Increasing efficiency of baculovirus-based r-protein production in orbitally shaken single-use bioreactors

Ina Dittler[a], Britta Badertscher[a], Katharina Blaschczok[a], Tim Bürgin[b], Tibor Anderlei[b], Peter Neubauer[c], Dieter Eibl[a] and Regine Eibl[a]

[a] Zurich University of Applied Sciences, School of Life Sciences and Facility Management, Institute of Chemistry and Biotechnology, Switzerland
[b] Kühner AG, Switzerland
[c] TU Berlin, Department of Biotechnology, Germany

Zurich University of Applied Sciences

zh aw Life Sciences and Facility Management

BIOCHEMICAL ENGINEERING AND CELL CULTIVATION TECHNIQUES

Introduction

Insect cells used in conjunction with the baculovirus-expression vector system provide a suitable production system for the development and manufacturing of recombinant protein products such as virus-like particle vaccines [1-2]. In order to speed up product development and manufacturing, new strategies for efficient baculovirus-based production technologies need to be investigated. Among these is the implementation of single-use (SU) bioreactors, which have been proven as cost-effective (48% less capital cost) [3]. Further time- and cost-savings can be achieved by combining SU bioreactors with the titerless infected-cell preservation and scale-up (**TIPS**) method [4-5] as an alternative to the classical **2-phase production process (2PP)**. The TIPS method enables a faster, scalable, large-scale protein expression up to 100 L cell culture volume with cryopreserved, baculovirus-infected insect cells (BIIC) in a single scale-up step [4-5].

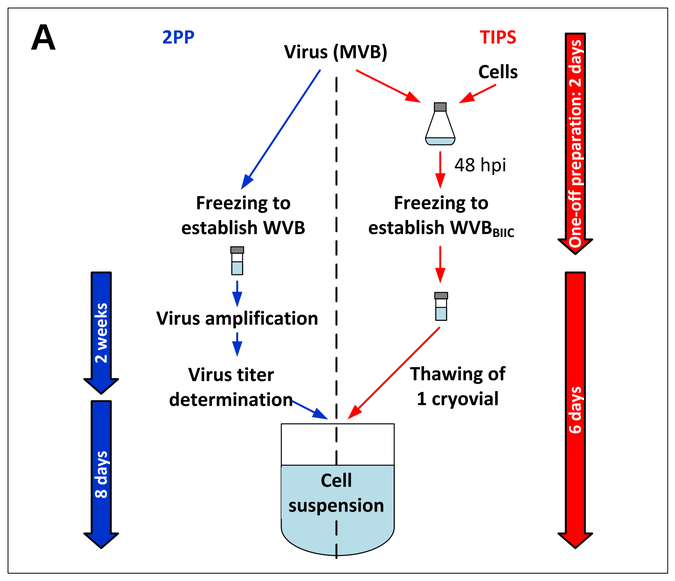

Fig. 1: Workflow of 2-phase production process and TIPS method (A), the cultivation systems used (125 mL (B), 3 L shake flasks (C), and SB10-X (D)).

Methods

	2PP	TIPS
Cell line:	*Spodoptera frugiperda* (*Sf*9)	
Expressed protein:	recombinant secreted alkaline phosphatase (rSEAP)	
Bioreactor:	125 mL and 3 L shake flasks, SB10-X (V = 30 mL, 1.5 L, 10 L)	
Cell counting device:	Cedex HiRes	
Analyzer:	BioProfile 100 Plus	
Scale-up parameter:	$k_La = 14\ h^{-1}$	
Virus:	rAcMNPV from WVB	rAcMNPV from WVB$_{BIIC}$
Cultivation time:	1. Growth phase: 2 days 2. Production phase: 6 days	Production phase: 6 days

Results

- **Infection studies using the 2-phase production process and TIPS method were successfully performed in orbitally shaken SU bioreactors** 125 mL and 3 L shake flasks, and SB10-X (see Fig. 2A).

- Maximum **viable cell densities (VCD_{max}) of 4.5-6.2x10^6 cells mL^{-1}** were obtained on day 2 post infection (pi).

2PP:

- 2 days after inoculation the cell suspension was diluted to introduce the production phase by adding the virus (**1. Phase: growth, 2. Phase: production**)
- **Cell sizes increased up to 12-14.5 %.**
- **Maximal rSEAP activities of 112-126 U mL^{-1}** were obtained on day 6 pi.

TIPS:

- The production process was directly initiated **without growth phase.**
- **Cell sizes increased up to 16-17 %.**
- **Maximal rSEAP activities of 164-168 U mL^{-1}** were obtained on day 5 pi.

Pros of the TIPS method:

1. **34% higher rSEAP activities**
2. **Production process reduced from 10 days to 6 days**

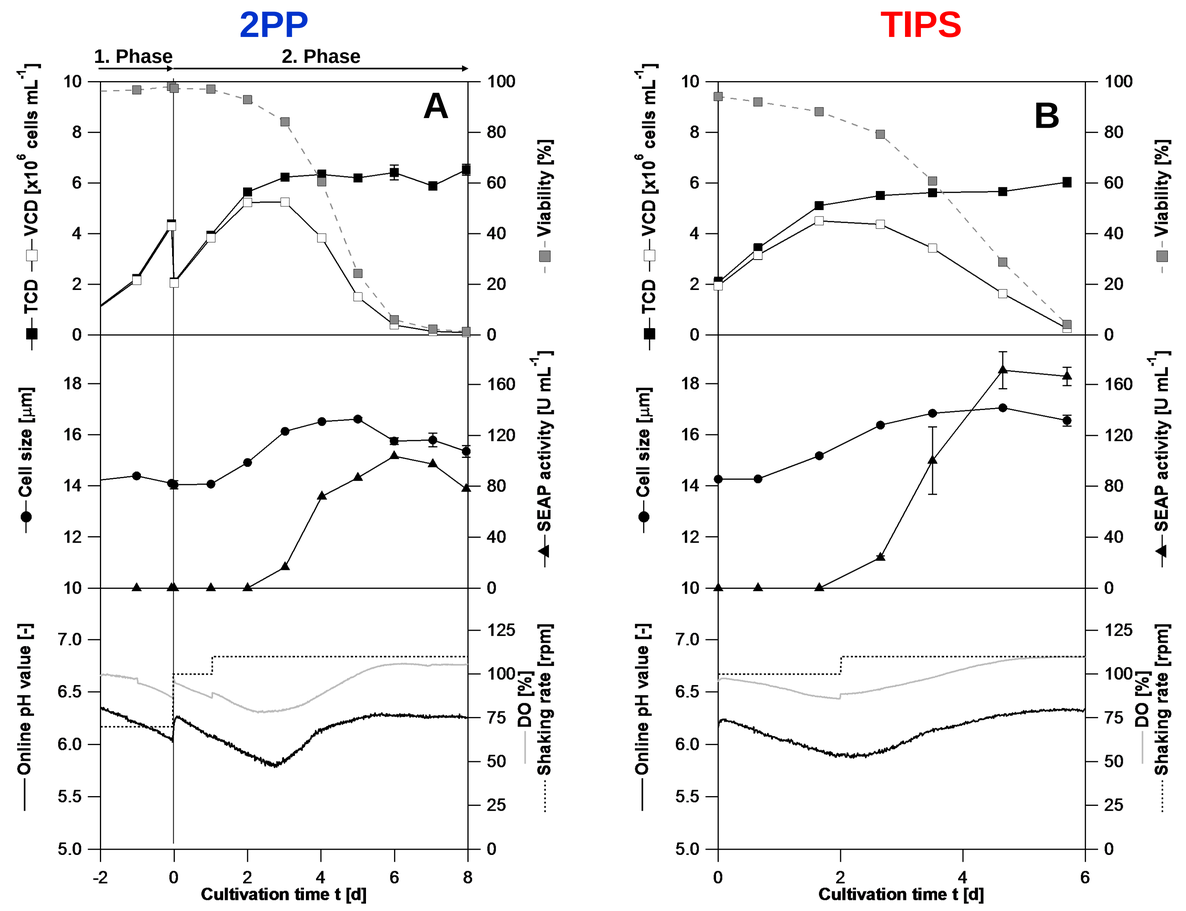

Fig. 2: Infection studies in SB10-X using the 2-phase production process (A) and the TIPS method (B).

Process	System	VCD_{max} [x10^6 cells mL^{-1}]	Cell-size increase [%]	SEAP activity [U mL^{-1}]
2PP	125 mL shake flask	6.16	11.54	111.96 ± 4.66
	3 L shake flask	6.10	13.49	125.63 ± 14.89
	SB10-X	5.30	14.33	125.68 ± 32.08
TIPS	125 mL shake flask	5.93	15.80	168.14 ± 6.05
	3 L shake flask	4.91	15.90	167.10 ± 15.40
	SB10-X	4.50	16.77	163.89 ± 10.40

Tab. 1: Comparison of both 2-phase production process and TIPS method in 125 mL and 3 L shake flasks and SB10-X.

Conclusion

Combining the TIPS method with orbitally shaken SU bioreactor technology was successfully demonstrated at mL- and L-scale. Compared to the 2PP-method, the efficiency of the production process was enhanced by using the TIPS method.

Acknowledgement: The authors would like to thank Prof. Dr. Martin Sievers (Zurich University of Applied Sciences, Switzerland) for providing the recombinant baculovirus.

[1] Eibl, R., Steiger, N., Wellnitz, S., Vicente, T., John, C., Eibl, D., Fast single-use VLP vaccine productions based on insect cells and the baculovirus expression vector system: influenza as case study. Disposable Bioreactors II, *Springer*, 99-125, 2014.
[2] Reid, S., Chan, L., van Oers, M.M., Production of entomopathogenic virus. Mass Production of Beneficial Organisms, *Elsevier*, 437-482, 2014.
[3] Lopes, A., Single-use in the biopharmaceutical industry: A review of current technology impact, challenges and limitations, *Food and Bioproducts Processing*, 93, 98-114, 2015.
[4] Wasilko, David J., et al., The titerless infected-cells preservation and scale-up (TIPS) method for large-scale production of NO-sensitive human soluble guanylate cyclase (sGC) from insect cells infected with recombinant baculovirus. *Protein expression and purification,* 65, 122-132, 2009.

Contact:
ZHAW, School of LSFM,
Ina Dittler
Grüental, CH-8820 Wädenswil
Mail: ina.dittler@zhaw.ch