 L and 10 L). Sf9 insect cells have been used as model organism for the growth characterisations in the bioreactors.



## RESULTS AND DISCUSSION

### Bioreactor characterisation



## 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 discoloration method using iodometry: thiosulfate redox reaction with iodine and a starch indicator.

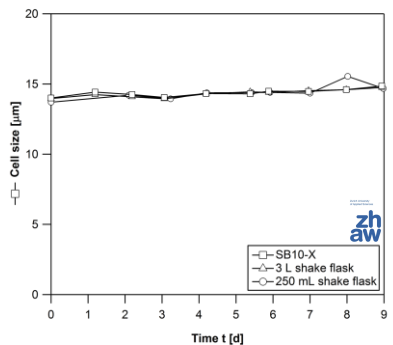
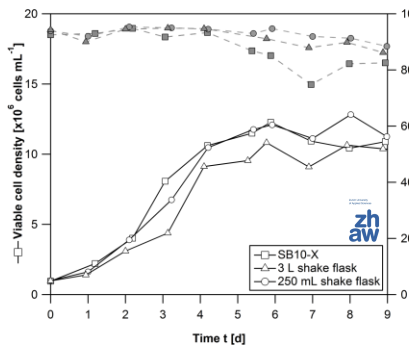
### Volumetric electric power input

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 (Sf9) 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 $k_La = 10 - 14 \text{ h}^{-1}$
Cell counting device	Cedex HiRes (Roche)
Analyser	BioProfile 100 Plus (Novo Biomedical)
Cultivation time	9 days

### Sf9 growth characterisation



	250 mL shake flask	3 L shake flask	SB10-X
VCDmax [ $\times 10^6$ cells $\text{mL}^{-1}$ ]	12.82	10.82	12.27
Growth rate $\mu$ [ $\text{h}^{-1}$ ]	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 Sf9 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 Sf9 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.