Benefits of off-gas analysis –

Using OUR and CER as parameters for reliable "Batch-End-Detection"

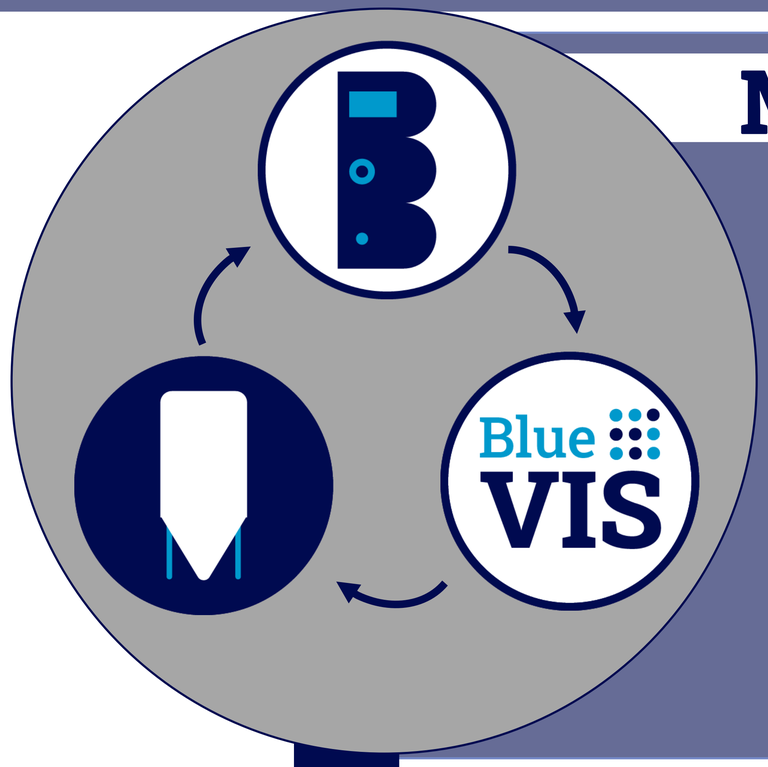
Smart Bioprocess Solutions

Nils Arto[1], *Garry Schulze*[1], *Matthias Wiltfang*[1], *Dr. Holger Müller*[1]

[1]*BlueSens gas sensor GmbH, Herten, North Rhine-Westphalia, Germany*

Introduction

Fed-Batch fermentation is often the favored mode of cultivation [1]. The most common method for detecting the end of the batch phase is the use of DO-Sensors. If a substrate depletion occurs, the DO-value will spike since cells will stop to perform respiration immediately [2]. However, DO-values provide only limited insight into the reactions taking place during cultivation. During a batch phase with different substrates (diauxic growth), the DO-value shows only small events if the metabolism is changing from one substrate to another. A combined use of OUR and CER can be used to trigger the feeding phase on one hand and give deeper insights into the metabolic activity on the other.



Methods

To ensure a reliable Batch-End-Detection (BED), the values of CER and OUR are used in combination to trigger the transition from batch to feed phase. *Saccharomyces cerevisiae* was used to test the conditions of the BED. This Crabtree-positive organism will produce ethanol during the batch phase due to high glucose concentration [3]. The BED was therefore prepared in a way that the feeding is started only after full depletion of all carbon sources, meaning that also ethanol has to be consumed.

1. Condition: Drop of OUR due to substrate limitation
Calculation of the smoothed dOUR value. The dOUR value has to drop below a given threshold of -0.075 mmol/L.

2. Condition: Rise of cumulative CER (cCER)
Maximum values for cCER under optimal growth conditions can be calculated based on the initial substrate concentration if biomass yields are known. A concentration of 10 g/L glucose was used as initial substrate leading to a theoretical maximum cCER of 163 mmol/L if glucose is solely used to produce energy and biomass ($Y_{X/S,\,max}$ = 0.51 g_X/g_S [4]).

Results

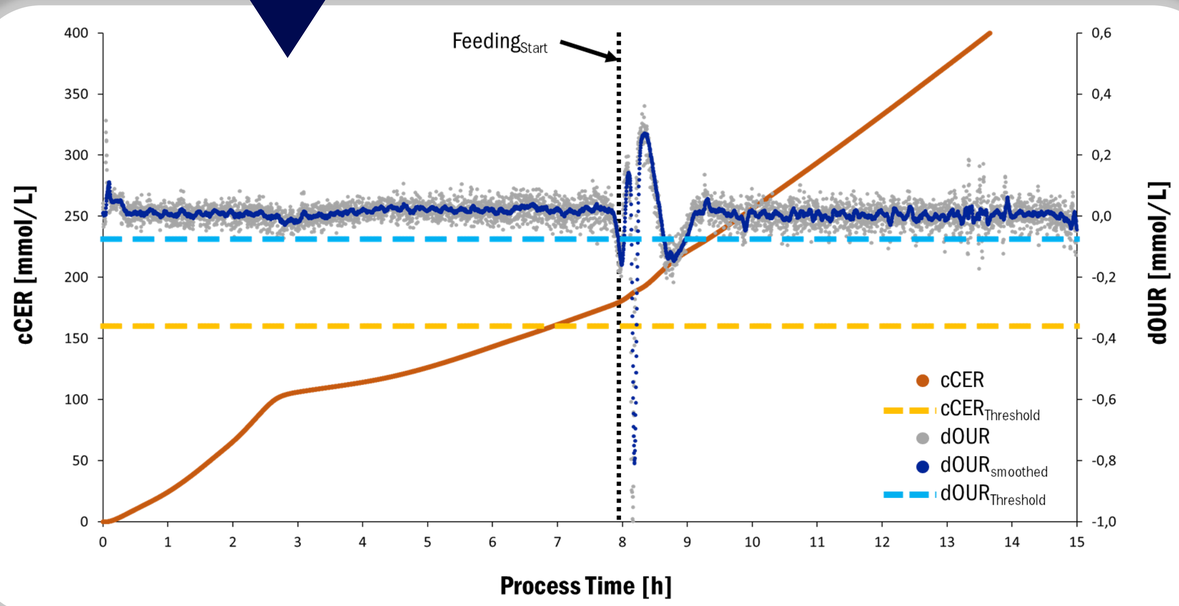


Figure 1: Illustration of the batch end detection based on dOUR and cCER; Calculated values are displayed as dots; Orange = cCER [mmol/L], Gray = dOUR [mmol/L], Dark blue = $dOUR_{smoothed}$ [mmol/L]; Thresholds are displayed as dotted line; Yellow = cCER [mmol/L], Light blue = dOUR [mmol/L]

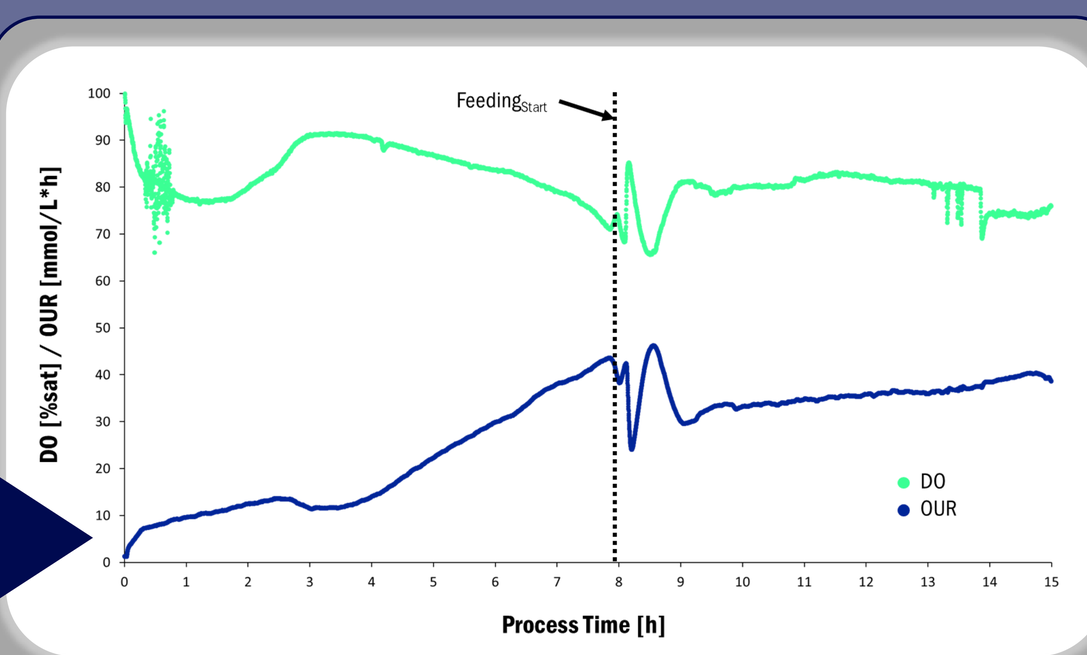


Figure 2: Illustration of the course of DO and OUR; Light green = DO [%sat], Dark blue = OUR [mmol/L*h]

The feeding was initiated after 7.9 h, when full depletion of carbon source occurred and the thresholds for both conditions were exceeded (Figure 1). The comparison of DO and OUR (Figure 2) shows that both values follow each other reciprocally and give only small hints about the metabolic activity. The RQ value, as a combination of OUR and CER, provides much more information about the processes taking place (Figure 3).

During the first segment of the batch phase, ethanol is produced due to high glucose concentrations, resulting in a RQ above 1. After 3 h the RQ drops to a value around 0.6, indicating glucose depletion and the switch to ethanol consumption [5]. This could be verified by measuring the ethanol concentration using an inline ethanol sensor (FMC Mini – Biotechnologie Kempe GmbH). The limitation of ethanol starts the addition of glucose feeding solution in a way that glucose is directly consumed, leading to a RQ of 1.

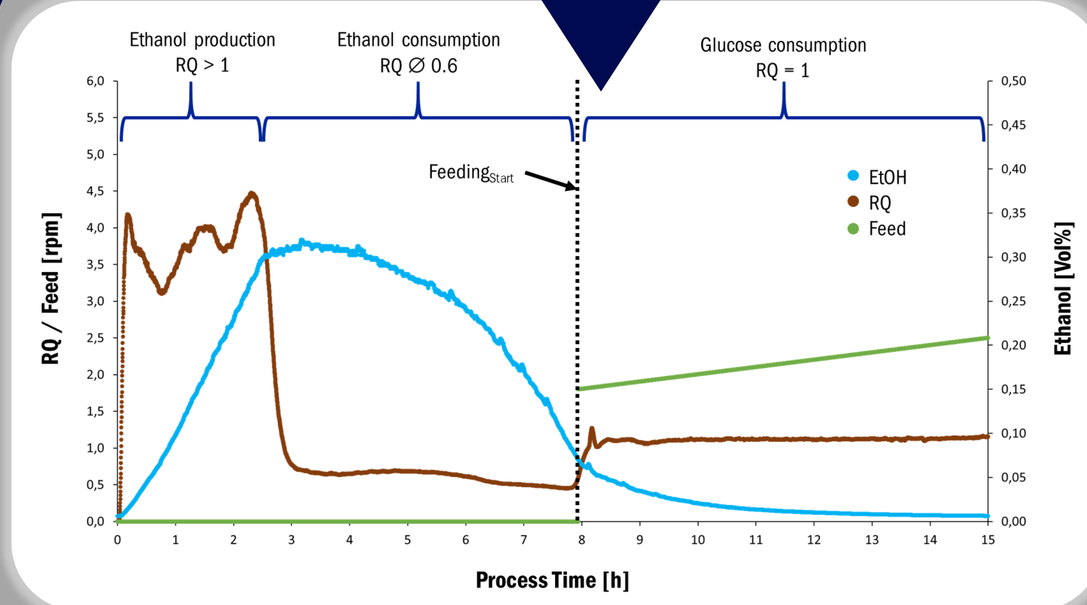


Figure 3: Illustration of the course of RQ, Feed and Ethanol concentration and division into different metabolic phases; Light blue = Ethanol [Vol%], Brown = RQ, Green = Feedrate [rpm]

Conclusion

It was possible to show in several experiments with different substrate concentrations that the transition from batch to feed phase can be triggered by a combination of CER and OUR. In Crabtree-positive yeasts in particular, it is possible to distinguish between glucose and ethanol depletion and to start feeding only if all carbon sources are fully depleted. Leading to a more efficient use of carbon sources. Using CER and OUR as control parameters, the RQ can be used to follow the metabolic activity of the organism and maintain substrate concentration in a glucose-limiting way to prevent further formation of ethanol. The same method can also be used for other processes in which diauxic growth occurs due to the composition of the medium or a metabolic overflow. Examples like this show how off-gas analysis can help to improve fermentation processes beyond their actual purpose.

BlueSens gas sensor GmbH
Snirgelskamp 25
45699 Herten
Germany

BlueSens Corporation
1001 Mittel Drive
Wood Dale, IL 60191
USA

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