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Hybridoma and CHO Cell Culture using the New Brunswick™ S41i, an Environmentally-Friendly, "Low Emission" Incubator Shaker

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Abstract

In this study, the New Brunswick S41i CO_2 incubator shaker's mammalian cell culture capability was first verified by culturing CHO cells. This was followed by a comparative performance evaluation against two leading incubator shakers on the market. The New Brunswick S41i provided equivalent performance on the growth rate and viability of mouse hybridoma cells. Comparison of CO_2 gas consumption was also conducted. Due to the superior "green" engineering and advanced control of critical parameters, the New Brunswick S41i demonstrated up to 10 times lower gas consumption compared to the competitive units while delivering uncompromised performance.

Introduction

Cars aren't the only source of CO_2 emissions; laboratory equipment, such as CO_2 incubators, could be releasing over 20,000 liters of CO_2 gas per year. Eppendorf® established the epGreen initiative to reduce the environmental impact of our products. Most of the CO_2 gas consumed by incubators is released to the environment. Eppendorf's new incubator shaker, the New Brunswick S41i, releases extremely low amounts of CO_2 under normal cell culture conditions without sacrificing performance. This study evaluates the New Brunswick S41i's performance culturing hybridoma and Chinese hamster ovary (CHO) cells. The study also investigates the New Brunswick S41i's CO_2 gas consumption compared to competitive units. The data reveals that the New Brunswick S41i consumes 5 to 10 times less CO_2 than competitors, resulting in a 5 to 10 times smaller carbon footprint. Superior engineering minimizes gas leakage with a tightly sealed inner glass door protected by sturdy outer door, tightly sealed motor drive boots as well as a sealed incubation chamber. The performance evaluation, based on the comparison of cell culture growth rates, cell densities, and percent viabilities, demonstrates the New Brunswick S41i's industry leading performance. This new CO_2 incubator includes a robust New Brunswick triple eccentric drive shaker for accurate and stable parameters required to grow non-adherent cells. The shaker drive is optimized for high performance within a humid and carbon dioxide rich environment.

Materials and Methods

Instruments

> New Brunswick S41i equipped with high-temperature disinfection
> CO_2 incubator shaker from competitor 1
> CO_2 incubator shaker from competitor 2
> Vi-CELL® analyzer (Beckman Coulter, Germany)
> YSI® 2700 analyzer (YSI Life Science, USA)
> New Brunswick Galaxy® gas analyzer
> Omega® FMA-1608A thermal mass flow-meter (Omega Engineering, USA)
> Eppendorf consumables
- Research® plus, single channel pipette
- epT.I.P.S®
- Easypet®

Media and cells

> DG44 CHO cell (Invitrogen)
> EX-CELL® CD CHO serum-free medium for CHO cells (Sigma)
> Hybridoma cell DA4-4; ATCC:HB57
> DMEM (ATCC)
> Fetal Bovine Serum 5% (Gibco)
> Penicillin-Streptomycin 100x (Gibco)

CHO culture protocol

CHO cells were grown in EX-CELL CD CHO serum-free medium supplemented with 1% penicillin-streptomycin antibiotic. Six 250mL Erlenmeyer flasks were each inoculated with 60mL of stock culture at a concentration of 3 x 10^5 cells/mL. All flasks were prepared from the same stock culture. Erlenmeyer flask was placed in six different locations on the shaker platform and the results were averaged. The flasks were incubated at 37°C in a mixture of 5% CO_2, 95% air and agitated at 130 RPM (4.69 rcf).

CHO cells were grown for a period of 14 days. A sample was taken on days 3, 5,7,10, 12 and 14 and was analyzed for glucose concentration, cell concentration and viability using YSI 2700 and Beckman Coulter Vi-CELL.

Hybridoma culture protocol

DA4-4 hybridoma cells were grown in DMEM medium supplemented with 5% FBS and 1% Penicillin-Streptomycin. Six 250mL Erlenmeyer flasks were each inoculated with 45mL of stock culture at a concentration of 2 x 10^5 cells/mL. All shake flasks were prepared from the same stock culture and were equally distributed in six different locations in the New Brunswick S41i, competitor 1 and competitor 2. The flasks were incubated at 37°C in a mixture of 5% CO_2 and 95% air. The units were agitated at 95 RPM (2.52 rcf).

Hybridoma cells were subcultured on day 2 and 4 to a concentration of approximately 2 x 10^5 cells/mL. A sample was taken every day from each of the flasks and analyzed for glucose concentration, cell concentration, and viability using YSI 2700 and Beckman Coulter Vi-CELL, respectively.

Gas consumption

The New Brunswick S41i and competitor incubator shakers 1 and 2 were programmed at 37°C, 95 RPM and 5% CO_2 and were allowed to equilibrate for at least 12 hours. Inline CO_2 gas pressures were set at the lowest values recommended by each manufacturer. An offline gas analyzer was used to verify the CO_2 levels within each incubator. A thermal mass flow-meter was used to record volumetric gas consumption over a time period of 48 hours on each unit. Tests were repeated three times and the average values are reported below.

Results

1.) Growth assessment of CHO and hybridoma

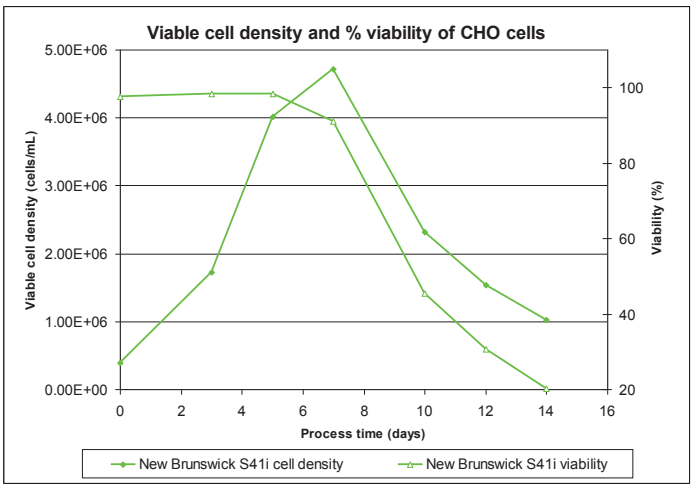

Figure 1: Average viable cell concentration and viability of CHO culture in the New Brunswick S41i.

Viable cell density of CHO cells reached a maximum of 4.72 x 10^6 cells/mL by day 7. The cell viability was maintained at approximately 98% up to day 5 and dropped steadily thereafter (Figure 1).

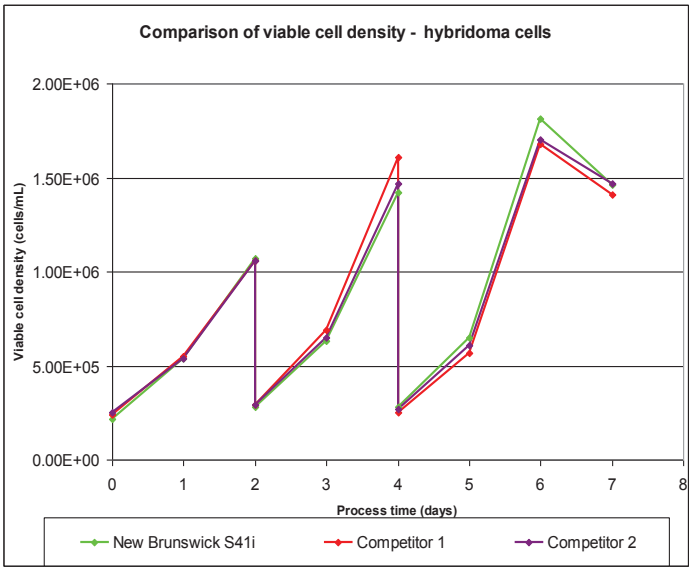

Figure 2: Comparison of average viable cell densities of hybridoma cultures grown in New Brunswick S41i and competitors 1 and 2.

In comparison to CHO cells, hybridoma culture was able to maintain a high average viability of approximately 95% through day 6, due to the subculturing of cells during log phase of growth on days 2 and 4. The maximum viable cell density of 1.81 x 10^6 cells/mL was achieved on day 6 (Figures 2 and 3).

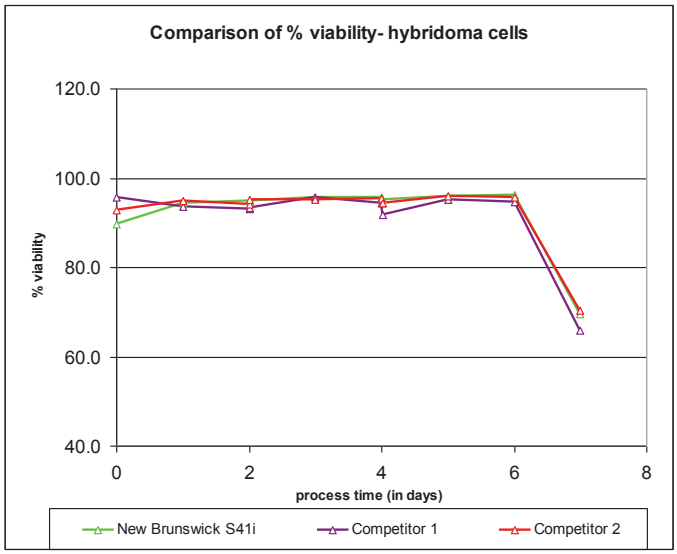

Figure 3: Comparison of average percentage viabilities of hybridoma cultures grown in New Brunswick S41i and competitors 1 and 2.

2.) Measurements of gas consumption

The measurement of CO_2 consumption at 5% CO_2 setpoint revealed that the competitive units evaluated consumes much higher CO_2 gas over the same period as compared to the New Brunswick S41i (Figure 4).

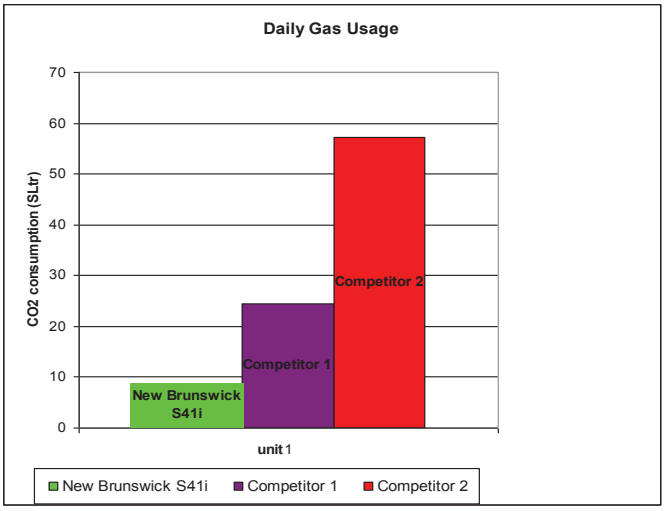

Figure 4: Average CO_2 gas consumption of tested units in standard liters (Sltr) over a 24 hour period

Discussion & Conclusion

The rising need to create more eco-friendly products and customer demand for higher efficiency were taken into consideration during the development of the New Brunswick S41i. This study validates the performance of the new CO_2 incubator with a New Brunswick shaker built inside by growing two cell lines which are very commonplace in research and production. Process and media were not optimized for either cell lines in this study.

In conclusion, the results show that the New Brunswick S41i is competent at growing mammalian cells while reducing environmental impact down to a minimum. The New Brunswick S41i combines a robust triple eccentric drive shaker within a CO_2 incubator, to provide accurate and stable parameters required for the growth of non-adherent cells.

Ordering Information

Description	International order no.	North America order no.
New Brunswick S41i CO_2 incubator shaker with high-temperature disinfection	S41I-230-0100	S41I-120-0100
Galaxy gas analyzer Electronic CO_2 gas analyzer	P0628-6150	P0628-6150
eppendorf Research® plus, adjustable Single channel pipette – 1-10 mL	3120 000.089	3120000089
Easypet® Pipette dispenser – suitable for pipettes from 0.1 to 100 mL	4421 000.013	022230204

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