

Cultivation of *Saccharomyces cerevisiae* CEN.PK113-7D in small-scale customizable single-use bioreactors

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ABSTRACT

Single-use bioreactors have become a widely established technology in bioprocesses development and commercial manufacturing fields because of the advantages they have over reusable bioreactors with respect to lower initial investments, faster setup and reduced cross-contamination risks. In this work the small-scale customizable single-use bioreactors from Getinge (AppliFlex ST) were tested. The strain *Saccharomyces cerevisiae* was cultivated in a 0.5 L microbial configuration version of this bioreactor and in a same-sized autoclavable microbial a same-sized autoclavable microbial configuration (Applikon 500 mL), and the growth in both was compared. The culture growth has been monitored by offline measurement of the optical density at 600 nm (OD₆₀₀), and the CO₂ concentration in the off-gas from both bioreactors. The results obtained show that the cultivation of *Saccharomyces cerevisiae* CEN.PK113-7D in the AppliFlex ST and Applikon bioreactors displays similar trends for the growth profile, CO₂ off-gas measurements, temperature, pH, and dissolved oxygen (dO₂). In conclusion, the AppliFlex ST can successfully be used for the cultivation of *Saccharomyces cerevisiae* CEN.PK113-7D.

MATERIALS AND METHODS

- The strain *Saccharomyces cerevisiae* CEN.PK113-7D used in this study was kindly donated by the Delft University of Technology. Shake flask cultures were grown at 30 °C in 500 mL flasks containing 100 mL YPD medium (deminerlized water, 10 g·L⁻¹ Bacto yeast extract, 20 g·L⁻¹ Bacto peptone, 20 g·L⁻¹ glucose, Carl Roth GmbH, Karlsruhe, Germany) in an incubator set at 200 rpm and 30 °C (Kuhner, Birsfelden, Switzerland). Frozen stocks were prepared by addition of glycerol (30% v/v) to exponentially growing shake-flask cultures of *S. cerevisiae* and stored aseptically in 1 mL aliquots at -80 °C.
- The 0.5 L single-use AppliFlex ST and autoclavable glass Applikon 500 mL, as presented in Figure 1 (Getinge Applikon, Delft, The Netherlands), were used to grow *Saccharomyces cerevisiae* CEN.PK113-7D, under the same culture conditions: 30 °C and pH 5.0 under aerobic conditions.
- The bioreactor configuration consisted of 2 rushton impellers, 1 L-sparger for the gas inlet, 1 gas outlet with condenser, 1 sampling pipe, several addition ports and a temperature pocket. Both vessels used the autoclavable AppliSens pH and dO₂ 8 mm mini sensors (Metroglas AG, Switzerland). In the autoclavable glass Applikon 500 mL the sensors were autoclaved together with the bioreactor, whereas in the single-use, the sensors were autoclaved separately and assembled afterwards on the vessel inside a laminar flow cabinet (KOJAIR, Vilppula, Finland).
- The my-Control was used to control the process. The temperature was two-sided controlled using a Peltier element, the pH was two-side controlled at 5.0 by the addition of base (2M KOH) and acid (1M H₂SO₄). The dissolved oxygen was kept above 40% by the aeration of the bioreactor at 2 vvm.
- In order to monitor the cell growth, samples were taken to measure the OD₆₀₀ with a spectrophotometer (Jenway 7200, Fisher Scientific, Leicestershire, UK). The maximum specific growth rates (μ_{max}) in the glucose phase were calculated via linear regression of the natural logarithm of at least seven OD₆₀₀ measurements.
- In addition to offline OD samples, the cell growth was also monitored online using CO₂ off-gas measurements done with a BlueSens BCP CO₂ sensor (BlueSens GMBH, Germany).



Figure 1. AppliFlex ST and Applikon (Applikon Getinge).

RESULTS

Growth rate

The growth rate of the strain was in the same range for both bioreactors as shown in Table 1.

1	<i>Saccharomyces cerevisiae</i> CEN.PK113-7D	μ _{max} (h ⁻¹)
	Applikon	0.48
	AppliFlex ST	0.47

Table 1. Growth rate of *Saccharomyces cerevisiae* CEN.PK113-7D in the Applikon and AppliFlex ST.

References

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CO₂ PROFILE

The off-gas CO₂ concentration profile in both bioreactors was similar, as can be observed in Figure 2. The first increasing CO₂ production phase results from glucose uptake and reaches a maximum value at around 9.5 h of the batch phase. The second CO₂ production phase occurs at around 12h and is due to the consumption of other nutrients. The differences in the values could be attributed to the use of different medium batch and different pre-cultures. In this regard, more experiments will be performed.

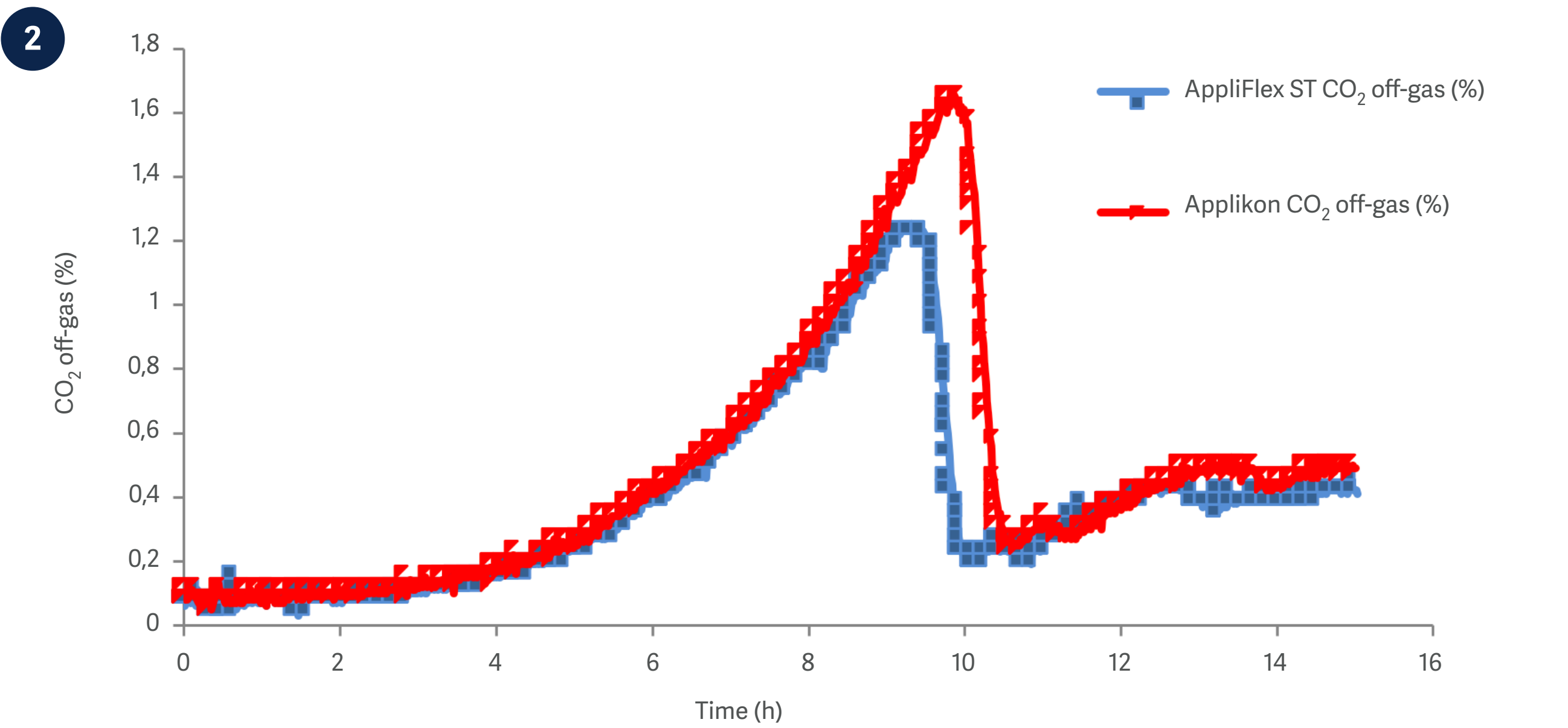


Figure 2. CO₂ off-gas profile of *Saccharomyces cerevisiae* CEN.PK in YPD in the Applikon and AppliFlex ST.

PARAMETER CONTROL

dO₂ and pH control

The dO₂ and pH trends showed the same profiles in the Applikon as well as the AppliFlex ST, as can be seen in Figure 3. pH was properly controlled at 5.0 in both bioreactors during the whole process. dO₂ was always held above 40% to prevent any O₂-deficiency that might invalidate the measurements.

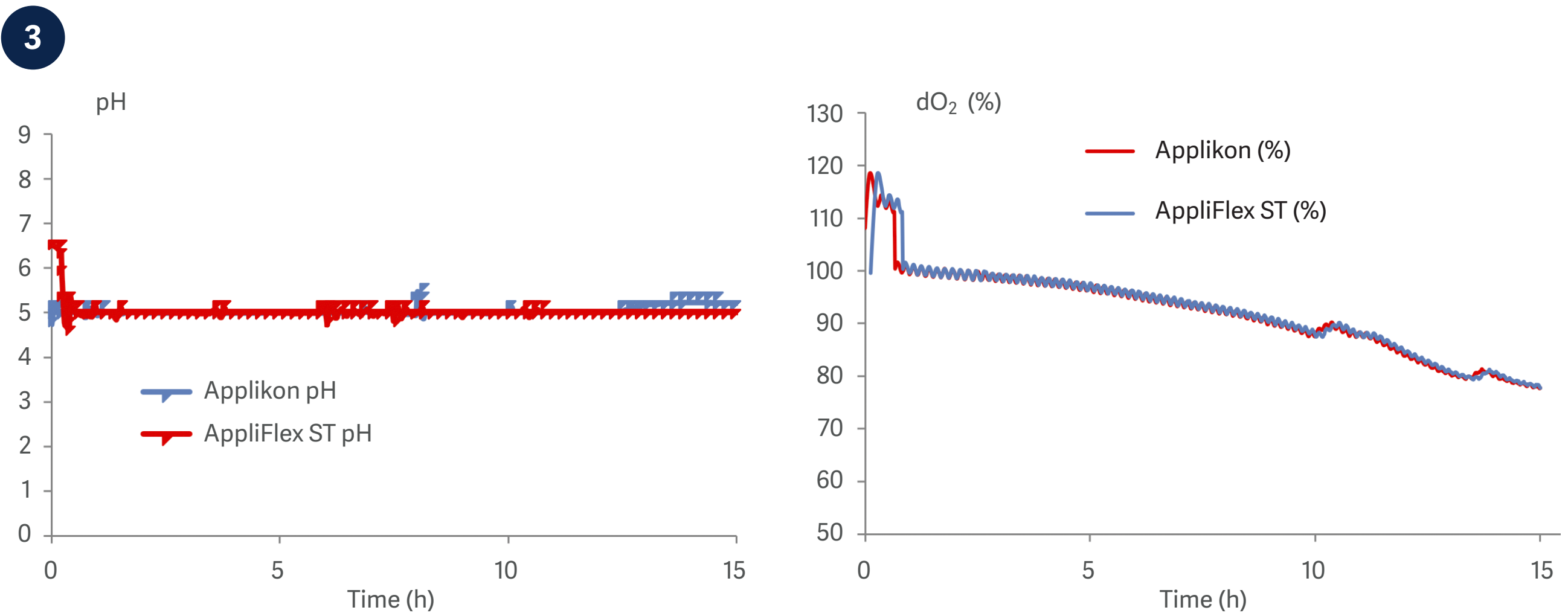


Figure 3. pH and dO₂ control profiles of *Saccharomyces cerevisiae* CEN.PK in YPD in the Applikon and AppliFlex ST.

TEMPERATURE CONTROL

A Peltier element held the temperature at 30 °C. As shown in Figure 4, the temperature was properly controlled in both the Applikon glass vessel and the AppliFlex ST plastic vessel.

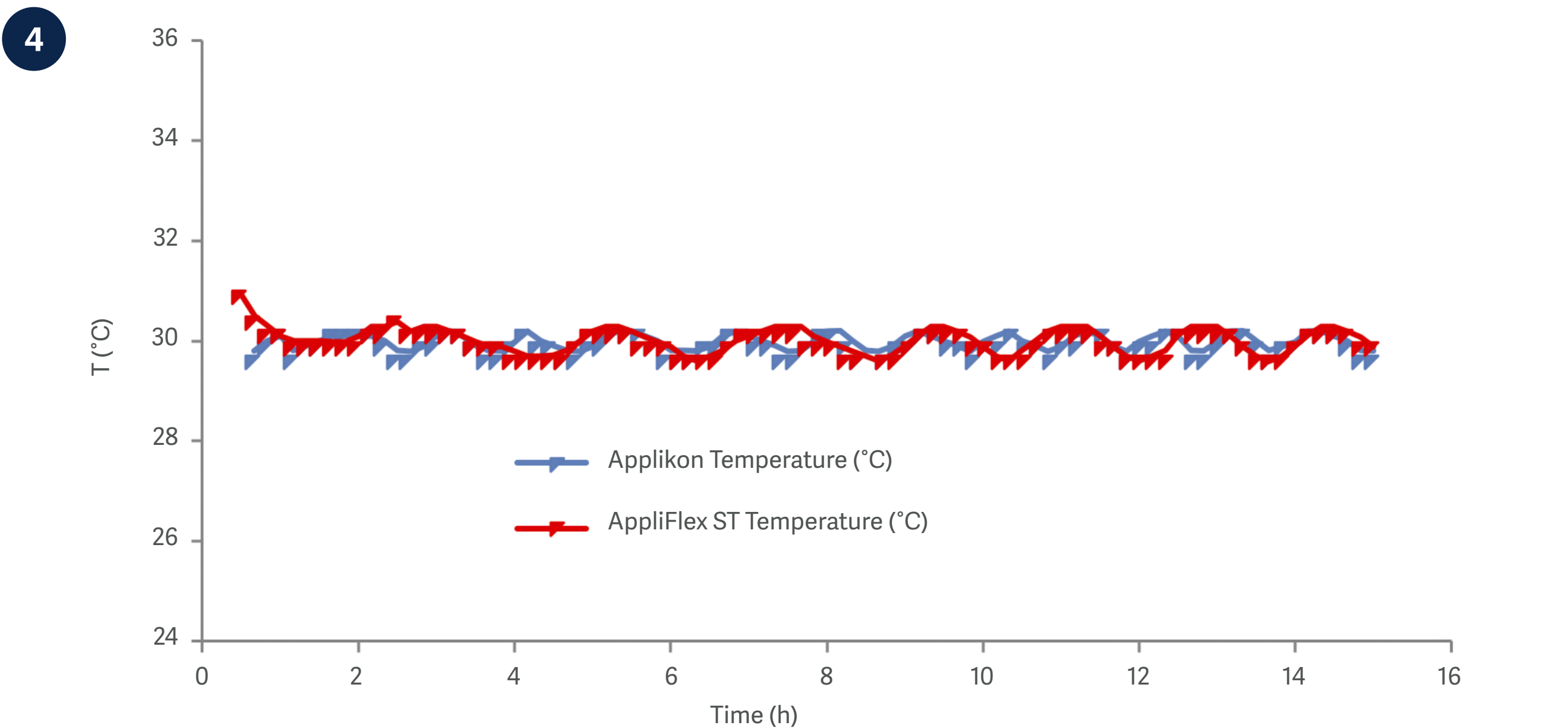


Figure 4. Temperature control profile of *Saccharomyces cerevisiae* CEN.PK in YPD in the Applikon and AppliFlex ST.

CONCLUSIONS

- The strain *Saccharomyces cerevisiae* CEN.PK was successfully grown in the Applikon and Appliflex ST with proper control of dO₂ (above 40%), pH (5.0) and T (30 °C).
- Saccharomyces cerevisiae* CEN.PK showed similar growth rates and CO₂ off-gas profiles in both the Applikon and Appliflex ST reactors.
- AppliFlex ST can be used for the cultivation of *Saccharomyces cerevisiae* CEN.PK113-7D; when comparing the growth profile of the strain, cultivation showed similar growth trends in both the Applikon and the AppliFlex ST single-use bioreactor.