HOW TO USE THE MICROBIAL RESPIRATION IN THE AEROBIC FERMENTATION PROCESS: OUR, CER, AND RQ DETERMINATION WITH BIONET bBREATH

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During fermentation, many phenomena related to cell metabolism take place. In the case of aerobic fermentation, the parameters related to respiration are direct indicators of the state of the culture. In aerobic fermentation, a certain amount of O_2 is needed to oxidize a relative quantity of carbon source into CO_2 and H_2O to obtain energy and for biomass formation. The next example is the stoichiometry relation of the glucose consumption:

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6H_2O + 6CO_2$$

Therefore, the measurement of the respiratory activity, and the corresponding parameters of microbial cultures in fermenters, is a powerful tool to control and optimize the process. One example is the identification of the different phases or the determination of points for the limiting substrate depletion (Fig. 1). In the case of mammalian cells or cell culture, the addition of CO_2 to control the pH must be taken into account.

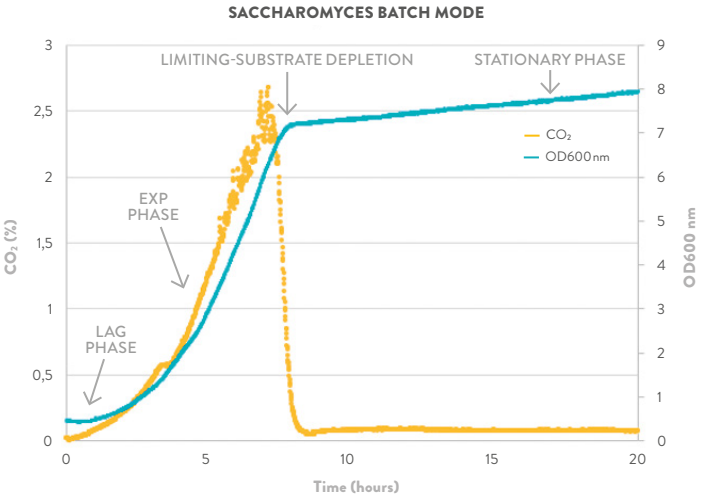

Fig. 1: Fermentation of the yeast *Saccharomyces cerevisiae* in batch mode with online measurement of the exhaust gas with the Bionet bBreath and online optical density probe.

Using an off-gas analyzer, such as the Bionet bBreath (Fig. 2), the value of the O_2 and CO_2 from the respiration in the exhaust gas can be analysed and monitored online.

The main parameters related to the exhaust O_2 and CO_2 are the Oxygen Uptake Rate (OUR), the Carbon dioxide Evolution Rate (CER), and the Respiratory Quotient (RQ). The se parameters can be calculated online with the Bionet bBreath and ROSITA SW.

Fig. 2: Bionet bBreath1

The formulas for the calculation of these parameters are:

- Oxygen Uptake Rate (OUR) in mol/l·h

$$OUR = \frac{Fgas \cdot P}{Vf \cdot R \cdot T} \cdot \left(O2\%in - \frac{1 - O2\%in - CO2\%in}{1 - O2\%out - CO2\%out} \cdot O2\%out \right)$$

- Carbon Dioxide Evolution Rate (CER) in mol/l·h

$$CER = \frac{Fgas \cdot P}{Vf \cdot R \cdot T} \cdot \left(\frac{1 - O2\%in - CO2\%in}{1 - O2\%out - CO2\%out} \cdot CO2\%out - CO2\%in \right)$$

- Carbon Dioxide Evolution Rate (CER) in mol/l·h

$$RQ = \frac{CER}{OUR}$$

Where:

- **Fgas** — The inlet gas flow rate in L/h
- **P** — Normal pressure 1.0133 bar
- **Vf** — Working volume in the bioreactor/fermenter in litters (L)
- **R** — Gases constant 8,314.10^{-2} bar·L/K·mol
- **T** — Temperature 273.15 K
- **$O_2\%_{in}$** — Oxygen composition in the inlet gas 20.95%
- **$CO_2\%_{in}$** — Carbon dioxide composition in the inlet gas 0.05%
- **$O_2\%_{out}$** — Oxygen composition in the outlet gas (bBreath)
- **$CO_2\%_{out}$** — Carbon dioxide composition in the outlet gas (bBreath)

In an optimal and balanced system, the ratio between carbon dioxide production and oxygen consumption, which is the respiratory quotient (RQ), should not exceed 1.0.

The variation of the dissolved O_2 concentration during the time in a fermentation process and the oxygen mass transfer can be calculated and correlated with the oxygen transfer rate (OTR) and OUR, as follows:

$$\frac{dC \cdot O2}{dt} = OTR - OUR$$

Where:

OTR = KLa·(C*$_{O2}$ − C$_{O2}$)

- **KLa** — Volumetric oxygen transfer coefficient (h−1)
- **C*$_{O2}$** — Saturated dissolved O2 concentration, maximum O_2 carrying capacity of the liquid in mol/L
- **C$_{O2}$** — Current dissolved O2 concentration in the liquid in mol/L

OUR = qO2·X

- **qO2** — specific oxygen uptake rate in mol/g·cell·h
- **X** — Biomass concentration in g·cell/L

The biomass formation X is related to the respiration according to the OUR and CER. As the CO_2 variation depends on the biomass formation, the specific growth rate (μ) of the cell can be calculated in a similar way to using other parameters, such as the optical density or dry cell weight. Indeed, there is a correlation between optical density and CO_2 production (Figure 3).

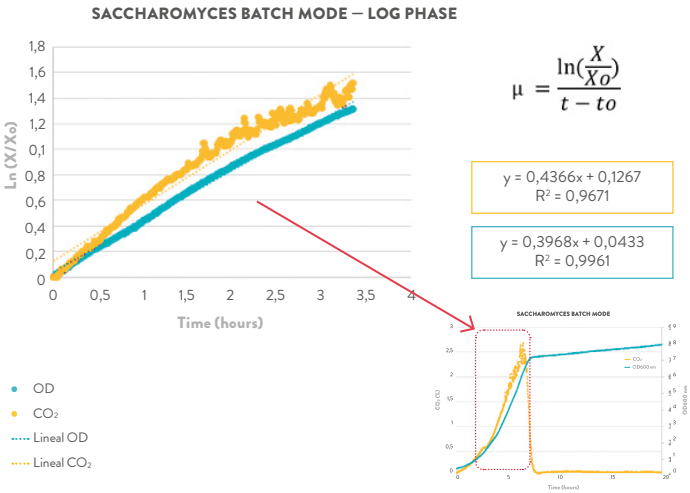

Fig. 3: Fermentation of the yeast *Saccharomyces cerevisiae* in batch. Calculation of the specific growth rate from the exponential phase using the OD at 600 nm and the CO_2 from the respiration.

Another use of respiratory indicators is to ensure correct process design and optimal scaling. Figure 4 shows how the OUR increases with the decrease in exhausted O_2 in a fed-batch mode process. With this characterization, a system can be designed with sufficient OTR to provide the O_2 that the cell needs. Usually, the OTR reached in large-scale equipment is typically lower than in bench-top bioreactors/fermenters. Therefore, in an early-stage characterization of the process and the strain, it is important to know the OUR in order to ensure the success and reduce uncertainties in the scale-up to industrial scale. Another important point observed in the graph of figure 4 is that despite maintaining the saturated dissolved oxygen value at an optimal 30±1 value, in reality, the culture is under a hypoxic state under 10% of O_2, with a high demand of O_2, which is why a strategy with oxygen enrichment or increasing the pressure in the fermenter would be convenient. Finally, with the cascade DO_2 control strategy it is not enough to maintain optimal O_2 values in the culture, as there is more information "masked" behind the typical profile of the agitation or the gas flow from the cascade.

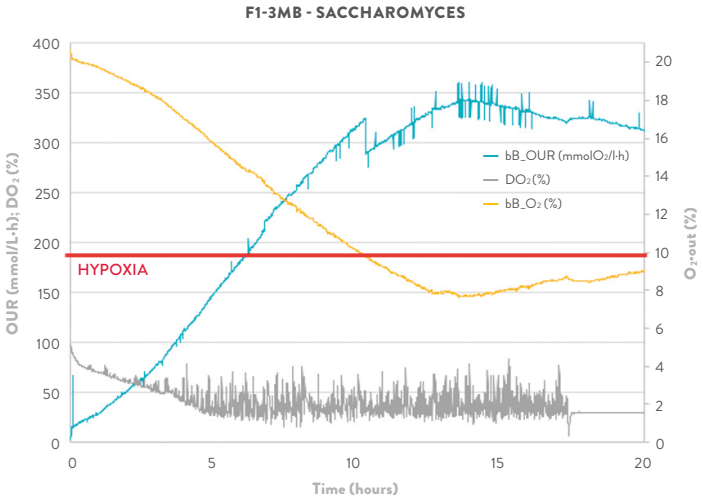

Fig. 4: Fermentation of the yeast Saccharomyces cerevisiae in fed-batch mode. Online calculation of the OUR according to the exhaust O2 and comparison with saturated pO_2 value.

Returning to the metabolic effect of the substrate, with the respiration parameters we have seen, the point at which the limiting substrate is depleted can be determined. There is a direct and immediate effect on the CO_2 values with the depletion of the substrate. As can be seen in figure 5, small pulsed additions generate an immediate effect, the response of the CO_2 being very sensitive.

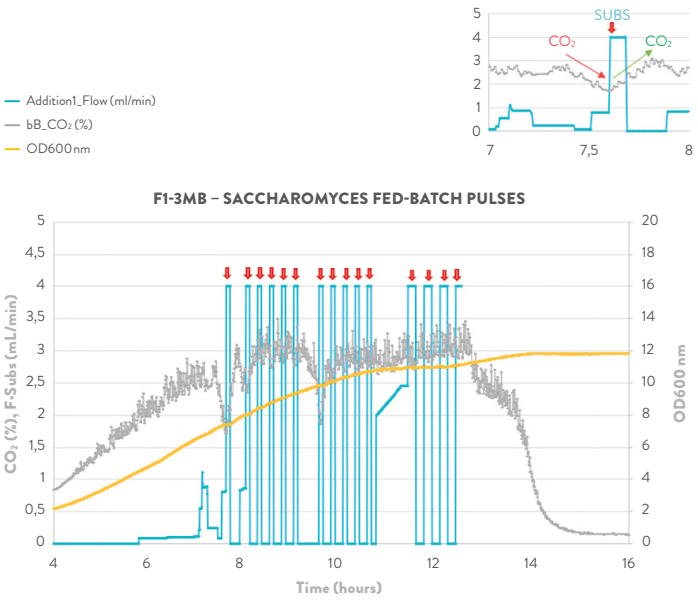

Fig. 5: Fermentation of the yeast Saccharomyces cerevisiae in fed-batch mode. Effect of the addition of substrate by pulses in the exhaust CO_2.

Therefore, the variation of the CO_2 trend can be used to trigger the automatic activation of the feeding pump for fed-batch strategies (Fig. 6). After a characterization in batch mode and in order to determine the maximum value of CO_2 reached until limiting substrate depletion, the addition pump can be activated automatically once a drop is measured in the exhaust gas CO_2 value from this maximum. In this case, an offset time must be included in the criteria for the transition between stages in the activation of the addition pump to avoid false triggers.

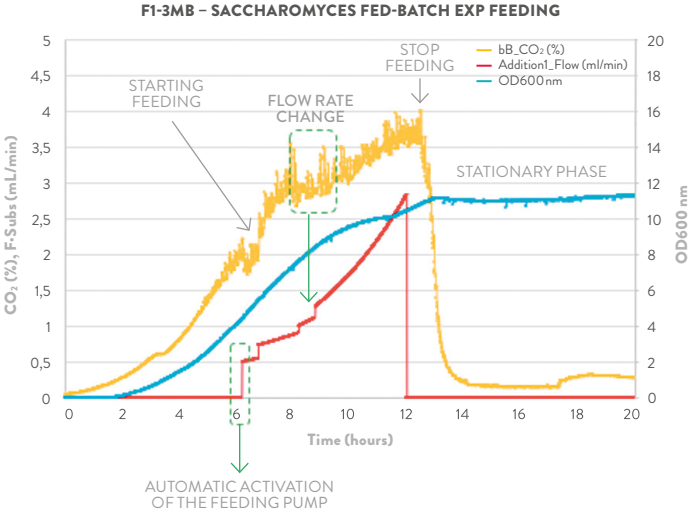

Fig. 6: Fermentation of the yeast *Saccharomyces cerevisiae* in fed-batch mode. Automatic activation of the addition pump using a recipe according to the values of the CO_2 and effect of the feeding in the evolution of the culture.

CONCLUSIONS

The conclusions about the possibilities of using an off-gas analyzer in a fermentation process:

- The respiration can be used to determine the depletion of the limiting substrate or an excessive concentration of substrate, which can inhibit the growth triggering a reduction of the CO_2 production or O_2 consumption.
- The Carbon dioxide Evolution Rate (CER) or exhaust CO_2 values can be used to identify the different fermentation stages (lag, exponential and stationary) and to calculate parameters related to the microbial growth kinetic as the specific growth rate (μ) of the cell.
- The evolution of the CO_2 can be used for optimal strategies of feeding and reduce byproducts formation.
- The values of the Oxygen Uptake Rate (OUR) can be used for an optimal design and scale-up of the process and the equipment (OTR) to ensure the meet of the O_2 needs of the cells.
- The relation of the parameters OUR and CER can be used to establish optimal and balanced conditions of growth using the Respiratory Quotient (RQ)