Supplementary File S1

"Towards Sustainable Carotenoid Bioproduction: An Exploratory Agent-Based Model of *Rhodotorula rubra* ICCF 220 in Batch Cultivation"

1. Description

This supplementary material provides the complete NetLogo multi-agent model developed to simulate batch cultivation of *Rhodotorula rubra* ICCF 220, including glucose consumption, biomass development, physiological state transitions, and carotenoid accumulation.

The model was developed using NetLogo 7.0.3 and is provided in .nlogox format.

2. Model Contents

The NetLogo file includes:

Initialization of 100 super-individual agents.

Glucose consumption and biomass growth calculations.

Physiological state transitions between active growth, transition, and stationary accumulation.

Carotenoid accumulation calculations.

Calibration procedures for glucose consumption, physiological thresholds, and carotenoid accumulation.

Sensitivity analysis of physiological phase factors.

Graphical visualization of simulation outputs.

3. Instructions for Reproducing the Simulation

Open the .nlogox file using NetLogo 7.0.3.

Click **Setup** to initialize the simulation.

Click **Calibrate glucose** to execute the calibration workflow, including glucose consumption, physiological thresholds, and carotenoid accumulation.

Allow the calibration procedure and final simulation to finish. The final simulation covers 120 h, with one tick representing one hour.

After calibration, click Sensitivity analysis to run the nine physiological phase-factor scenarios. The baseline configuration is restored when the analysis finishes.

Examine the numerical outputs and graphical results displayed in the NetLogo interface.

The glucose calibration uses eight experimental measurements at 0, 10, 14, 18.5, 24, 48, 74, and 120 h. The carotenoid calibration target is 52.39 μg/g dry biomass at 120 h. The experimental glucose concentration at 120 h was 2.97 ± 0.05 g/L (mean ± SD of triplicate determinations).

4. Reference Outputs

The calibrated baseline simulation produced the following reference outputs:

Parameter	**Value**
Glucose consumption coefficient	0.0212 h^{-1}
Glucose fitting RMSE	1.8345 g/L
Glucose fitting R^2	0.9715
Predicted specific carotenoid content at 45 h	0.904 μg/g
Predicted specific carotenoid content at 90 h	15.791 μg/g
Predicted specific carotenoid content at 120 h	52.39 μg/g

The carotenoid content of 52.39 μg/g dry biomass was specified in the model as the experimental calibration target at 120 h. The values predicted at 45 and 90 h are simulation outputs and should not be interpreted as independently validated experimental measurements.

The reported values correspond to the baseline simulation obtained with the supplied model and calibration dataset. Glucose calibration statistics were independently recalculated from the eight experimental mean values. The carotenoid values listed above were confirmed from the NetLogo interface following the 120 h calibration run.

5. Reproducibility

The complete NetLogo source code, model parameters, calibration algorithms, physiological state transition rules, and graphical interface are provided to facilitate independent inspection and reproduction of the computational results.

The accompanying model file allows researchers to examine the implemented equations, reproduce the calibration workflow, visualize the simulated glucose and carotenoid profiles, and explore the effects of model parameters.

The supplementary material is intended to improve the transparency, accessibility, and reproducibility of the multi-agent modeling approach.