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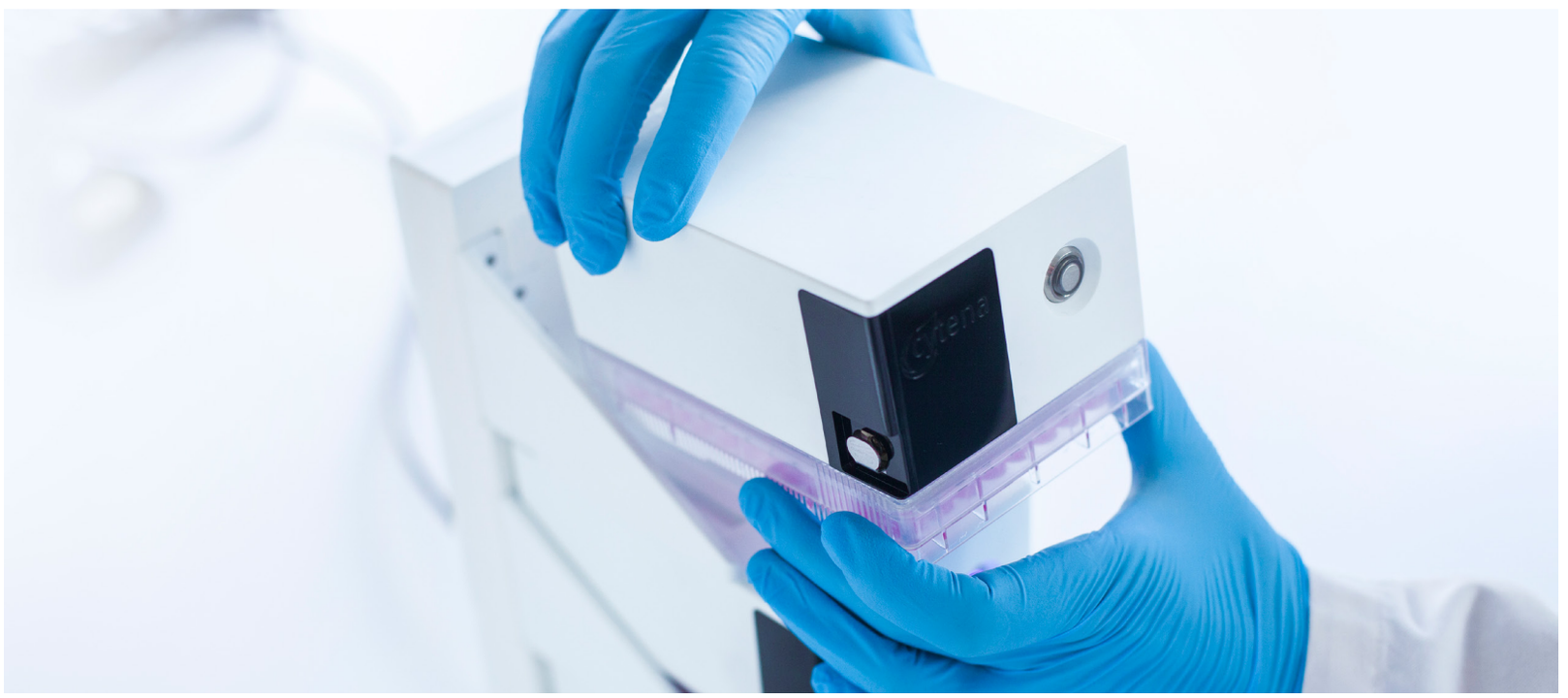
Improving Oxygen Transfer in Standard 96-well Plates Using the C.BIRD™ Microbioreactor

Paul Andrew Lee, BSc, Da-Han Kuan, MSc
CYTENA Bioprocess Solutions, Taipei City, Taiwan



Abstract

In most aerobic bioprocesses, oxygen deficiency occurs because aerobic organisms consume oxygen faster than the rate at which oxygen enters aqueous medium. Most conventional solutions to this limitation only apply to large-scale culturing. Our study demonstrates that the C.BIRD system improves oxygen transfer in standard 96-well plates by allowing controllable mixing that increases oxygen transfer into aqueous medium. An extracellular oxygen assay was used to measure oxygen levels inside 96-well plates by comparing glucose oxidation after three different mixing conditions: static, C.BIRD 50-second and C.BIRD 25-second mixing periods. Results show that the C.BIRD significantly increased the oxygen transfer into the aqueous medium in 96-well plates, providing a better culturing environment for cells and other aerobic organisms.

Introduction

Oxygen transfer is a limiting factor in aerobic bioprocesses because of the relatively low solubility of oxygen in aqueous medium (Place, 2017). This can lead to oxygen depletion during cell culture or microbial growth. Agitation or mixing in bioreactors is often used to provide better oxygen transfer conditions and improve cell or microbial growth and protein production. In most large-scale bioreactors, oxygen is supplied by blowing air bubbles into the culture medium through a sparger. However, this method is restricted to large-scale bioreactors.

In this study, we demonstrated that the C.BIRD microbioreactor enables controlled mixing in standard 96-well and 24-well plates. The C.BIRD is compact and fits in most standard incubators. Its docking station can hold three C.BIRD control units (**Figure 1A**) in horizontal orientations. Each control unit is composed of two parts: an autonomous control box (**Figure 1B**) on the top and a consumable C.BIRD lid with 96 or 24 cylindrical tubes (**Figure 1C**). These tubes serve as fluidic channels, allowing the suction and expulsion of air into and out of each well in a standard cell culture plate. Pneumatic connection with these channels and actuation by the control system enable continuous reciprocal mixing at different mixing periods in each well (**Figure 1D**). With the C.BIRD, mixing is possible with volumes as small as 150 to 200 µL in 96-well plates or 1000 to 1600 µL in 24-well plates. This can provide a better culturing environment that improves culture growth and viability.

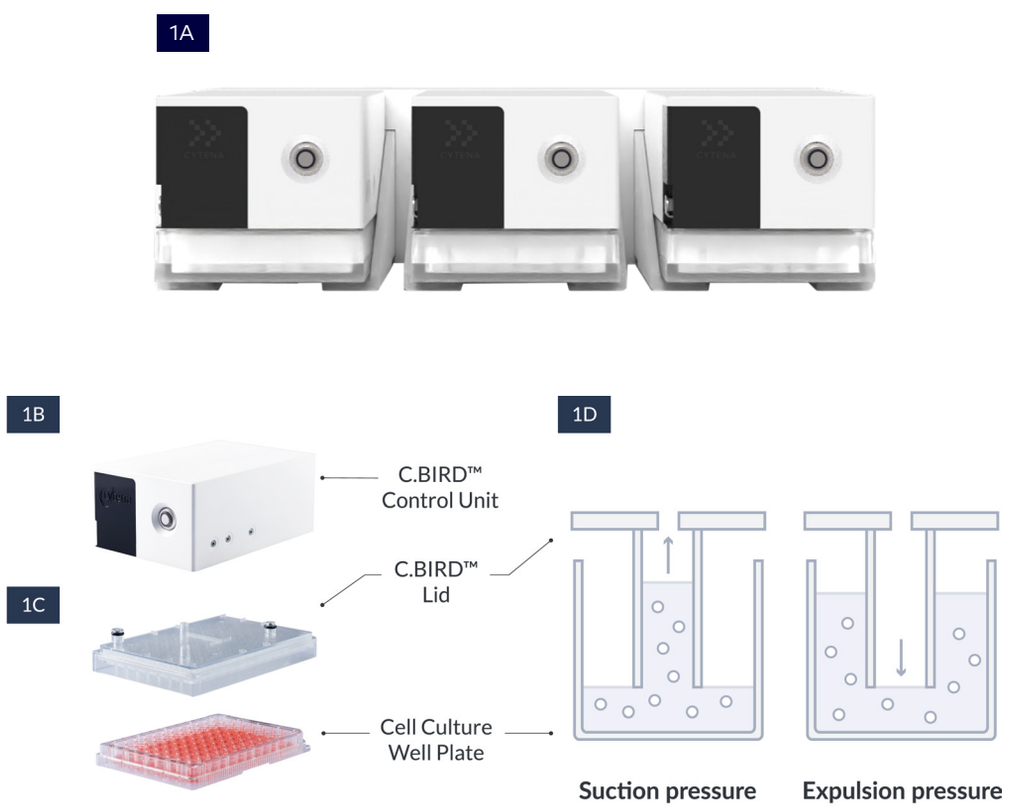


Figure 1. A) *The C.BIRD system includes a docking station for three control units. Each control unit is composed of* ***B)*** *a control unit and* ***C)*** *a consumable lid and a standard 96-well plate.* ***D)*** *The principle of reciprocal mixing provided by the C.BIRD.*

Materials and methods

Oxygen levels were measured using an extracellular oxygen consumption assay (AB197243, Abcam) and a kinetic fluorescence microscope (Olympus IX73). The fluorescent dye contained in the assay kit is quenchable by oxygen and fully reversible. This means that an elevated fluorescence signal would indicate depleted oxygen content in the sample. 10 µL of the assay's probe were added into a 150 µL double distilled water (ddH_2O) solution containing 10 mM glucose and 5.549 × 10-3 units/µL glucose oxidase. Probes containing pure ddH_2O were used as blank samples. Standard 96-well plates (0030730011, Eppendorf) were used in these experiments.

Olympus cellSens software was used to measure the fluorescence intensities. A 340 to 390 nm excitation filter and a 692/40 nm emission filter were used. Samples were exposed for 340 milliseconds and were read three times per interval. C.BIRD's mixing cycle was stopped 15 seconds before each reading to ensure the same fluid level for every measurement. Comparison studies were performed with samples measured in static conditions and in the C.BIRD at different mixing periods (**Figure 2**).

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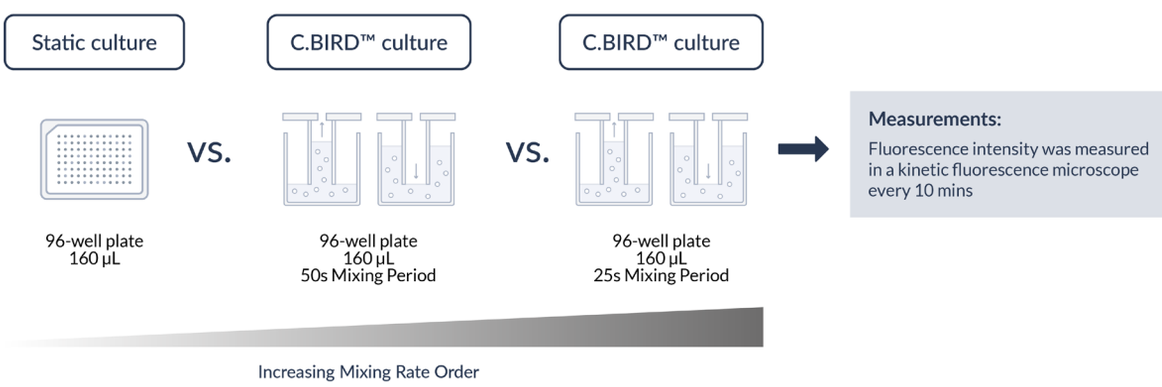

Figure 2. *Experiment design showing comparison between different mixing rates.*

Results and discussion

The oxidation of glucose catalyzed by glucose oxidase was observed and compared under the fluorescence microscope for different mixing conditions: static, C.BIRD 50-second mixing period and C.BIRD 25-second mixing period. The fluorescent dye in the assay is quenched by oxygen; therefore, the decrease in fluorescence signal reflects an increase in oxygen content. Upon the addition of glucose in the mixture, glucose reacts with the existing oxygen in the mixture (**Figure 3A**), exhausting the dissolved oxygen, which results in a high fluorescence signal (Linek, 1981). Since glucose continuously reacts with oxygen as oxygen enters the medium, glucose will eventually be depleted, making it the limiting reagent. Oxygen content will then continue to increase as glucose decays, resulting in a decrease in fluorescence intensity.

Fluorescence intensity was recorded after 1 hour of mixing to allow the stabilization of glucose oxidation reaction. Each glucose-containing sample's fluorescence signal was then subtracted from its own respective blank sample's fluorescence signal. The resulting difference was then normalized to each mixing condition's highest signal difference and plotted against time passed (**Figure 3B**). We observed that the decrease in fluorescence intensity shows an exponential decay. This exponential decay shows the depletion of glucose and simultaneously reflects the increase in oxygen. Results show that the C.BIRD system's mixing conditions produced a greater exponential curve. By fitting these curves in an exponential decay equation, we were able to identify their time constant (**Table 1**), which is also the time it takes for them to reach approximately 36.8% of their original intensity. Results show that the C.BIRD can significantly decrease the time it takes to consume glucose by as much as 7 times. The C.BIRD 25-second mixing period sample required only around 35 minutes compared to the static condition's 255 minutes. The C.BIRD 50-second mixing period sample's time constant on the other hand was almost half of the static condition's time constant. All time constants indicate a high coefficient of determination (R2), with R2 values of at least 0.999.

Since the exponential decay of glucose is directly proportional to oxygen transfer, we can compare each mixing rate's oxygen transfer relative to one another using the derivatives of their respective exponential decay curves. By normalizing the absolute value of their derivatives to the static's, we can see how oxygen transfer in the C.BIRD 25-second condition is 7 times faster than that of the static condition (**Figure 3C**). The oxygen transfer under C.BIRD 50-second condition is almost double that of the static. Our results provide convincing evidence that the C.BIRD's controllable mixing can effectively improve oxygen transfer and can allow users to modify oxygen transfer rates with different mixing rates.

3A

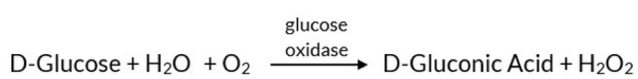

Figure 3A. *Enzymatic oxidation catalyzed by glucose oxidase.*

3B

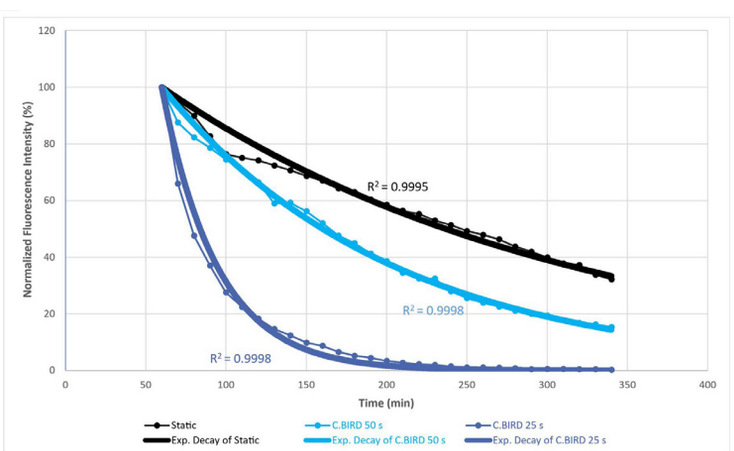

Figure 3B. *The exponential decay of glucose as represented by the normalized fluorescence intensity at different mixing rates.*

Mixing Condition	Time Constant
Static	255 min
C.BIRD 50s	145 min
C.BIRD 25s	35 min

Table 1. *Time constants of the exponential decay of glucose at different mixing rates.*

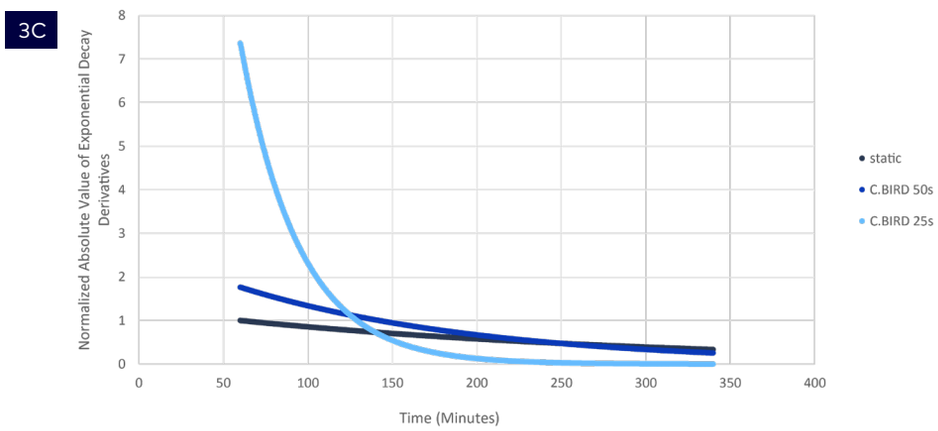

Figure 3C. *Absolute value of the exponential decay derivatives normalized to static condition.*

Conclusion

This study shows that the C.BIRD improves oxygen transfer in standard 96-well plates by comparing glucose depletion and oxygen recovery in different mixing conditions. The exponential decay observed in the experiment showed that the time constants were shorter for C.BIRD 50-second and C.BIRD 25-second conditions than for the static condition. This translates into an oxygen transfer rate that is twice as fast in C.BIRD 50-second and up to 7 times faster in C.BIRD 25-second when compared to the static condition.

In conclusion, the C.BIRD allows controllable mixing that increases oxygen transfer into aqueous medium. This is beneficial in alleviating the limitation caused by the low solubility of oxygen into aqueous medium and the high oxygen demand of aerobic bioprocesses. With improved oxygen transfer, the C.BIRD can provide a better environment for cell cultures or the cultivation of other aerobic organisms.

Reference

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Edited version: August 2023 | CBS_PUB_CBIRD_App-Note-04_Digital

info@cytena-bps.com | +886 2 27206135 | www.cytena-bps.com