

Cultivation of entomopathogenic fungi in orbitally shaken bioreactors – Investigation of respiratory activity in scale-up experiments

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Introduction

Scale-up implementations are an ongoing challenge in industrial scale biotechnological production processes that include biological pest control. From the laboratory concept to the production plant, a pilot scale is generally necessary to gain a better understanding of the process and to identify potential challenges. Bioengineering parameters, such as oxygen transfer, mixing time and power input, are usually used to determine important scale-up factors. In addition, process control that employs off-line, at-line or on-line measurements are also performed [1]. Analysis of cellular respiration provides improved understanding of cell metabolism and allows the cultivation state to be determined online. Thus, prompt action can be taken if there are sudden changes due to stress, deprivation or contamination, resulting in minimal losses. Additionally, off-line measurements can be implemented for automated process control strategies in fed-batch or continuous cultivations [2, 3].

Material and Methods

- Batch cultivations of *Metarhizium brunneum* in 500 mL shake flasks and a single use OrbShake system SB10-X (Kuhner Shaker) were performed.
- Basic medium supplemented with ground barley at 200 mL and 6 L working volumes, respectively, were utilized.
- Working volume and cultivation parameters were set to values that result in similar theoretical oxygen transfer rates (OTR).
- No regulation of pH or dissolved oxygen.
- Data were obtained using off-line gravimetric dry mass, HPLC and on-line exhaust gas (KuhnerTOM and BlueInOne) measurements.

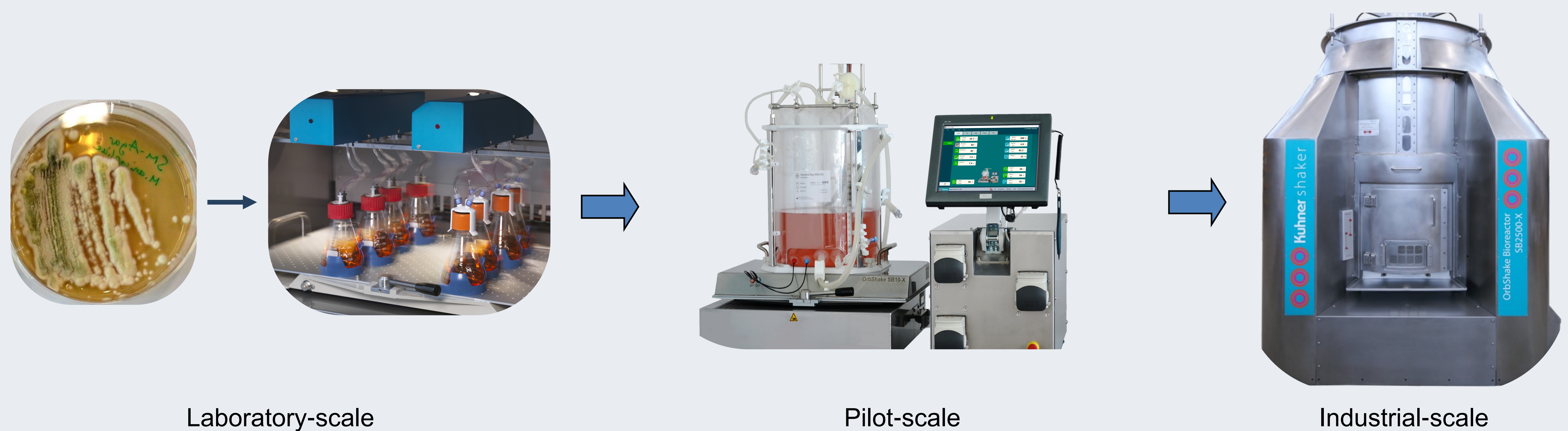


Figure 1: Scale-up flow chart of the batch cultivation biotechnological production processes.

Inoculum production was prepared in petri dishes for both, lab-scale and pilot-scale cultivations, at a spore density of $1 \cdot 10^5$ spores per mL cultivation volume. Daily sampling was performed for biomass and total sugar determination. Off-gas measurements were recorded using KuhnerTOM for lab-scale and BlueInOne for pilot-scale. Utilizing the same procedure, industrial scale production can be performed.

Results and discussion

- High reproducibility was achieved in shake flasks and comparable respiratory and sugar consumption profiles were observed in the SB10-X.
- Off-line biomass determination in the pilot-scale SB10-X is not reproducible due to high viscosity of the culture broth and unsuitable sampling port for this cultivation type as the system is designed for mammalian cell culture.
- OTR and CTR data indicate an exponential growth phase 1, a limited growth phase 2 and a stationary phase 3 in both scales.
- Maximum carbon dioxide transfer rates (CTR) of $3.89 \text{ mmol} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ at 113.9 h and $5.96 \text{ mmol} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$ at 51.9 h were achieved in shake flasks and the SB10-X system, respectively.
- The sum of carbon dioxide produced in shake flasks and the SB10-X system resulted in $563.0 \text{ mmol} \cdot \text{L}^{-1}$ and $532.9 \text{ mmol} \cdot \text{L}^{-1}$, respectively, after 240 h of cultivation. Sugar depletion occurred after 163.5 h and 189.5 h of cultivation, respectively.

Conclusion

We present a reliable process monitoring strategy that can be easily applied in lab- and pilot-scale cultivation processes, with simplified process monitoring and contamination detection. The indications given by the OTR and CTR profile regarding the cultivation state, can be used for tailored control strategies in fed-batch or continuous cultivations over various scales. Differences in process progression due to scale-up phenomena can be detected and optimisation strategies designed. Understanding the respiratory activity of entomopathogenic fungi during cultivation enables further improvement of production processes and makes the commercialisation of biological pest control agents economically more attractive.

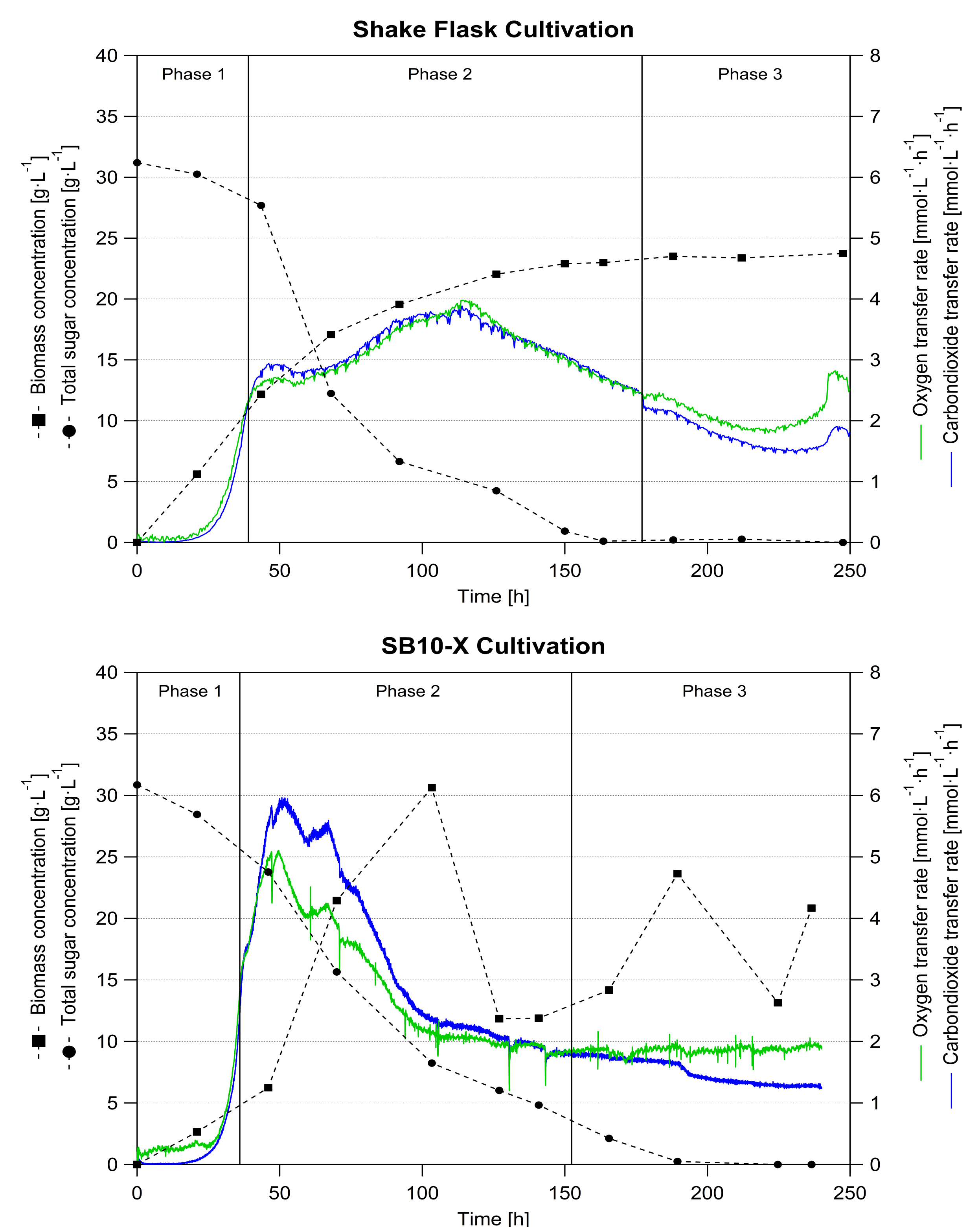


Figure 2: Biomass, total sugar, OTR and CTR profiles for the cultivation in shake flasks and SB10-X.

Comparison of off-line and on-line measurements for lab- and pilot-scale cultivation. The OTR and CTR online profiles and biomass and sugar offline measurements are shown.

References

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