National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Vero cell growth in the CELL-tainer® disposable bioreactor

Introduction

Rapid and simple cell cultivation can currently be carried out using disposable bioreactors. Various reports have described growth of adherent cells in rocking-type bioreactors which rely on the rocking motion for both mixing and mass transfer. The CELL-tainer® (CELLution Biotech BV) disposable bioreactor (CT) is a rocking motion-type bioreactor which not only has vertical movement (like the BIOSTAT® CultiBag (Sartorius Stedim Biotech) (RM)) but horizontal displacement as well (Figure 1). For the RM Vero cell cultivation protocols have been described previously (*1*, *2*). To transfer the cultivation protocol from the RM to the CT the mixing characteristics of both bioreactors were determined.

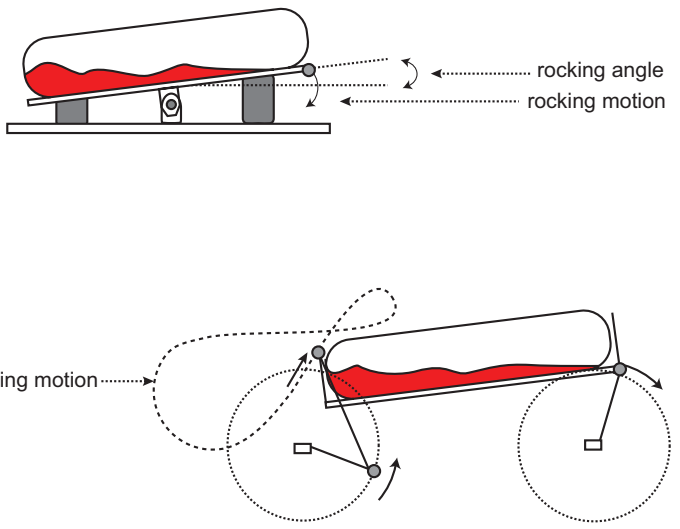

Figure 1. Schematics of the motions of rocking motion type bioreactors. A: the Cultibag (RM) bioreactor (figure adapted from (3)); B: the CELL-Tainer (CT) bioreactor (figure adapted from (4))

Results

To develop models for mixing times and microcarrier homogeneity in the CT and RM a design of experiments approach was chosen. A response surface model design (CCF) was setup. The resulting model predictions are given in Figure 2. Applied conditions for Vero cell growth in the RM were angle 8° and rocking speed 16 rpm. These conditions correspond with a mixing time of 89s (Figure 2A) and microcarriers that are in suspension (Figure 2C). Based on comparable mixing times and microcarrier homogeneity, the settings for cultivation of Vero cells in the CELL-tainer® bioreactor were chosen. For 3L cultivation in the CT a predicted mixing time of 88s can be obtained by applying a vertical movement of 30% and a rocking speed of 15 rpm (Figure 2B). In addition the horizontal displacement should be 40% as was based on the model for microcarrier homogeneity (Figure 2D).

Vero cells growing adherent to Cytodex 1 microcarriers were cultivated in the RM and CT. Cells were grown in animal component free (ACF) media. Under controlled (T; pH; DO) conditions Vero cell culture in both bioreactors was comparable with respect to the growth characteristics and main metabolite production and consumption rates (Table 1).

Table 1. Growth characteristics and metabolic rates for adherent Vero cell growth in a conventional rocking motion bioreactor (RM) and in the CELL-Tainer (CT).

	CultiBag (RM)	CELL-tainer® (CT)
start cell concentration (10^6 cells.mL^{-1})	0.2	0.2
cell concentration at 95h (10^6 cells.mL^{-1})	0.8	0.9
µmax (h^{-1})	0.017	0.027
accumulation of lactate (mM)	26	27
accumulation of ammonia (mM)	2.3	2.0
q_{Glc} (mmol.10^9 cells^{-1}.h^{-1})	0.5	0.5
q_{Gln} (mmol.10^9 cells^{-1}.h^{-1})	0.1	0.1
q_{Lact} (mmol.10^9 cells^{-1}.h^{-1})	1.0	1.3
q_{NH3} (mmol.10^9 cells^{-1}.h^{-1})	0.1	0.1
$Y_{Lact/Glc}$	1.9	2.0
$Y_{NH3/Gln}$	1.0	1.0

Vero cells grown in the RM or CT were infected with polio virus to assess virus production capabilities of cells grown in these rocking motion type bioreactors. Cells were infected at the end of the exponential phase using a multiplicity of infection of 0.01. Virus culture was complete when 100% cytopathic effect was observed. Results (Table 2) show that virus production capabilities in both bioreactors are comparable.

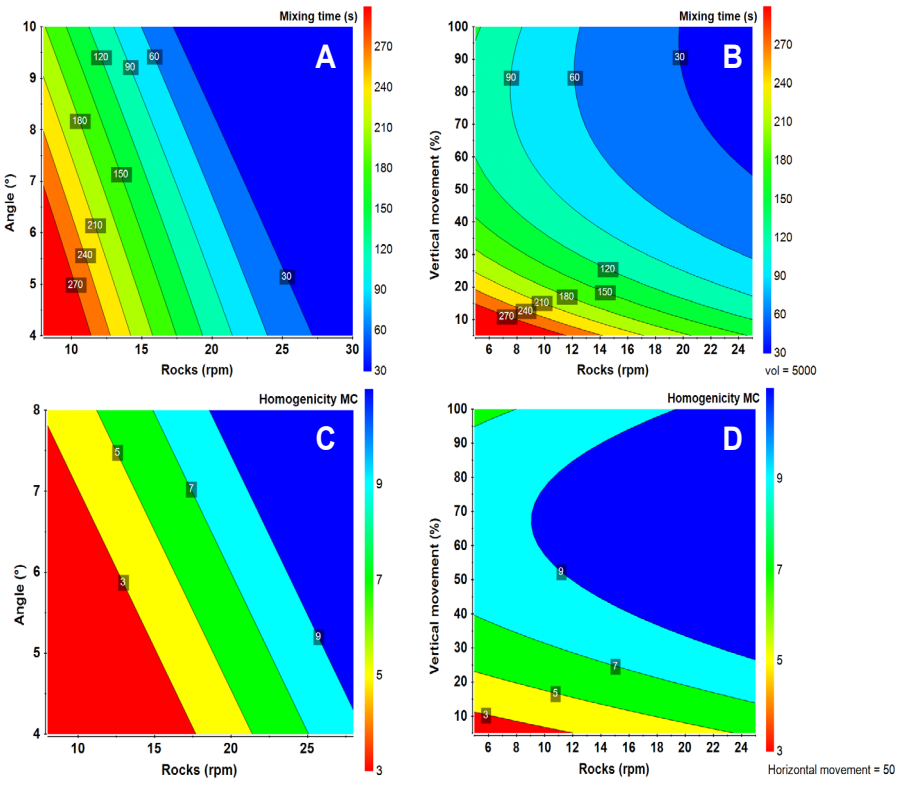

Figure 2. Model predictions for mixing times based on pH (A&B) and microcarrier homogeneity (C&D) in the Cultibag (RM) (A&C) and CELL-Tainer (CT) (B&D). From these models the settings for the Vero cell growth in the CELL-tainer were derived.

Table 2. Polio virus (Sabin type 1) production in rocking motion type bioreactors

	Cell concentration at infection (10^6 cells.mL^{-1})	Virus culture time (h)	Virus titer (10log $TCID_{50}$.mL^{-1})	D-antigen content (DU.mL^{-1})
Cultibag (RM)	0.81	76	8.3	75
CELL-Tainer (CT)	0.80	72	8.3	77

Conclusions

The applied strategy to select cultivation conditions for implementation of a new rocking-type bioreactor based on comparable mixing times was successful. The CELL-tainer® supports growth of Vero cells adherent to microcarriers comparable to conventional rocking-type bioreactors.

References

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Published by
National Institute for Public Health and the Environment
P.O. Box 1 | 3720 BA Bilthoven
www.rivm.com

002486