APPLICATION NOTE 90646

The Cytomat Automated Incubator Series and the Air Purging System

Optimizing cell culture conditions

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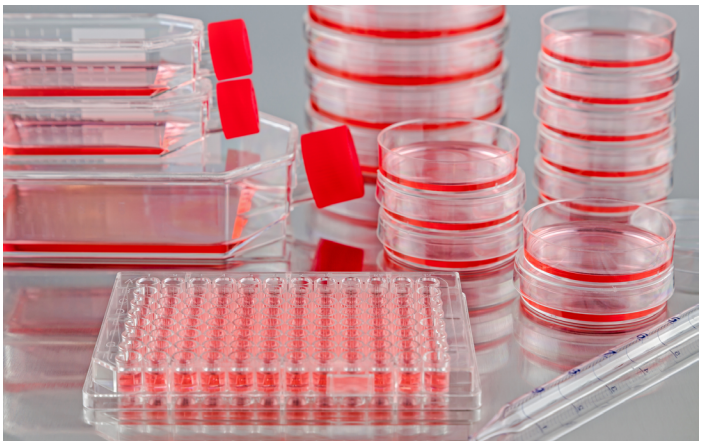

Goal

To provide an optimum environment for growth and protein expression in yeast in the Thermo Scientific™ Cytomat™ 2 C450-LiN ToS Automated Incubator Series using the Air Purging System (APS).

Introduction

Microbial cultures such as yeast and bacteria are important tools in the biopharmaceutical industry. The need to screen large quantities of microbial clones for relevant recombinant proteins has increased the demand for high capacity automated incubation, which provides optimum environmental conditions during the growth phase of these clones. Enclosed automated incubators, such as the Cytomat series, are optimized for mammalian cell culture growth and offer robust, scalable and reproducible environmental conditions. For microbial growth, Cytomat incubators with integrated TRUE orbital shakers have been used for many years to enable efficient gas exchange of growth media, in deep well plates and 24-well plates. A specially constructed gate minimizes gas exchange with the environment, even if plates are loaded and retrieved with high frequency. However, the oxygen (O_2) content during several days of growth in these enclosed environments has never been investigated. Initial tests showed that microbial metabolism inside incubators leads to a reduction in O_2 levels of up to 15% per day, affecting growth rates negatively. This phenomenon is accompanied by an increase of carbon dioxide (CO_2) concentration. In this paper we present an effective method to maintain oxygen and carbon dioxide at optimal atmospheric levels during yeast cell culturing over several days.

Equipment and methods

These experiments used the latest generation of Cytomat 2 C450-LiN ToS incubators equipped with two individual tower shaker stackers (3 mm shaking diameter; max. 1.200 rpm).

This system works within a temperature range of 4°C to 50°C, with a temperature deviation of +/- 0.2°C at 30°C. In addition, an integrated infrared (IR)-sensor (which has been reliably installed in the Thermo Scientific™ Heracell™ incubator systems thousands of times) is used to measure and control the CO_2 concentration inside the incubation chamber (ranging from ambient to 20 vol%).

In this investigation, the Cytomat system was equipped with the newly developed air purging system (APS) which is used to control the atmospheric gas inside the incubation chamber by adding/purging it with filtered ambient air. APS consists of a software-controlled air pump with a maximum flow rate of 6 L/min. When the IR-sensor detects a deviation from the target CO_2 concentration, the air pump will automatically adjust the flow rate equivalent to the size of the deviation. When the target value is reached, the pump will stop. The ambient air used for purging ensures that both O_2 and CO_2 values are restored in parallel.

In the following experiments, the CO_2 target value for the IR-sensor was set to 0.5 vol%.

Starting conditions

O_2 depletion and an increase in CO_2 was mimicked by flooding the incubation chamber with pure CO_2 until a concentration of 16 vol% was reached. This correlated with a drop in O_2 concentration down to 17 vol%. Following this, the APS function was activated for 70 hours with 10 second sensor read intervals. Physiological experiments with yeast cells have been conducted by Adam Penson (GSK, Stevenage, UK) with and without use of APS (Data withheld).

Results

At the start of the experiment, the adjusted CO_2 levels were 16 vol% and the O_2 levels were 17.7 vol%. After activation of the APS system, the incubator showed a steep decline in the concentration of CO_2 and an increase in O_2. Once environmental gas levels were reached, they remained constant during the next 60 hours until the experiment ended. The temperature remained at 30°C for the entire duration of the experiment (Figure 1).

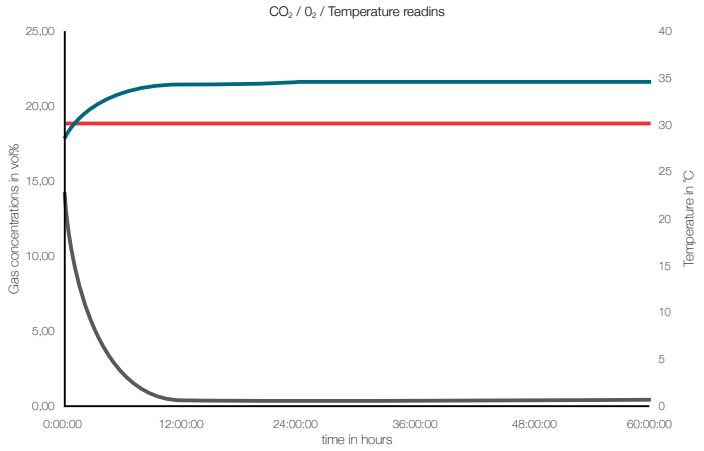

Figure 1: Sensor reads of the Cytomat 2 C450-LiN ToS incubation chamber. A graph showing gas concentration (vol%) and temperature (°C) against time (sec). The red line indicates temperature, the blue line shows the percentage of O_2 and the black line represents the percentage of CO_2 in the chamber. Air purging starts at t = 0 sec.

A close-up view of the same graph shows, that after approximately four hours, 85% of the CO_2 had been removed, reaching normal environmental conditions after approximately 12 hours. In parallel O_2 levels returned to its normal value of 21 vol% (Figure 2).

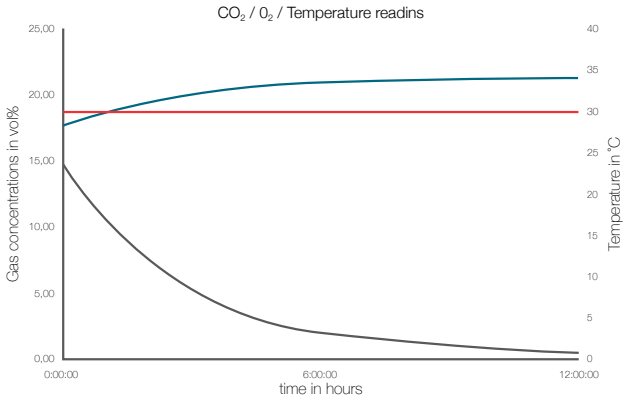

Figure 2: Sensor reads of chamber conditions – a close up view. A graph showing gas concentration (vol%) and temperature (°C) against time (sec). The red line indicates the temperature, the blue line shows the percentage of O2 and the black line represents the percentage of CO2 in the chamber. Air purging starts at t = 0 sec.

Physiological experiments performed in the lab of Adam Penson, GSK, showed that without APS, low O_2 and CO_2 levels caused yeast cell cultures to reduce their metabolic activities and start to die. When APS is used, growth levels and metabolic activities remain at normal levels.

Discussion

When culturing highly metabolically active yeast in incubation chambers without an APS system, a rapid increase in CO_2 and a correlating O_2 depletion is observed. This results in a toxic atmosphere, cell death and a significant reduction in yields (Adam Penson, Senior Scientist of GSK Stevenage, data withheld).

In this study, the Cytomat 2 C450-LiN ToS series coupled with an accompanying APS was tested, to see whether the optimum environmental conditions could be maintained. As soon as the CO_2 sensor detected a deviation from the target CO_2 value, the APS was activated to normalize gas levels. The APS demonstrated that it was capable of normalizing extremely high deviations in gas concentration, in a relatively short time. Under normal experimental conditions, the APS was activated as soon as the slightest deviation of gas concentration was observed – keeping gas levels stable from the offset. Using filtered environmental air to adjust gas levels, normalization of the CO_2 concentration was accompanied with a normalization of O_2 concentration. This is another important advantage of the APS.

The Cytomat series combined with the APS system, maintained CO_2 levels below 1% in the incubation chamber. Temperature and humidity were not affected by purging, however the purging gas volume was much higher than under normal runtime conditions. Normal runtime conditions start with a CO_2 value of 0.3 vol% causing much lower purge rates than shown in these experiments.

Using the CO_2 sensor (IR-Sensor) – a default Cytomat incubator unit sensor – eliminates additional cost and enables users the option of retrofitting the APS when required.

Conclusion

Typically, during yeast cell incubation, O_2 levels decrease and CO_2 levels increase. O_2 depletion can result in a reduction in the cell proliferation rate since microorganisms require oxygen for optimal growth. The optional “Air Purging System” can maintain the incubator atmosphere at optimum gas levels with help from an integrated CO_2 sensor.

The Cytomat 2 C450-LiN ToS with APS enables scientists to regulate CO_2 and O_2 levels during incubation whilst keeping the required temperature stable from the very beginning of their experiment.

Find out more at **thermofisher.com/cytomat**

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