Application Note

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Cedex Bio HT Analyzer as a tool for high-throughput analysis for fast physiological characterization of microbial bioprocesses

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Introduction

Bioprocess development is a laborious and expensive task, taking several years from lab scale to production scale. In particular, the physiology of the applied cell factories and their physiological response to varying process parameters must be investigated in detail for sound science-based process development and scale-up. In order to accelerate development times, several approaches have been applied in recent years. On the one hand, multi-bioreactor systems have gained in importance. These systems enable the user to perform multiple parallel fermentations to investigate the influence of various process parameters on the performance of the cells. A second approach is the use of dynamic experiments, where process parameters are changed dynamically during a single experiment; the influence of the parameters on the cells at changing levels can be investigated during a single cultivation. Here, high measurement frequencies are important to properly resolve process events and the physiological response of the cells to the investigated process parameters in order to improve process understanding. Both approaches result in a high number of samples, making the fast analysis of the key metabolites a bottleneck in process development. Accurate high-throughput devices are needed to yield the required measurement data at a time adjacent to the process for subsequent characterization of the strain physiology. In this article, we describe the application of the Roche Cedex Bio HT Analyzer for the fast and robust analysis of extracellular metabolites during bioprocesses of recombinant protein-producing *E. coli*. We demonstrate the applicability for fast analysis of microbial batch and fed-batch processes, and show the advantage of the Cedex Bio HT Analyzer when quickly dealing with a high number of samples for highly time-resolved analysis of a complex dynamic experiment to determine key physiological parameters.

For use in quality control/manufacturing process only.

Materials and Methods

We used an industrial strain of *Escherichia coli* overexpressing a recombinant protein, and performed batch cultivations in shake flasks at 37 °C as well as fed-batch fermentations and dynamic pulse-feeding experiments at 35 °C using the DASGIP Microbiology PD system (DASGIP GmbH, Jülich, Germany). For all cultivations, a chemically defined medium was used containing glucose as the sole carbon source. Induction of recombinant protein production was performed by addition of IPTG to the culture.

Samples were taken in regular intervals to monitor biomass formation and metabolite exchange rates. Sampling of batch and fed-batch cultivations was performed manually. For the highly time-resolved sampling of culture supernatant from dynamic experiments, samples were constantly removed from the bioreactor using a membrane probe connected to a peristaltic pump at a pump speed of 0.2 ml/min combined with the OAS-3x00TXRS Autosampler (Thermo Scientific, Dreireich, Germany).

To determine biomass formation, cell pellets of 2 ml samples from bioreactors were washed twice with deionized water and dried for 48 hours at 105°C. Dry cell weight (DCW) was determined gravimetrically. The uptake and production of the metabolites glucose, acetate, ammonia, and the important salts phosphate and magnesium were determined photometrically using the Cedex Bio HT Analyzer. Samples from the bioreactors were centrifuged at 13,000 × *g* for 5 minutes to remove cells from the culture supernatant. Small sample volumes (200 µl) of culture supernatant were sufficient to perform the determination of the above-described metabolites. The obtained data were used to calculate specific rates of uptake and production of metabolites, yielding important physiological information.

Results

3.1 Application to batch and fed-batch applications of *E. coli*

Initially, batch cultivations in shake flasks were performed to estimate the performance of the Cedex Bio HT Analyzer when dealing with samples from microbial cultivations. Batch cultivations were performed in triplicate. Approximately 3.1 g/l of biomass were produced during the exponential growth phase (Figure 1A). The time course of metabolite concentrations for glucose, acetate, ammonium, phosphate, and magnesium was determined from supernatant samples analyzed via the Cedex Bio HT Analyzer, and is shown exemplarily for one shake flask in Figure 1A and Figure 1B. Due to the easy and fast analysis of culture supernatants with the Cedex Bio HT Analyzer, the progression of metabolites could be monitored almost in real-time for three parallel shake flasks. After 7.5 hours of cultivation, glucose was completely consumed while 1.2 g/l actetate had been produced by the cells. The acquired data were used to calculate important physiological parameters, namely, the specific growth rate µ, the biomass yield $Y_{X/S}$, the specific rate of acetate production qAc, and the specific consumption rates of glucose q_{Glc} and ammonia q_{NH3}. The results are depicted in Table 1. Due to the high accuracy of the Cedex Bio HT Analyzer measurements, a very low error between cultivations was observed.

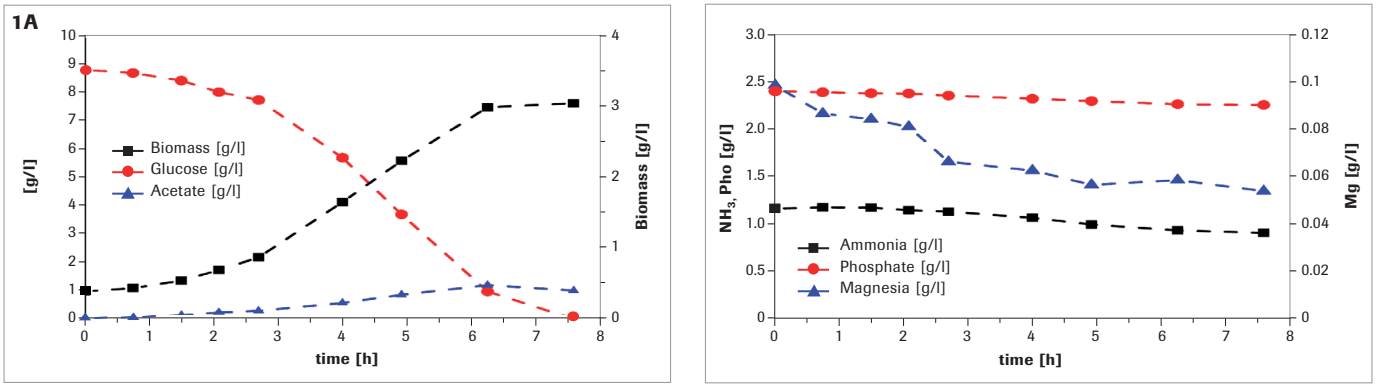

Figure 1: Application of the Cedex Bio HT Analyzer for monitoring of *E. coli* batch cultivations in shake flask for A) glucose consumption and acetate production; B) consumption of NH_3, magnesium and phosphate.

Results

Next, we wanted to demonstrate that the Cedex Bio HT Analyzer is also applicable to high cell density fed-batch cultivations, which are routinely performed during bioprocess development.

We performed a cultivation of an induced recombinant protein-producing *E. coli* strain. The bioprocess was divided into two phases: an initial fed-batch phase for biomass formation followed by an induction phase with lowered feeding rate for product formation. Cumulative amounts for biomass formation and glucose addition are depicted in Figure 2A. The fermentation supernatant was analyzed using the Cedex Bio HT Analyzer. During the feeding and the induction phase, the culture was glucose-limited, as can be seen from the low residual concentration of glucose during the cultivation (Figure 2B). No acetate accumulation occurred during the process, as demonstrated from the very low acetate level detectable in the culture supernatants. Further, ammonia, phosphate, and magnesium were analyzed to verify that none of these metabolites became limiting during the cultivation, and also at high cell densities over 30 g/l (Figure 2B). We used these results to calculate the physiological parameters μ, q_s, and $Y_{X/S}$ for the feeding phase, and the induction phase (Table 1). During the feeding phase, the growth rate was kept at 0.20 h^{-1} and switched to 0.025 h^{-1} for the induction phase. This resulted in a qs of 0.08 g/(gh) and 0.01 g/(gh) during the feeding and the induction phase, respectively. During the induction phase, the biomass yield $Y_{X/S}$ decreased constantly from 0.42 g/g to a final value of 0.31 g/g. Thus, prolonged recombinant protein production posed an increasing burden to the cell, reflected in the decreasing biomass yield.

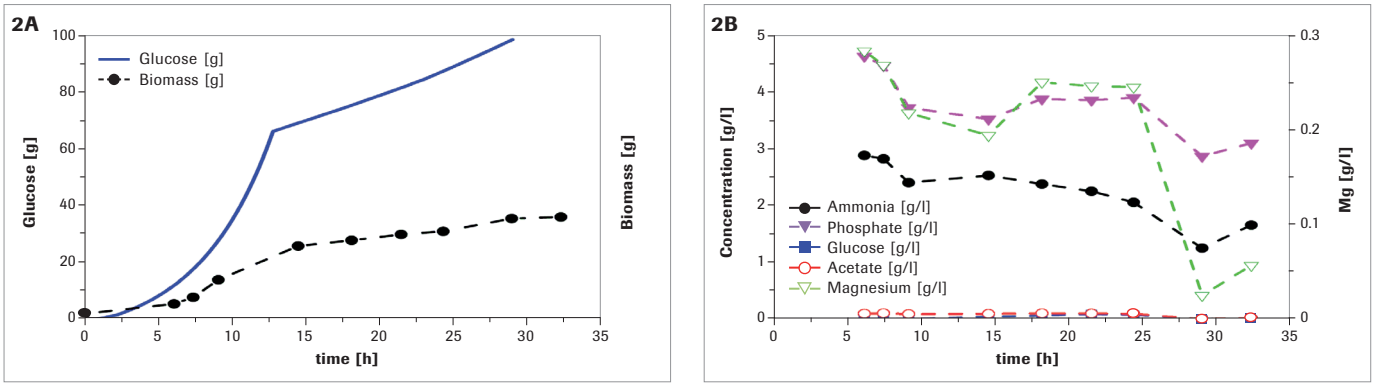

Figure 2: Application of the Cedex Bio HT Analyzer for monitoring of recombinant protein-producing *E. coli* fed-batch cultivations in bioreactors for A) glucose consumption and acetate formation; B) levels of magnesium and phosphate.

	Batch	Fed-Batch: Feeding phase	Fed-Batch: Induction phase
μ [h^{-1}]	0.41 ± 0.00	0.20	0.022 ± 0.00
$Y_{X/S}$ [g/g]	0.33 ± 0.01	0.42	0.31
q_{Glc} [g/(gh)]	1.10 ± 0.02	0.08	0.01
q_{Ac} [g/(gh)]	0.17 ± 0.01	-	-
q_{NH3} [g/(gh)]	0.042 ± 0.001	0.023	0.004

Table 1: Physiological characteristics of the applied *E. coli* strain during batch and fed-batch cultivations. Average values over the respective phases are given for: specific growth rate μ; biomass yield from glucose $Y_{X/S}$; specific production rate of acetate q_{Ac}; specific uptake rates of glucose q_{Glc} and NH_3 q_{NH3}. Average values with standard deviations obtained from triplicate measurements are shown.

3.2 Application to highly time-resolved dynamic experiments

Dynamic experiments are powerful tools to elucidate the physiological response of microbial cultures to changing conditions in a single experiment. Using an induced *E. coli* culture, the physiological changes due to the increasing metabolic burden of recombinant protein production on the physiology of the culture were investigated. The cells were cultivated in batch to a cell density of 3.8 g/l before glucose was depleted. Next, three subsequent pulses of glucose were added to the bioreactor (Figure 3A) to evaluate the effect of recombinant protein production on μ_{max}, $q_{s,max}$, and q_{Ac}.

A prerequisite for reliable characterization of these effects is highly time-resolved monitoring of the consumption and production of key metabolites. Such monitoring makes it possible to observe small but significant changes and to detect process events important for bioprocess development.

To realize highly time-resolved monitoring of the process, cell-free culture supernatants were automatically sampled from the bioreactor. Using the Cedex Bio HT Analyzer for metabolite analysis of the automatically collected samples, we measured the main metabolites (glucose, acetate, phosphate, magnesium, and ammonia) of 86 samples, resulting in 430 measurements in less than 2 hours. This enabled us to monitor consumption of glucose and production of acetate for all three glucose pulses with a time resolution between 20 and 30 minutes (Figure 3A). Moreover, by measuring phosphate and magnesium, we could verify that that both important salts were never limiting during the experiment, also at high cell densities of over 20 g/l (Figure 3B). A final biomass density of 24 g/l was determined from manually collected cultivation samples.

The highly time-resolved monitoring of the cultivation using the high-throughput Cedex Bio HT Analyzer enabled us to observe various changes in culture physiology with increasing duration of recombinant protein production.

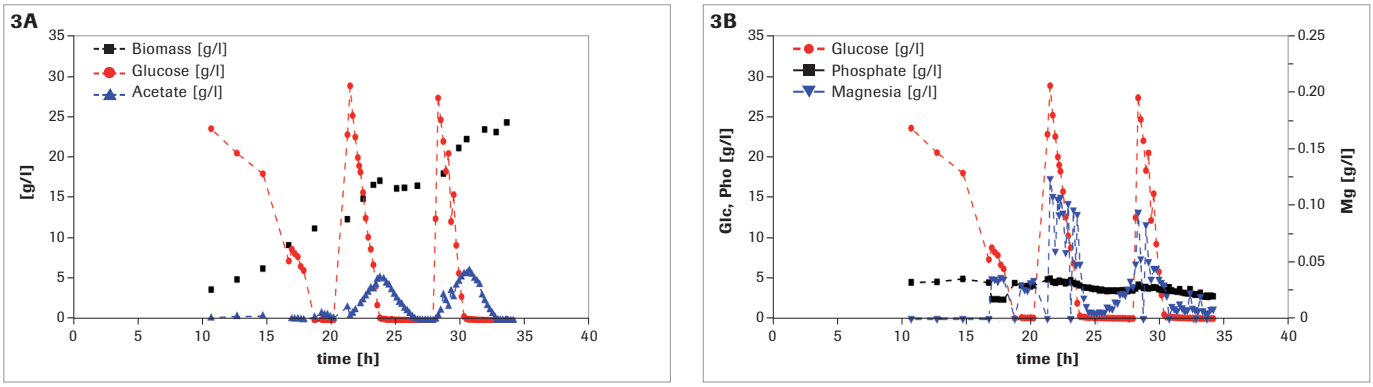

Figure 3: Application of the Cedex Bio HT Analyzer for monitoring of recombinant protein-producing *E. coli* during bioreactor cultivations with addition of three subsequent glucose pulses. A) glucose consumption and acetate formation; B) levels of magnesium and phosphate.

Results

The obtained data were used to calculate μ_{max}, $q_{Glc,max}$, and q_{Ac}, as well as the biomass yield $Y_{X/S}$ and yield of acetate from glucose $Y_{Ac/Glc}$ for each pulse. The specific growth rate decreased after the first pulse from 0.140 h^{-1} to 0.120 h^{-1} for the third pulse. The biomass yield also decreased with each pulse. Thus, prolonged metabolic burden of recombinant protein production led to a decrease of the maximum cellular growth capabilities reflected in $Y_{X/S}$ and μ_{max}. At the same time, however, $q_{s,max}$ increased with each pulse, indicating a higher demand of the cell for carbon. Moreover, with each pulse, acetate formation increased as well as $Y_{Ac/Glc}$.
This indicated a strong redistribution of the metabolism, most likely as an effect of cellular stress to meet the increased metabolic burden associated with high-level recombinant protein production.

	μ [h^{-1}]	$Y_{X/S}$ [g/g]	q_{Glc} [g/(gh)]	q_{Ac} [g/(gh)]	$Y_{Ac/Glc}$ [g/g]
Pulse 1	0.140	0.312	0.445	0.022	0.050
Pulse 2	0.142	0.190	0.767	0.107	0.139
Pulse 3	0.119	0.120	1.08	0.151	0.140

Table 2: Physiological parameters derived from a dynamic experiment using three subsequent glucose pulses. Shown are: the specific growth rate μ; the biomass yield from glucose $Y_{X/S}$; the specific production rate of acetate q_{Ac}; and specific uptake rate of glucose q_{Glc}.

Conclusions

- The Cedex Bio HT Analyzer from Roche was successfully applied to analyze the key metabolites glucose, acetate, ammonia, phosphate, and magnesium produced and consumed during microbial *E. coli* bioprocesses.
- Concentration profiles of these metabolites during batch and fed-batch fermentations and dynamic experiments of recombinant protein producing *E. coli* were recorded and used to calculate key physiological parameters for fast process and strain characterization.
- The obtained data were highly reproducible due to the high accuracy of the Cedex Bio HT Analyzer measurements, which was reflected in the low error on the derived physiological parameters.
- Highly time-resolved monitoring of a dynamic pulse experiment of an induced *E. coli* culture could be performed in very short time using an automated sampling unit and the high-throughput Cedex Bio HT Analyzer.

The Cedex Bio HT Analyzer is a powerful tool for high-throughput analysis of key metabolites of microbial bioprocesses. Using this tool, analytical bottlenecks due to high sample numbers can be easily overcome.

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