micro-Flask

CHO cell culture in 24-deep square well MTP vs 24-standard round well MTP format using micro-Flask by Duetz covers

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Introduction

Scale-down models of mammalian cell culture processes are essential for generating more efficient protocols and ultimately reduce the cost of biopharmaceuticals (Barrett *et al.,* 2010). The microtiter plate format has been established as a suitable format for the cultivation of mammalian cell lines and successfully used as a scale-down model for shake flasks (Micheletti and Lye, 2006; Silk *et al.* 2010). However, plastic lids are often used as covers for the plates, leading to inconsistent evaporation and insufficient sealing when used on an orbital shaker. Applikon's micro-Flask (Figure 1) combines the microtiter plate technology with a lid system that allows for a clearly defined gas exchange and reduced evaporation. The specific composition of the lid defines the gas exchange and thus liquid evaporation. The micro-Flask lid system consists of: i) silicone seal, which provides identical gas exchange per well and prevents well-to-well cross contamination ii) ePTFE and microfibre layers, which act as a sterile barrier and limit diffusion and iii) stainless steel cover, to hold all layers and provide rigidity.
The micro-Flask accommodates various plate formats ranging from 6 to 96 wells, available in regular and low evaporation lid format. In this work, a 24-standard round well (SRW) was used in combination with a regular and low-evaporation lid and 24-deep square well (DSW) microtiter plate with a regular evaporation lid.
It has been shown that the geometrical structure of a well can be adapted to recreate the effect of baffles within microtiter plates; e.g. a square well providing more turbulent mixing, thus promoting higher transfer of oxygen into the liquid (Funke *et al.,* 2009). However, mammalian cells tend to be more sensitive than microbial cells to a more turbulent system since they are more susceptible to mechanical stresses.

The aim of the present work was to compare the growth and production kinetics of IgG4 expressing CHO cells in the 24-DSW to the 24-SRW, which is already well-characterised for mammalian cell culture.

Materials and methods

Preculture

The IgG4 expressing CHO cell line GS-CY01 was used in this work. The cells were thawed and expanded in a shake flask with a vent cap (Corning Life Sciences, USA) for 7 days in CD-CHO medium (Life-Technologies, UK) with 25 µM MSX (Sigma-Aldrich). The shake flask was mounted on an orbital shaker (Sartorius, UK) at 37°C, 5% CO_2, and 70% humidity.

Batch culture in 24-SRW and 24-DSW

The micro-Flask lids were autoclaved and left inside the autoclave to dry for 12 hours. A suspension with a final concentration of $0.3 \cdot 10^6$ CHO cells/mL was prepared using the appropriate amount of CD-CHO medium. This suspension was then used to fill the wells of the 24-SRW (Corning Life Sciences, USA) to a working volume of 1 mL and the wells of the 24-DSW (Applikon, The Netherlands) to a working volume of 1 mL and 3.5 mL. Two different lids (regular and low-evaporation) were used in the 24-SRW, thus creating two systems with different gas exchange (1.1vvm and 0.25vvm respectively). In the case of 24-DSW only one regular evaporation lid was used with 2 working volumes meaning two different gas exchange systems (0.8 vvm for 3.5 mL and 2.5 vvm for 1 mL). All the micro-Flask systems were mounted onto an orbital shaker (Sartorius, UK) with an orbital diameter of 25 mm and a shaking speed set to 220 rpm (Figure 1, A and B). The micro-Flask clamp was used to hold the plates in place and they were incubated for 8 days at 37 °C, 5 % CO_2, and 70 % humidity. Samples were first taken daily and later every second day on a sacrificial basis. Upon sampling, the cell suspension inside the wells was extracted and weighed in order to determine the volume of the evaporated liquid. The cell concentration was then determined using the Vi-CELL XR and the remaining cell suspension was centrifuged at 1000 g (2.0 mL) or 16100 g (0.5 mL) for 5 min. The supernatant was stored at -18 °C for later analysis.

Analytical methods

Viable cell concentration and viability were determined using the Vi-CELL XR (Beckman Coulter, UK), which is based on the Trypan Blue staining method. Samples were diluted 1:1 with Phosphate Buffered Saline (PBS) (Thermo Fisher Scientific, UK). Metabolite concentrations were determined using the NOVA Bioprofile Flex (nova biomedical, UK). Samples were diluted 1:1 with Milli-Q water. IgG4 quantification was done using an Agilent 1200 (Agilent Technologies, UK) high-performance liquid chromatography (HPLC) with a 1 mL HiTrap Protein G HP column (GE Healthcare, UK). A buffer of 10 mM NaH_2PO_4 (Fluka, Cat No. 71642-1KG) and 10 mM $NaH_2PO_4 \cdot H_2O$ (Sigma-Aldrich, Cat No. 71507-1KG) adjusted to pH 7 and a buffer of 20 mM glycine (VWR, Cat No. 101196X) adjusted to pH 2.8 was used to operate the column. The elution peak was recorded via UV at 260 nm and a standard was used to calculate the resulting IgG4 concentration. Where applicable, the analytical results were corrected for evaporation.

Figure 1A | Representative well of a microtiter plate, covered with a layered lid-system comprising of stainless steel, microfiber, expanded teflon, and soft silicone. Some of the layers are perforated to allow for adequate gas exchange whilst maintaining sterility.
Figure 1B | The micro-Flask sandwich cover consists of silicone seal, ePTFE and microfibre layers and the stainless cover.
Figure 1C | The microtiter plates are mounted on an orbital shaker using a clamp system. The clamp also ensures a tight closure between lid and the microtiter plate. In order to use the incubator space most efficiently, the plates can be stacked.

Results and discussion

Growth kinetics

The growth kinetics for the CHO cells in the 24-SRW (1 mL working volume with regular and low evaporation covers) and the 24-DSW (1 mL and 3.5 mL working volume) are depicted in Figure 2. All the conditions presented similar growth profiles. After 8 cultivation days the highest cell density of $6 \cdot 10^6$ cells/mL was achieved with the 24-DSW and 1 mL fill volume. In contrast, the lowest cell density of $3.5 \cdot 10^6$ cells/mL was obtained in the 24-SRW with the low-evaporation lid. The low-evaporation lid promotes a limited gas exchange, thereby resulting in a lower growth rate and maximum cell density. The viability was in all cases close to 90-100 % during the 8 days of cultivation, showing no significant differences between the individual values.

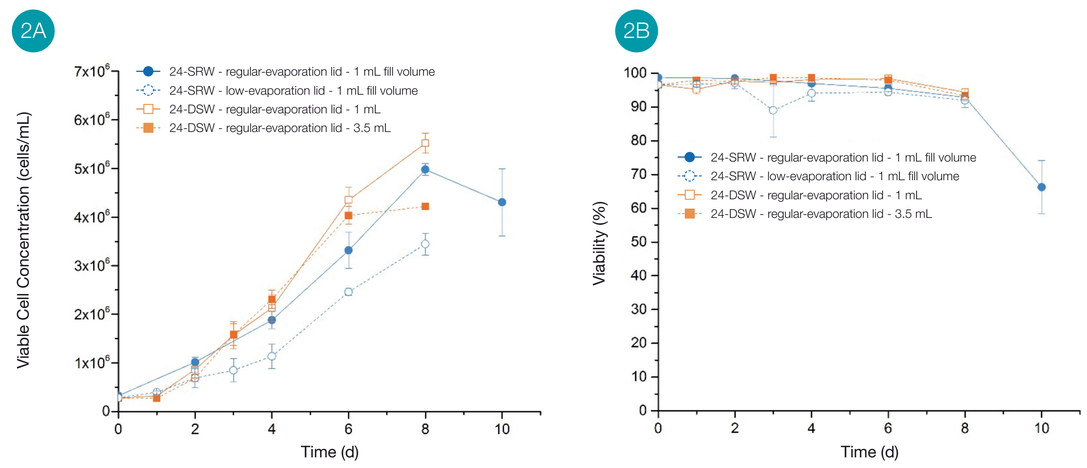

Figure 2A | Growth kinetics and:
Figure 2B | viability for CHO cells grown in 24-SRW using either the regular-evaporation (—●—) or the low-evaporation (-◌-) lid system and in the 24-DSW with either 1 mL (—□—) or 3.5 mL (-■-) fill volume. Error bars represent range (n = 2).

Production kinetics

Product formation progresses similarly for both working volumes in the 24-DSW and the 24-SRW with regular-evaporation lid and reaches a titer of between 0.4 - 0.45 g/L (Figure 3). In line with the cell growth, product formation also decreased for the low-evaporation lid system and only reaches a titer of 0.3 g/L on day 8. This reduction is likely caused by the lower cell concentration resulting from this particular setup. However, an extended cell culture duration might prove desirable in order to compare final titers.

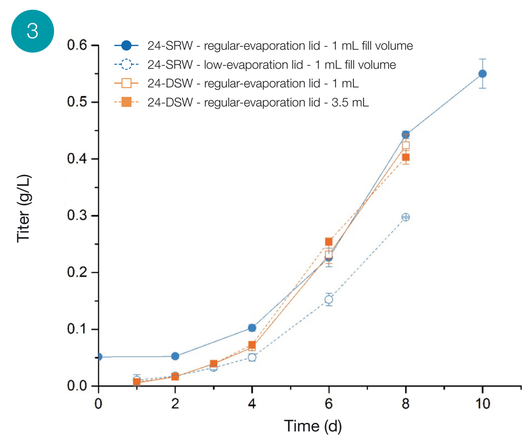

Figure 3 | Production kinetics for CHO cells grown in 24-SRW using either the regular-evaporation (—●—) or low-evaporation (-◌-) lid system and in the 24-DSW with either 1mL (—□—) or 3.5 mL (-■-) fill volume. Error bars represent range (n = 2).

Metabolic profile

The glucose consumption (Figure 4A) is very similar for all formats and conditions. A linear decrease of the glucose concentration is followed by glucose depletion after approximately 8 days of cultivation. The rate of consumption is highest for the 24-SRW with regular evaporation lid and lowest for the 24-SRW with low evaporation lid. Correspondingly, lactate production (Figure 4B) progresses following a similar pattern in all cases, but at a slightly elevated level for the 24-DSW compared to the 24-SRW. Maximum concentrations range from 1.2 g/L to 1.6 g/L for the 24-SRW with regular-evaporation lid and 24-DSW with 1 mL fill volume, respectively. The peak is followed by a gradual consumption of lactate, predominantly visible for the runs in the 24-SRW. Such a consumption of lactate is only suggested in the case of the 24-DSW.

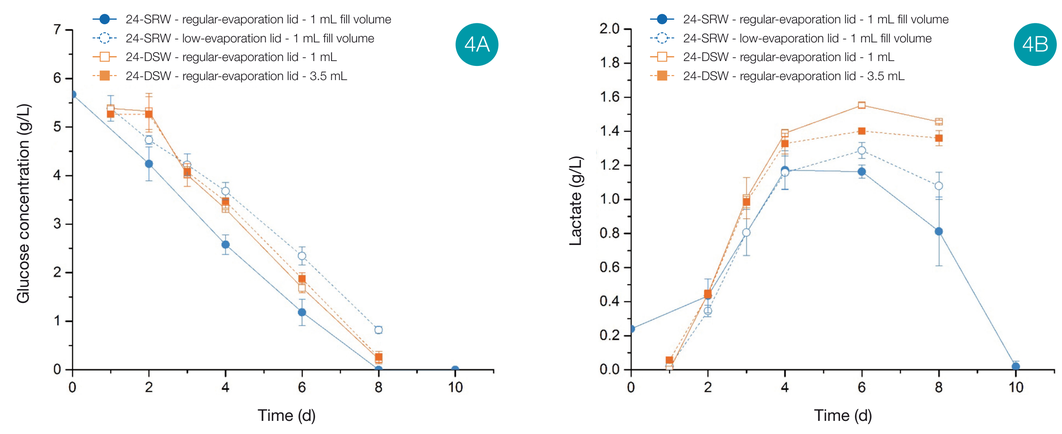

Figure 4 | Glucose consumption and B) Lactate production / consumption for CHO cells grown in 24-SRW using either the regular-evaporation (—●—) or the low-evaporation (-◌-) lid system and in the 24-DSW with either 1 mL (—□—) or 3.5 mL (-■-) fill volume. Error bars represent range (n = 2).

Evaporation

Evaporation is greatest for 24-SRW with regular-evaporation lid and the 24-DSW with 1 mL fill volume, where approximately 33% and 25% respectively of the well content are evaporated on day 8 (Figure 5). In both cases the rate of evaporation is similar. For the 24-SRW with the low-evaporation lid and the 24-DSW with 3.5 mL fill volume evaporation progresses almost identically, and in both cases little over 10% of the well content is evaporated after 8 days.

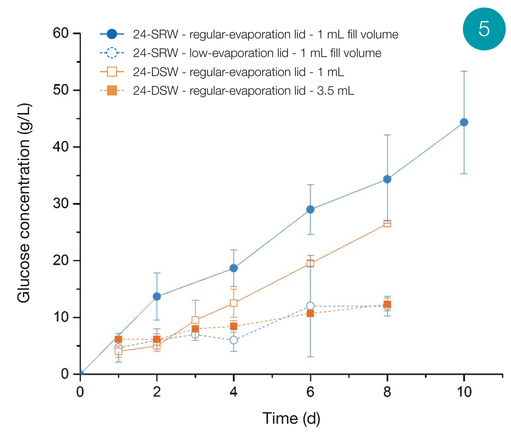

Figure 5 | Percent of well content evaporated for CHO cells grown in 24-SRW using either the regular-evaporation (—●—) or the low-evaporation (-◌-) lid system and in the 24-DSW with either 1 mL (—□—) or 3.5 mL (-■-) fill volume. Error bars represent range (n = 2).

Conclusions

- The square well format does not impair cell growth and is therefore suitable for CHO cell culture applications.
- Both culture formats deliver highly comparable growth, production, and metabolic profiles.
- The low gas exchange rate is unfavorable for GS-CHO CY01 under the current shaking conditions.
- The potential for a higher working volume in the 24-DSW is useful if larger sample volumes are required or if long run times compel a lower relative rate of evaporation.
- Higher working volumes in the 24-DSW should be accompanied by increased shaking speeds to compensate the growth limitations at higher cell densities.

References

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