Microaerobic Fermentation of *Lactobacillus acidophilus* within Gut Microbiome Physiological Conditions by BioFlo® Bioprocess Control Stations

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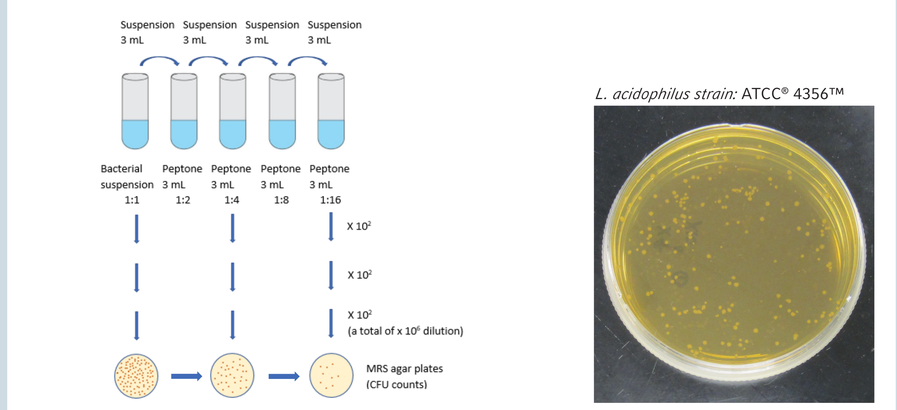
Abstract

There is a growing interest among food & feed and biofuel industries for microaerobic fermentation, a fermentation process at close to anaerobic condition but still requiring small amounts of oxygen. The dissolved oxygen level is rather low, often less than 5 %. Also, there is a huge interest in probiotics production due to their great health benefits, and many probiotics are beneficial bacteria that naturally thrive in the human gut microbiome under microaerobic conditions. In this application note, we successfully performed microaerobic fermentation of a probiotic strain, *Lactobacillus acidophilus*, at a very low oxygen level (< 1 %, representing the natural physiological condition of human gut microbiome) using BioFlo® 120 and 320 control stations. The microaerobic fermentation was carried out in BioBLU® 3f Macrosparge Single-Use Vessels. Through automatic gas mix and control, we demonstrated the feasibility of precisely controlling the dissolved oxygen (air saturation) at 4 % throughout the fermentation process by both BioFlo 120 and 320. We showed robust growth of *Lactobacillus acidophilus* which reached a biomass concentration of 3.13 x 10^9 CFU/mL by BioFlo 120 and 3.73 x 10^9 CFU/mL by BioFlo 320.

Scope

Providing a good reference for probiotic and microbiome studies under microaerobic conditions.

Process Parameter and Set-up



L. acidophilus strain: ATCC® 4356™

Figure 1: Serial dilution of the *Lactobacillus acidophilus* suspension for colony forming units (CFU) counting. The CFUs appeared visible 48 hours post inoculation

Table 1: Bioprocess control stations set-up.

Parameter	Configuration
Vessel	BioBLU 3f
Inoculation density	1 % (v/v), 30 mL inoculum to a 3 L working volume
Dissolved oxygen (DO)	4 % air saturation, ~ 0.8 % oxygen environment
Agitation	Magnetic drive, 150 rpm
Gassing	Automatic gas flow and mix
Temperature	37 °C, cooling controlled by a single stainless-steel cooling finger
pH	6.5 ± 0.1 for the first 4 hours, then 5.0 ± 0.1 for the remaining of the culture, controlled by external 4 mol/L sterile sodium hydroxide solution and 2 mol/L sterile hydrochloric acid solution
Impeller	Three Rushton impellers
Sparger	Macrosparger

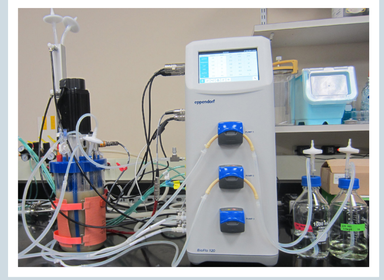


Figure 2: BioFlo® 120 control station with a BioBLU 3f Single-Use Vessel.

Figure 3: BioFlo® 320 control station with a BioBLU® 3f Single-Use Vessel.

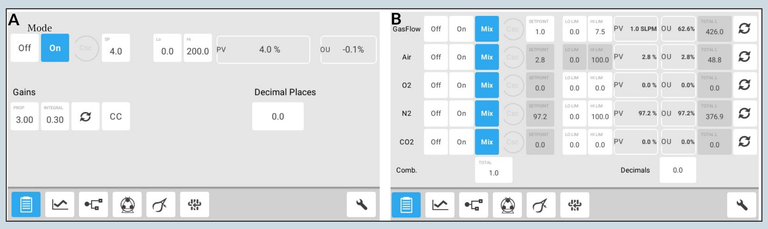


Figure 4: DO (A) and sparge (B) parameter setup in BioFlo 120.

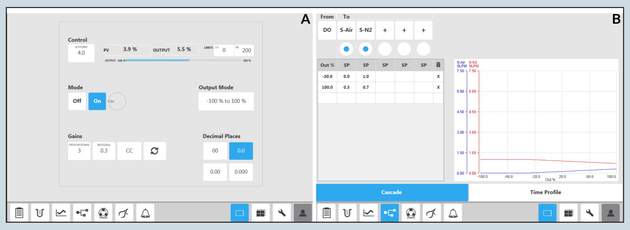


Figure 5: DO (A) and sparge (B) parameter setup in BioFlo 320.

Results

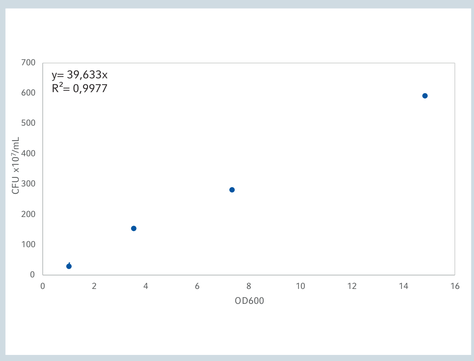


Figure 6: Standard curve correlating OD_{600} and CFU of *Lactobacillus acidophilus*.

> Number of CFU (N) on each agar plate are counted

> Volumetric CFU calculation: colony-forming units per milliliter = N x 10^6 / 0.1 mL = N x 10^7 CFU/mL.

> The calculated correlation is: CFU/mL = 39.63 x OD_{600} x 10^7.

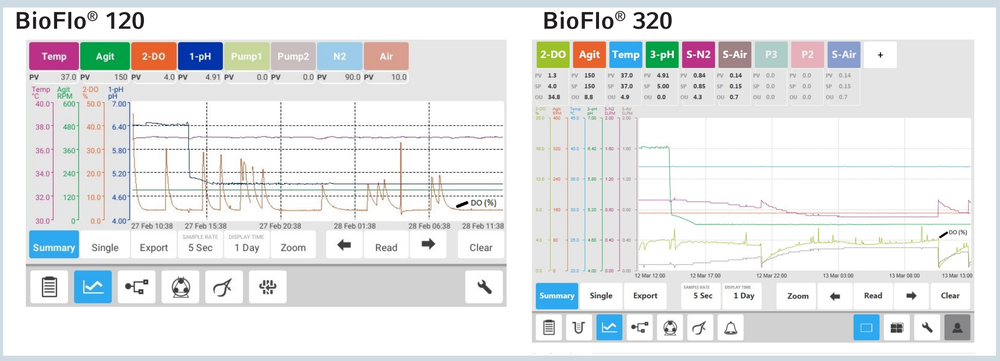


Figure 7: Trends for the first 24 hours after inoculation:

> pH adjustment form 6.5 to 5.0 at t= 4h

> DO-level was maintained at 4 %

> Thanks to the 4 integrated TMFCs, DO control of the BioFlo 320 was more stable and precise

> The DO peaks for BioFlo 120 is due to that PID (proportional-integral-derivative) modulates the combined impact of control through a single TMFC of nitrogen and air.

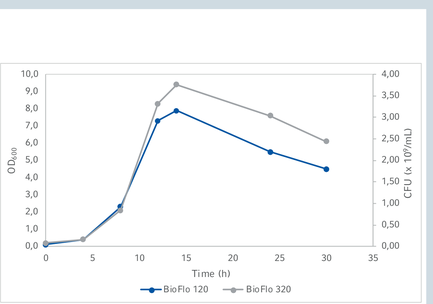


Figure 8: Growth curves of *Lactobacillus acidophilus*.

> Exponential growth phase between 8 and 12 h for both runs.

> Maximum OD_{600} at t=14 h, with an OD_{600} of 7.9 for the BioFlo 120 and an OD_{600} of 9.4 for the BioFlo 320 control station.

Conclusion

> The BioFlo 120 and BioFlo 320 control stations are feasible to maintain dissolved oxygen level at 4 % which equals an oxygen concentration of 0.8 %.

> *L. acidophilus* shows a robust growth under both control stations, yielding in a maximum biomass of 3.13 x 10^9 CFU/mL and 3.73 x 10^9 CFU/mL, respectively.

> Probiotics available on the market are often packed with 20 to 40 billion CFU per capsule. With the assumption of 30 billion Lactobacillus CFU per capsule and *L. acidophilus* is the only strain, one single run produced 313 capsules per BioBLU 3f vessel controlled by BioFlo 120, and 373 capsules by BioFlo 320.

This application note shows a detailed example of precisely controlling the DO at 4 % to support microaerobic fermentation, and will be very helpful for peers in the field of microbiome to apply to their specific bioprocesses.