HyPerforma Glass Bioreactor for fermentation of *Saccharomyces cerevisiae*

Summary

Here we describe the cultivation of *Saccharomyces cerevisiae* in the Thermo Scientific™ HyPerforma™ Glass Bioreactor with a maximum working volume of 2.0 L. Using the chemically defined medium D, it was possible to produce up to 5.33 g/L of dry biomass using 40 g/L glucose. The cells were grown under aerobic conditions with a specific growth rate of up to 0.4/hr and a yield coefficient for the biomass of 0.13 gCDW/gGluc.

Introduction

The stirred HyPerforma Glass Bioreactor has been designed for mammalian cell culture and microbial fermentation in benchtop-scale applications. The bioreactor, with a maximum working volume of 2.0 L, can be controlled with the Thermo Scientific™ HyPerforma™ G3Lab™ Controller in conjunction with the Thermo Scientific™ TruBio™ software powered by the Emerson™ DeltaV™ system. The industry-standard, cGMP-validated software offers state-of-the-art graphics, database management, and real-time control algorithms. It also includes preconfigured parameters for controlling bioprocesses such as pH, dissolved oxygen (DO), temperature, and agitation.

The yeast *S. cerevisiae* can metabolize glucose both aerobically and anaerobically, although the chosen method does affect the efficiency of metabolization [1]. Aerobic metabolism requires sufficient mixing and aeration.

The scope of the study was to demonstrate the viability of the bioreactor for growing a yeast strain under aerobic conditions. The combination of a modified Rushton turbine (bottom-mounted) and a three-bladed segment impeller with a blade angle of 45° (top-mounted) was tested to examine any effect this might have on oxygen mass transfer and cell growth. Some modifications were compared to standard mammalian cell cultivation processes. To improve mixing, three baffles were used. A cooling coil (cold finger), controlled via an external thermostat, was installed to reduce the temperature inside the vessel to counteract excessive heat production.

Figure 1. The HyPerforma Glass Bioreactor, and HyPerforma G3Lab Controller with TruBio software.

Materials and methods

Medium

The chemically defined medium D with a glucose concentration of 40 g/L was used for both inoculum production and cultivation [2]. During the cultivation process, emulsion C (Sigma-Aldrich, 10 g/L) was added as an antifoam agent.

Inoculum preparation

The inoculum production was performed in baffled shake flasks (5 x 500 mL total volume). Several single colonies of *S. cerevisiae* were transferred from agar plates (casein soyabean digest agar) into the shake flasks by use of an inoculation loop. The flasks were incubated in a shaking incubator (Infors HT, Switzerland) at 34°C and 140 rpm (50 mm amplitude) for about 20 hours.

Bioreactor preparation

The pH sensor was calibrated using pH 4.01 and pH 7.0 buffers (Mettler Toledo, Germany). DO and pH probes were installed in the vessel and it was filled with 800 mL PBS before sterilization (30 min, 121°C). After sterilization, the DO probe was calibrated for 0 and 100% air saturation. Subsequently, the bioreactor was placed in a laminar flow cabinet and two bottles, one with a sterile NaOH solution (1 M) and the other with an antifoam agent (Emulsion C, 10 g/L), were connected to the bioreactor via luer lock connections. The PBS solution used for sterilization was removed by means of a peristaltic pump and the bioreactor was then filled with 1.9 L of fresh medium. A single-use Clave™ sampling port was also installed.

Inoculation procedure

To achieve the desired initial biomass concentration of 0.5 g/L dry biomass, the cell suspensions from the shake flasks were pooled in four sterile plastic bottles with total volumes of approximately 200 mL, before being centrifuged for 15 minutes at 5,000 rpm (Eppendorf™ 5417C centrifuge). The supernatant was removed; each pellet was resuspended in approximately 10 mL fresh medium and pooled in a single plastic bottle. A sample was taken to measure the inoculum cell density using the NucleoCounter™ NC-3000 system (ChemoMetec, Denmark).

The required inoculum volume was added to the bioreactor in a sterile working cabinet via a funnel. The funnel was then rinsed with additional medium to bring the total volume to the desired initial volume of 2 L. Afterwards, the bioreactor was reconnected to the control unit and the control loops were started. The main cultivation parameters are shown below.

Culture conditions	
Culture volume	2 L
Agitation speed	500 rpm
pH value	5.9
pH regulation	Sodium hydroxide (1 M)
Temperature	34°C (heating blanket)
Air flow rate	1.15 slpm (sparger)
Initial biomass	0.5 g/L
Cultivation time	7.5 hours

Sampling and analysis

Samples of at least 6 mL were taken every 30 minutes using sterile syringes connected to the CLAVE sampling port. Analyses of the biomass concentration and total cell density were performed by means of photometrical measurements using a Novaspec II photometer (Amersham Pharmacia Biotech) at 620 nm and a NucleoCounter NC-3000 system respectively. The substrate and metabolite concentrations (glucose, ammonia, and lactate) were analyzed using a BioProfile 300 chemistry analyzer (Nova Biomedical, USA). The glucose and ethanol concentrations were also analyzed by HPLC (Shimazu, Japan) using a refractive index (RI) detector. Finally, the oxygen and carbon dioxide concentrations in the bioreactor exhaust air were analyzed by a BlueInOne gas analyzer (BlueSens, Germany).

Results

The initial cell density was 15.5 x 10^6 cells/mL, corresponding to a dry cell weight of 0.5 g/L. The cells grew exponentially with a mean growth rate of 0.38/hr, corresponding to a doubling time of 1.8 hr. No lag phase was observed. These values were comparable to literature data for the growth of *S. cerevisiae* on glucose [3,4]. After approximately 7 hr, when the main substrate glucose had been completely consumed, the dry biomass concentration in the bioreactor was about 5.8 g/L, corresponding to a cell density of 180 x 10^6 cells/mL (Figure 2). Thus, 5.3 g/L dry biomass was produced and the yield coefficient for the biomass was 0.13 gCDW/gGluc. As expected, the carbon dioxide concentration in the off-gas decreased rapidly, while the oxygen concentration slightly increased when the glucose substrate had been completely consumed (Figure 3). Besides biomass, ethanol and acetate were also produced from the glucose. The maximum concentrations of ethanol and acetate were about 13 g/L and 11 mmol/L, respectively (Figure 4). Hence, the yield coefficients for the biomass and for the ethanol were comparable with literature data for *S. cerevisiae* [1,3-5].

After reaching the set temperature point at the beginning of the fermentation process, the temperature was well-regulated at 34°C by means of a heating blanket. No cooling was required because of the relatively low heat production in the culture. The pH was maintained at 5.9 by the addition of NaOH; in total, approximately 20 mL of NaOH solution was added. No acid addition was required, due to the acid formation and CO_2 accumulation in the culture.

The accumulated pumped volume leveled off after about 6.5 hr, which was an additional indication that the stationary growth phase had started. The DO concentration in the bioreactor was above 94% for the entire process, and therefore, oxygen limitation could be excluded. The ethanol production can be explained by the high glucose concentrations, known as the Crabtree effect [1, 2].

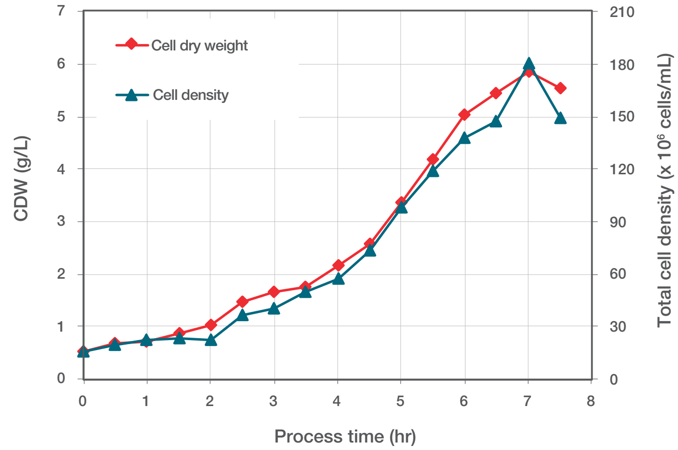

Figure 2. Cell dry weight (CDW) and total cell density of *S. cerevisiae* culture in HyPerforma bioreactor.

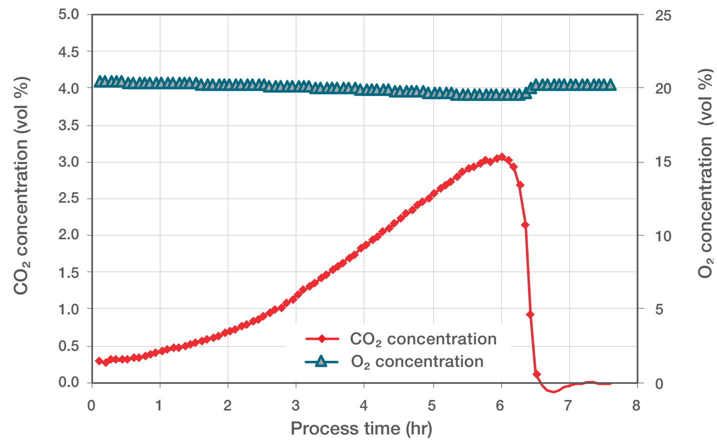

Figure 3. CO_2 and O_2 concentrations in the exhaust air.

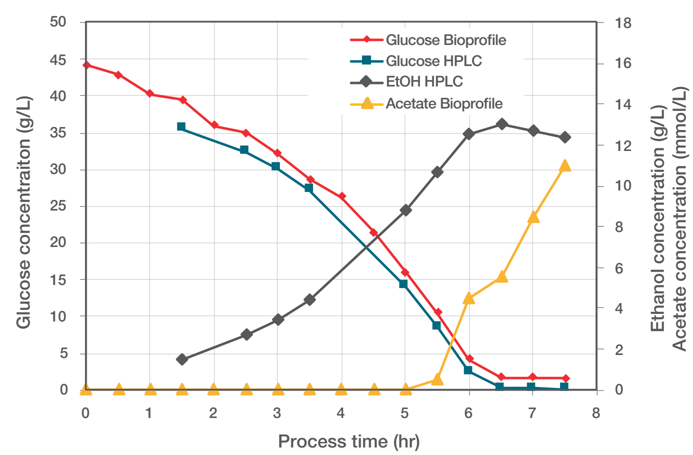

Figure 4. Concentrations of glucose, ethanol, and acetate.

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References

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Authors

Nadezda Perepelitsa, GMP Process Engineer, Johnson & Johnson, Basel, Switzerland

Stephan C. Kaiser, Staff Scientist, Research and Development, Thermo Fisher Scientific, Santa Clara, USA

Dieter Eibl, Professor, ZHAW School of Life Sciences and Facility Management, Centre for Biochemical Engineering and Cell Cultivation Technique, Wädenswil, Switzerland

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