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Linear Correlation between Online Capacitance and Offline Biomass Measurement up to High Cell Densities in *Escherichia coli* Fermentations in a Pilot-Scale Pressurized Bioreactor

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To yield high concentrations of protein expressed by genetically modified *Escherichia coli*, it is important that the bacterial strains are cultivated to high cell density in industrial bioprocesses. Since the expressed target protein is mostly accumulated inside the *E. coli* cells, the cellular product formation can be directly correlated to the bacterial biomass concentration. The typical way to determine this concentration is to sample offline. Such manual sampling, however, wastes time and is not efficient for acquiring direct feedback to control a fed-batch fermentation. An *E. coli* K12-derived strain was cultivated to high cell density in a pressurized stirred bioreactor on a pilot scale, by detecting biomass concentration online using a capacitance probe. This *E. coli* strain was grown in pure minimal medium using two carbon sources (glucose and glycerol). By applying exponential feeding profiles corresponding to a constant specific growth rate, the *E. coli* culture grew under carbon-limited conditions to minimize overflow metabolites. A high linearity was found between capacitance and biomass concentration, whereby up to 85 g/L dry cell weight was measured. To validate the viability of the culture, the oxygen transfer rate (OTR) was determined online, yielding maximum values of 0.69 mol/l/h and 0.98 mol/l/h by using glucose and glycerol as carbon sources, respectively. Consequently, online monitoring of biomass using a capacitance probe provides direct and fast information about the viable *E. coli* biomass generated under aerobic fermentation conditions at elevated headspace pressures.

Keywords: Capacitance measurement, *Escherichia coli*, high cell-density cultivation, online signal

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Using *E. coli* in high cell-density cultures (HCDC) is one of the most widely used techniques for producing heterologous proteins [26]. Because most protein products generated from recombinant *E. coli* strains are accumulated intracellularly, the protein concentration is one important parameter that is strongly correlated to biomass concentration [4]. HCDC are normally grown under nutrient-limiting (carbon-limiting) conditions. However, simple feeding strategies such as constant-rate feeding, a stepwise increase in the feeding rate, and exponential feeding have been all used to obtain high cell densities and high space-time yields of *E. coli* in fed-batch cultures. The last strategy (*i.e.*, exponentially increased feeding rates) can be used as a simple yet efficient method to minimize overflow metabolites such as acetate, provided that the specific growth rate for *E. coli* is maintained between 0.1 h^{-1} and 0.3 h^{-1} [15]. For this, many authors [9, 12, 14, 15, 26] take advantage of an equation [see Eq. (1)] derived from a simple mass balance with the assumption of a constant cell yield on the used substrate. Consequently, with this equation, a predetermined, open-loop, exponentially increasing amount of feeding solution is fed into the reactor without feedback from the biology.

With regards online measurement techniques for detecting the viability and biomass concentration, Fyferling *et al*. [8] recommend online monitoring of the oxygen transfer rate (OTR). Understanding the interaction between oxygen transfer and the microbial activity is a key step in achieving high yields of the target protein and improving bioprocess strategies. In particular, most industrial fed-batch fermentations are limited by the transfer of oxygen into the broth [24]. Various authors [2, 8, 11, 12] reported about high cell-density fermentations with *E. coli* using online measurement of oxygen consumption, such as OTR, in order to validate biomass growth and characterize cell viability.

Using electrical capacitance for ascertaining viable biomass, Markx and Davey [18] explained the electrical properties of biological cells. Various authors have already generated datasets for fermentations with bacterial species (*e.g.*, *Streptomyces* or *Lactobacillus* [3, 7, 22]), yeast species (*e.g.*, *Saccharomyces* or *Arxula* [19–21, 27]), as well as animal cell cultures such as hybridoma cells [23] and Chinese hamster ovarian cells [5]. With respect to high *E. coli* densities generated in fed-batch culture, Kaiser *et al.* [11] have reported reliable capacitance values for viable cell density up to biomass concentrations of 60 g/l dry cell weight.

The aim of this study was to investigate on a pilot scale the progression of an impedance signal in high cell-density fermentations of *E. coli* having dry cell weights of up to 85 g/l. In order to ensure fully aerobic conditions over the entire fermentation time, high cell-density fermentations are performed at a relatively moderate specific power input (3–6 kW/m^3) using a pressurized stirred tank bioreactor [12, 16]. Furthermore, in this study, the *E. coli* cultures were cultivated in fed-batch mode at a low constant specific growth rate (μ=0.17 h^{-1}) to ensure carbon-limiting conditions, thereby minimizing the production of overflow metabolites. As the optimization of the fermentation was not the main focus of this work, a simple, well accepted, and robust open-loop feeding strategy was used.

Materials and Methods

Microbial Strain

In this study, the *Escherichia coli* K12-derived strains W3110 (ATCC No. 27325) and VH33 were used. Strain VH33 is a Δ*ptsH*, *ptsI*, *lacI*, *lacZ*::*lox*P W3110 derivative that lacks PTS activity. In order for glucose to pass the plasma membrane, the galactose permease (GalP) as an alternative uptake mechanism was amplified on a genetic level [6].

Culture Media

For the first preculture, TB media (Terrific Broth) was used containing, in g/l, yeast extract (Roth, Karlsruhe, Germany), 24; peptone from casein (Roth, Karlsruhe, Germany), 12; KH_2PO_4, 4.72; K_2HPO_4, 11.36; and glucose, 10 or glycerol, 10. For the second preculture, the media PAN-SF contained, in g/l, KH_2PO_4, 8.66; K_2HPO_4, 23.74; triNa-citrate·2H_2O, 1; $ZnCl_2$, 0.1; $FeSO_4$·7H_2O, 0.15; $(NH_4)_2SO_4$, 5; $CaCl_2$·2H_2O, 0.02; $MgSO_4$·7H_2O, 0.3; glucose, 20 or glycerol, 20; PAN trace element solution, 1 ml/l; and thiamine solution, 1 ml/l. For the main culture, the media PAN-F consisted of, in g/l, KH_2PO_4, 17; triNa-citrate·2H_2O, 1.5; $ZnCl_2$, 0.1; $FeSO_4$·7H_2O, 0.15; $(NH_4)_2SO_4$, 5; $CaCl_2$·2H_2O, 0.02; $MgSO_4$·7H_2O, 3; glucose, 5 or glycerol, 5; PAN trace element solution, 1.5 ml/l; and thiamine solution, 2 ml/l. The PAN trace element solution contained, in g/l, $Al_2(SO_4)_3$·18H_2O, 2; $CoSO_4$·6H_2O, 0.75; $CuSO_4$·5H_2O, 2.5; H_3BO_3, 0.5; $MnSO_4$·H_2O, 24; Na_2MoO_2·2H_2O, 3; $NiSO_4$·3H_2O, 2.5; $ZnSO_4$·7H_2O, 15; and H_2SO_4 (30%), 2 ml/l. The thiamine solution consisted of 5 g/l.

The feed solution contained glucose, 650 g/l (still soluble at room temperature) or glycerol, 870 g/l (prefabricated solution, Applichem, Darmstadt, Germany); PAN trace element solution; 1.5 ml/l; and thiamine solution, 2 ml/l.

Cultivation

The organism was stored in complex medium (TB) containing 33% (v/v) glycerol at –80°C before the two preculture steps were begun to inoculate the main culture in the bioreactor. All precultures were performed in 1,000-ml Erlenmeyer shake flasks at shaking frequencies of 300 rpm and 50 mm shaking diameter (Kuhner shaker, ISF1-X, Basel, Switzerland) at 37°C. The applied media and shaking conditions were first investigated by using a Respiration Activity MOnitoring System (RAMOS) [1] equipped with 250-ml RAMOS shake flasks, to avoid depletion of the carbon source over the entire preculture time and to ensure a viable broth for inoculating of the main culture. Thereafter, the shaking conditions (filling volume, shaking frequency, and shaking diameter) were converted from the 250-ml to the 1,000-ml shake flask scale, applying the method described by Maier *et al.* [17] (data not shown).

Escherichia coli VH33 was cultivated in the first preculture step in 50 ml of TB medium for 4 h. In the second preculture step, 1.2 l of PAN-SK medium was inoculated with the total amount of the first preculture, and this broth was devided among 12 shake flasks. Then, the bacteria in the flasks were cultivated for 16 h. Upon applying glucose or glycerol as a C-source for the main culture in the batch and fed-batch phases, the preculture was cultivated using the identical C-source, respectively.

High cell-density cultivations in the main culture were performed in a 50-l stirrer tank bioreactor (LP 351; Bioengineering AG, Wald, Switzerland), which is described in detail in Maier *et al.* [16] and Knoll *et al.* [12]. Starting in batch mode, 14 l of the main culture medium (PAN-F) with the respective carbon source (glucose, or glycerol) was inoculated with 1.2 l of the second preculture. After the carbon source was depleted – indicated by a sharp increase in the DOT signal – the fed-batch phase was started. To obtain a constant growth of *E.coli* during the fed-batch phase under carbon limitation, an exponential feeding rate was applied that was calculated according to Eq. (1):

$$F = \left(\frac{\mu_{set}}{Y_{X/S}} + m_s\right) \cdot \rho_F \frac{c_{XF} \cdot V_{LF}}{c_{SF}} \cdot e^{\mu_{set} \cdot (t - t_F)} \quad (1)$$

The numerical values used for Eq. (1) were entered in the algorithm before the respective fermentations (main culture) had been started. Thereby, numerical values used for calculating F are presented in the caption of Fig. 1 and Fig. 2, respectively. Here, values for the biomass concentration at the beginning of the fed-batch phase (c_{XF}) and the biomass yield ($Y_{X/S}$) were determined from preliminary batch fermentations in the stirred tank reactor, applying identical conditions also for the preculture (*e.g.*, growth on minimal medium; data not shown). Using these preliminary batch fermentations, the length of the batch phase t_F could be estimated and the maximum specific growth rate μ_{max} was determined at a value of 0.21 h^{-1}. As a result, for all fermentations, the specific growth rate μ_{set} was arbitrarily chosen below μ_{max} at 0.17 h^{-1} to guarantee carbon limitation and to avoid overflow metabolism [15].

To suffice the oxygen demand of the fermentations and to prevent an oxygen limitation, the headspace pressure of the bioreactor was incrementally increased to a maximal overpressure of 9.0 bar

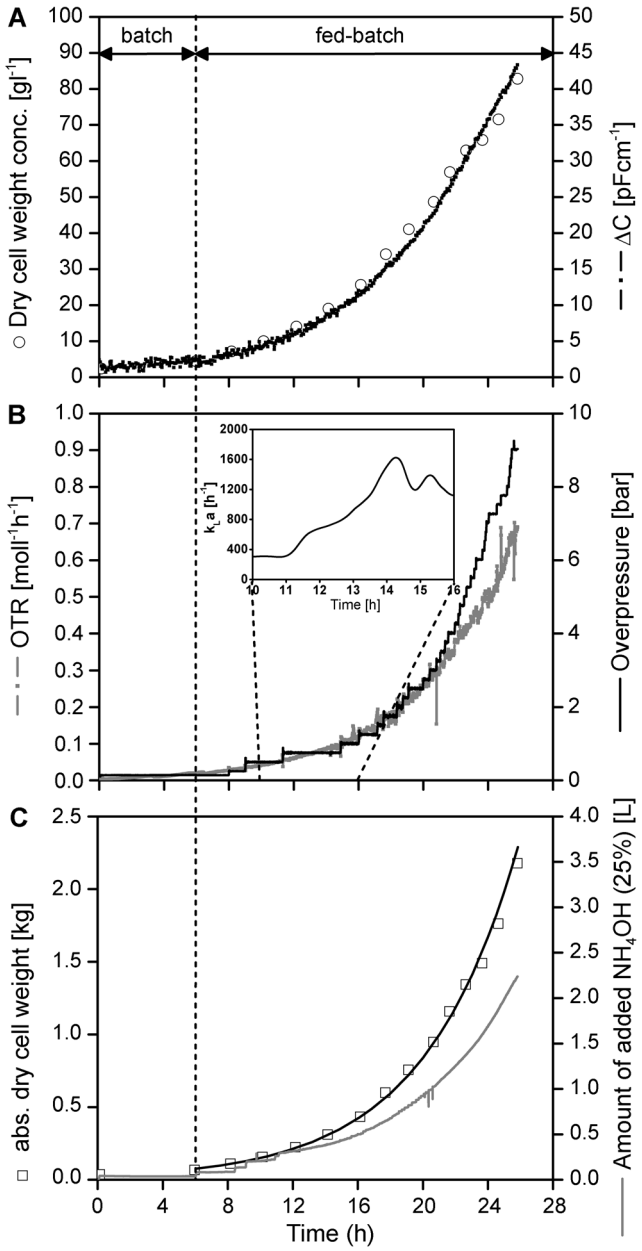

Fig. 1. High cell-density cultivation of *E. coli* VH33 in minimal medium with glucose as the C-source.
(**A**) Dry cell weight concentration, ΔC (capacitance increment); (**B**) OTR, overpressure, k_La value (inlay); (**C**) absolute dry cell weight (open squares) and a curve fitted with μ_{exp}=0.172 h^{-1}, amount of added NH_4OH solution (25%) for pH titration; initial glucose concentration 5 g/l, superficial gas velocity 0.00179 m/s, temperature 37°C, pH 7.0±0.1, constant stirrer speed of 500 rpm. DOT was above 30% air saturation during the whole fermentation time. Parameters for the calculation of the feed rate, according to Eq. (1): reactor filling volume at beginning of fed-batch 15.2 l, concentration of biomass at beginning of fed-batch 4.4 g/l, biomass yield 0.44 g/g, concentration of glucose in feed solution 650 g/l, density of feed solution 1,210 g/l, setpoint of specific growth rate 0.17 h^{-1}.

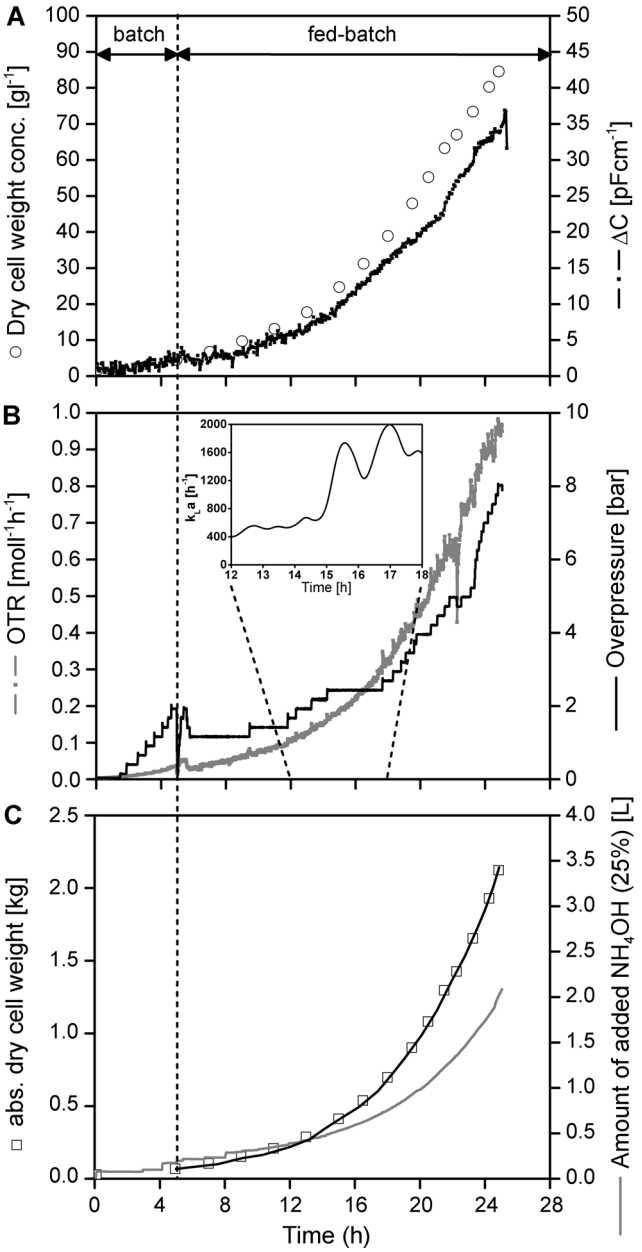

Fig. 2. High cell-density cultivation of *E. coli* VH33 in minimal medium with glycerol as the C-source.
(**A**) Dry cell weight concentration, ΔC (capacitance increment); (**B**) OTR, overpressure, k_La value (inlay); (**C**) absolute dry cell weight (open squares) and a curve fitted with μ_{exp}=0.170 h^{-1}, amount of added NH_4OH (25%) for pH titration; initial glycerol concentration 5 g/l, superficial gas velocity 0.00179 m/s, temperature 37°C, pH 7.0±0.1, constant stirrer speed of 650 rpm. DOT was above 30% air saturation during the whole fermentation time. Parameters for the calculation of the feed rate: reactor filling volume at beginning of fed-batch 15.2 l, concentration of biomass at beginning of fed-batch 4.0 g/l, biomass yield 0.48 g/g, concentration of glucose in feed solution 870 g/l, density of feed solution 1,230 g/l, setpoint of specific growth rate 0.17 h^{-1}.

(0.90 MPa) to keep the DOT above a minimal value of 30% air saturation. Simultaneously, the aeration rate was increased to realize a constant superficial gas velocity inside the bioreactor [13] at a value of 0.00179 m/s. The pH was set to a value of 7 by adding

25% NH_4OH. During the cultivation using glucose as the C-source, the stirrer velocity was kept constant at a value of n=500 rpm. For the fermentation using glycerol as the C-source, the stirrer velocity was maintained at a value of n=650 rpm.

The specific volumetric power input P/V_L was calculated according to Eq. (2) by measuring the torque using a sensor installed at the bottom of the stirrer shaft [16].

$$\frac{P}{V_L} = \frac{2\pi n M}{V_L} \tag{2}$$

Analytical Methods

Capacitance measurement. An Aber Probe of 25 mm diameter, which incorporated four annular ring electrodes for detecting the capacitance of the broth, was connected to a Biomass Monitor 210 (Aber Instruments, Aberystwyth, Wales, UK) for determining the viable cell concentration. Placed in an Ingold connector at the side of the vessel, the probe was integrated in the *in situ* sterilization process of the reactor (121°C, 20 min). Capacitance values were calculated online and resulted from the difference between two measurement points made at two frequencies according to Eq. (3) [20]. The capacitance increment (ΔC) values calculated in the dual-frequency mode were transmitted to a computer using an automatic data acquisition system (ADC-16, PicoRecorder; Pico Technology Limited, UK).

$$\Delta C = C^{\beta} - C_{\infty} \tag{3}$$

whereby C^{β} (f = 1.0 MHz); C_{∞} (f = 15.649 MHz)

Offline detection of biomass concentration. The biomass concentration was determined as dry cell weight by centrifuging four preweighed Eppendorf tubes, each having a filling volume of 2 ml of culture broth. After centrifugation (18000 × RFC, 10 min, Sigma 1-15 microcentrifuge, Sartorius, Germany), the resulting biomass pellets were separated from the supernatant and stored at 80°C until the mass, weighed with an electronic precision balance (SBC 31, Scaltec, Göttingen, Germany), remained constant. The STY was derived from the mass of product m_X (in this case, dry biomass), the reactor filling volume V_L, and the associated cultivation time t, as follows:

$$STY = \frac{m_X}{V_L \cdot t} \tag{4}$$

O_2 and CO_2 measurement in the offgas for the calculation of OTR. The O_2 and CO_2 concentrations in the offgas were measured by means of an exhaust gas analyzer (Rosemount NGA 2000; Emerson Process Management GmbH&Co. OHG, Haan, Germany) by applying a paramagnetic analyzer and an infrared analyzer, respectively. According to Eq. (5), the O_2 consumption of the bacteria was measured *via* the oxygen transfer rate (OTR):

$$OTR = \frac{\dot{V}_G}{V_L \cdot V_{molar}} \cdot \left(y_{O_2,in} - \frac{1 - y_{O_2,in} - y_{CO_2,in}}{1 - y_{O_2,out} - y_{CO_2,out}} \cdot y_{O_2,out} \right) \tag{5}$$

Offline determination of glucose, glycerol, and acetate. To determine the glucose, glycerol, and acetate concentrations, the supernatant was measured by means of a HPLC with an organic acid resin column (CS-Chromatographie, Langerwehe, Germany) using a 5 mM sulfuric acid solution as eluent and a refractive index detector and a photodiode array.

Results and Discussion

High Cell-Density Cultivation with Glucose as C-Source

In the experiment shown in Fig. 1A, the culture broth was inoculated with *E. coli* VH33 at a starting concentration of 2.4 g/l dry cell weight. After the glucose had been totally consumed in the batch phase lasting 6 h, the feeding was begun. According to Eq. (1), the feeding rate F of a 650 g/l glucose solution was calculated using the parameters presented in the caption of Fig. 1. Starting with a dry cell weight concentration of 4.4 g/l, the biomass concentration increased exponentially to a maximum value of 83 g/l until the end of the fermentation (26 h). As a result, the STY was calculated at a value of 3.21 g/l/h. During the fed-batch phase, glucose and acetate were not detectable in the supernatants of the respective samples. Nearly congruently to the increase in dry cell weight, the capacitance increment ΔC rose to a maximum value of 44 pF/cm (Fig. 1A). The noise level of ΔC decreased from 2 pF/cm at the beginning to approx. 0.2 pF/cm at the end of the fermentation. This increasingly smoother course of ΔC could be attributed to a decreasing conductivity (start: 31 mS/cm; end: 15 mS/cm) of the broth (progression is not shown).

As shown in Fig. 1B, the OTR curve increased exponentially from 0.02 mol/l/h at the beginning of the fed-batch phase to a maximum value of 0.69 mol/l/h at the end of the fermentation. To our knowledge, the OTR_{max} value presented in Fig. 1B was the highest OTR value ever measured in a microbial fermentation using glucose as C-source. The continuous increase in the OTR signal indicated a viable biomass up to a concentration of nearly 90 g/l as well as aerobic culture conditions during the entire fermentation time. A sufficient oxygen supply for aerobic culture conditions in the batch phase could be guaranteed by maintaining the rotation speed of the stirrer at 500 rpm and an aeration rate of 0.5 vvm at ambient pressure. However, with increasing biomass concentration in the fed-batch phase, the headspace pressure was increased incrementally to a maximum of 9 bar overpressure (Fig. 1B). Starting at approx. 11 h, a plateau in the overpressure curve lasting ca. 4 h could be observed at 1 bar. By keeping the DOT above a minimal value of 30% air saturation, the overpressure did not have to be increased in this time range. Owing to the increasing reactor filling volume, the surface of the broth eventually contacted the second six-blade standard Rushton turbine [12] at a fermentation time of approx. 11 h. As a result, the k_La value increased (inlay of Fig. 1B), and the overpressure could be maintained constant for a time period of approx. 4 h. A Henry coefficient of 909 bar/l/mol was used to calculate the k_La value. The k_La value started at approx. 300 h^{-1} and remained constant at this value until it rose to

approx. 1,400 h^{-1} beginning at a fermentation time of ca. 11.4 h. After the second turbine had been completely covered by broth at a fermentation time of approx. 15 h, the overpressure had to be increased, according to Fig. 1B, so as to maintain the DOT above a minimal value of 30% air saturation.

As shown in Fig. 1C, the mathematical fitting of the absolute dry cell weight resulted in an experimentally determined specific growth rate μ_{exp} of 0.172 h^{-1}. It was necessary to calculate μ_{exp} from the absolute dry cell weight over the fermentation time rather than from the dry cell weight concentration, to exclude dilution effects of the broth due to the increasing reactor filling volume V_L during the fed-batch phase. The μ_{exp} of 0.172 h^{-1} almost completely agreed with the predetermined setpoint of the specific growth rate μ_{set} of 0.17 h^{-1} used for the calculation of the feeding rate [see Eq. (1)]. The amount of added NH_4OH (25%) started to increase exponentially at the beginning of the fed-batch phase at approx. 6 h fermentation time to maintain the pH at a constant value of 7 (Fig. 1C). The amount of added NH_4OH reached a maximum value of 2.24 l at the end of the fermentation. As a result, the yield coefficient $Y_{X/N}$ was calculated at 3.52 g/g. This value fell in the range of already reported literature data for *E. coli* of $Y_{X/N}$=1.65–4.94 g/g [4]. The NH_4OH curve (Fig. 1C) increased in parallel to the curve of the dry cell weight concentration (Fig. 1A). The consumption of base during the cultivation of *E. coli* could thus be used as a valuable signal for an online estimation of biomass and the specific growth rate [10, 25].

High Cell-Density Cultivation with Glycerol as C-Source

In the experiment shown in Fig. 2, glycerol was applied as C-source. According to Fig. 2A, the dry cell weight concentration in the batch phase increased from an initial concentration of 1.9 g/l to a concentration of 4.2 g/l. Once the glycerol was totally consumed within this time period of 5.5 h, the fed-batch phase was initiated. The feeding rate F of a 870 g/l glycerol solution was calculated according to Eq. (1) using the parameters presented in the caption of Fig. 2. After the fed-batch phase started, the biomass concentration increased exponentially to a maximum of 85 g/l until the end of the fermentation (25 h). As a result, the STY was calculated at 3.40 g/l/h. During the fed-batch phase, glycerol and acetate were not detectable in the supernatants of the respective samples. As shown in Fig. 2A, the curve of ΔC rose to 4 pF/cm at the end of the batch phase. After that, an exponential increase followed. At the end of the fermentation, ΔC reached a maximum of 37 pF/cm. The noise level of ΔC decreased from 2 pF/cm at the beginning to approx. 0.3 pF/cm at the end of the fermentation. The decrease in the noise level of ΔC could be explained, as in Fig. 1, by the decreasing conductivity (start: 31 mS/cm; end: 12 mS/cm) of the fermentation broth (progression not shown).

As shown in Fig. 2B, the OTR in the batch phase rose to a maximum of 0.06 mol/l/h until the glycerol had been totally depleted. To avoid oxygen limitation in this time period, the rotation speed of the stirrer had to be maintained at a value of 650 rpm from the beginning of the fermentation, and the overpressure had to be increased stepwise starting at 1.8 h fermentation time to a maximum value of 2.0 bar at the end of the batch phase. Although both fermentations shown in Fig. 1 and 2 were initiated with the same amount of carbon source (5 g/l glucose or glycerol, respectively), the overpressure had to be increased already in the batch phase using glycerol, as this carbon source is more reduced than glucose and requires more oxygen for complete oxygenation. This led to a higher OTR and higher values for the overpressure and the rotation speed of the stirrer, respectively. As shown in Fig. 2B, during the fed-batch phase, the OTR increased exponentially to a maximum of 0.984 mol/l/h. This value was, to our knowledge, the highest respiration activity ever detected in a microbial fermentation. To reach these high OTR values, the overpressure had to be increased incrementally to a maximum of 8 bar at the end of the fermentation. Here, this maximum value was approx. 1 bar lower than the overpressure needed in the fermentation using glucose as C-source (Fig. 1B). Thus, the rotation speed of the stirrer was maintained at 650 rpm over the entire fermentation time compared with 500 rpm using glucose. Again, the overpressure curve remained at a plateau of approx. 2.5 bar in the time period between 14 h and 18 h (Fig. 2B), comparable to the plateau observed during the fermentation using glucose as a carbon source in the time range between 11 h and 15 h (see Fig. 1B). During this time range of 14 h and 18 h (Fig. 2B) the OTR curve rose continuously. Here, an increase in the k_La value could be observed (inlay of Fig. 2B) that was caused by the surface of the broth contacting the second blade of the six-blade standard Rushton turbine, resulting from the rising reactor volume in the fed-batch mode. The k_La value started at approx. 400 h^{-1} to 600 h^{-1} and remained constant at this range until it rose to approx. 1,700 h^{-1} beginning at a fermentation time of ca. 15 h. Compared with the fermentation using glucose as C-source (Fig. 1B), the plateau was delayed for approx. 3 h, as shown in Fig. 2B, although a nearly congruent pattern in biomass concentration of both fermentations could be observed (Fig. 1A and 2A). Thus, this time shift could be attributed to the increased substrate concentration in the feed applying glycerol (870 g/l) compared with that with glucose (650 g/l). Consequently, the reactor filling volume rose slower, thereby delaying the broth touching the second turbine blade. Because an antifoam agent had to be added to counteract foam formation at ca. 22 h, a short decrease in OTR could be

observed at this time. Therefore, the continuous increase in overpressure was disrupted between the fermentation times of 22 h and 23 h, and a plateau lasted for approx. 1 h.

The experimentally determined specific growth rate μ_{exp} was calculated at 0.170 h^{-1} according to a mathematical fitting of the absolute dry cell weight vs. fermentation time (Fig. 2C). The μ_{exp} fitted precisely to the predetermined setpoint of the specific growth rate μ_{set} at 0.17 h^{-1} that was used for calculating the feeding rate [see Eq. (1)]. In order to maintain the pH value at 7 for the entire fermentation time, the amount of added NH_4OH (25%) was increased exponentially until a maximum of 2.08 l was attained at the end of the fermentation (Fig. 2C). Thus, the yield coefficient $Y_{X/N}$ was calculated at 3.69 g/g, which was comparable to the value of the fermentation using glucose. Even by using glycerol as C-source, a parallel increase in the added amount of NH_4OH (Fig. 2C) and the dry cell weight concentration (Fig. 2A) could be observed. The online determined slope for the NH_4OH consumption agreed with the direct online estimation of biomass (abs. dry cell weight) and the specific growth rate [10, 25].

Specific Power Input

According to Eq. (2), the specific volumetric power input P/V_L was approx. 3 kW/m^3 for the fermentation using glucose as C-source, and ranged from 5 to 6 kW/m^3 for the fermentation using glycerol as C-source over the entire cultivation time. It should be noted that both fermentations were maintained at different stirrer velocities (glucose, 500 rpm; glycerol, 650 rpm).

Correlation of Capacitance and Dry Cell Weight

According to Fig. 3, the correlations between ΔC and dry cell weight showed linear patterns for both fermentations independent of the C-source used (glucose or glycerol). Beginning at the origin, both curves stay linear over the entire biomass concentration range. For both curves, the coefficient of determination R^2 indicated a high agreement of the proportionality between the online measured capacitance and the offline determined dry cell weight concentration. Depending on the C-source used (glucose or glycerol), both curves only depicted marginal differences in their slopes. Upon using glucose as C-source, the slope (0.498) of the line is higher than the slope (0.406) for glycerol as C-source. Above biomass concentrations of 65 g/l dry cell weight, both curves deviate slightly from the linear correlation. Presumably, this phenomenon was caused by an increasing foam accumulation and the subsequent addition of antifoam agent.

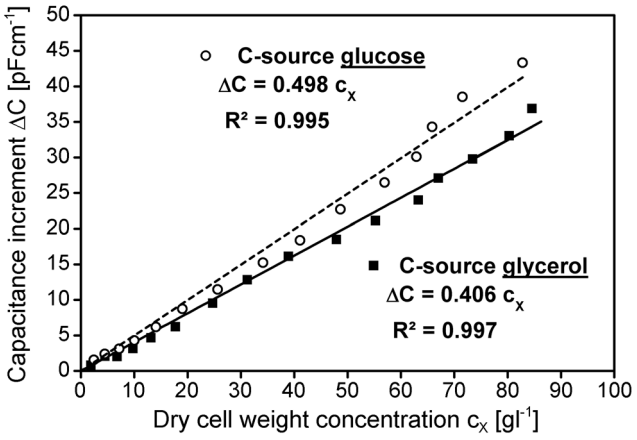

Fig. 3. Correlation between ΔC (capacitance increment) & dry cell weight concentration of *E.coli* VH33 in minimal medium with a glucose and a glycerol feed.

Online monitoring of biomass using a capacitance signal is an effective tool to follow the cell concentration of *E. coli* during high cell-density fermentations. In this study, biomass concentrations of up to 85 g/l dry cell weight and space time yields of up to 3.40 g/l/h could be achieved. Thereby, the cultures grew under carbon-limiting conditions in a simple open-loop fed-batch control strategy using different carbon sources (glucose, glycerol), respectively. Closed-loop feedback feeding strategies based on for example DOT measurement should be preferred, if maximal cell yield or product concentrations is the main task. However, a capacitance signal to detect biomass online can basically contribute to a better and faster understanding of bioprocesses [21, 23]. Other authors [11] investigating capacity measurements on *E. coli* only reached a dry cell weight concentration of 60 g/l by using only the carbon source glucose. The aforementioned cell concentrations represent, to our knowledge, the highest ever detected biomass concentrations of *E. coli* using an online impedance signal. In this investigation, the progression of capacitance as a function of dry cell weight concentration showed a highly linear correlation independent of the applied carbon source. This is presumingly attributed to the continuous growth of the cultures by controlling the feed at a constant specific growth rate (μ=0.17 h^{-1}). Furthermore, to validate the bacterial viability online, the OTR was additionally monitored, whereby very high OTR_{max} values of 0.691 mol/l/h and 0.984 mol/l/h were formed using glucose and glycerol as carbon sources, respectively. Similarly, these values represent, to our knowledge, the highest respiration activities ever detected in *E. coli* fermentations and prove the viability of the bacteria even at the high concentration range discussed here. The online determined ammonia consumption also proved the cell viability at high cell densities with congruent increases in biomass and at comparable $Y_{X/N}$ values. In conclusion, the online capacitance measurement can simplify bioprocesses in stirred tank fermenters by reducing offline sampling.

Acknowledgments

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Abbreviations

ΔC	increment between capacitance values at low and at high frequencies [pF/cm]
C^{β}	capacitance value at low frequency [pF/cm]
C_{∞}	capacitance value at high frequency [pF/cm]
c_X	concentration of dry biomass [g/l]
c_{XF}	concentration of dry biomass at the beginning of the fed-batch [g/l]
$c_{X,max}$	maximal concentration of biomass in a fermentation process [g/l]
c_{SF}	concentration of substrate in the feed reservoir [g/l]
dcw	dry cell weight [g/l]
DOT	dissolved oxygen tension [%]
F	feed rate [g/h]
f	frequency of alternating current [s^{-1}]
HCDC	high cell-density cultivation
k_La	specific mass transfer coefficient [h^{-1}]
M	torque [Nm]
m_S	maintenance coefficient for substrate [g/g/h]
m_X	total mass of dry cell weight [g]
n	agitation rate [s^{-1}]
OTR	oxygen transfer rate [mol/l/h]
P	power input of the stirrer [W]
STR	space time yield [g/l/h]
t	time [h]
t_F	time at the beginning of the fed-batch [h]
$\dot{V}_G$	standard gas flow rate [NL/min]
V_L	reactor filling volume [l]
V_{LF}	reactor filling volume at the beginning of the fed-batch [l]
V_{molar}	molar gas volume [l]
$Y_{X/N}$	yield coefficient of biomass per amount of nitrogen [g/g]
$Y_{X/S}$	yield coefficient of biomass per amount of substrate [g/g]

Greek Letters

μ_{exp}	specific growth rate determined experimentally [h^{-1}]
μ_{set}	setpoint of the specific growth rate [h^{-1}]
ρ_F	density of feed solution [g/l]

References

1. Anderlei, T., W. Zang, M. Papaspyrou, and J. Büchs. 2004. Online respiration activity measurement (OTR, CTR, RQ) in shake flasks. *Biochem. Eng. J.* **17:** 187–194.
2. Andersson, L., L. Strandberg, and S. O. Enfors. 1996. Cell segregation and lysis have profound effects on the growth of *Escherichia coli* in high cell density fed-batch cultures. *Biotechnol. Prog*. **12:** 190–195.
3. Arnoux, A. S., L. Preziosi-Belloy, G. Esteban, P. Teissier, and C. Ghommidh. 2005. Lactic acid bacteria biomass monitoring in highly conductive media by permittivity measurements. *Biotechnol. Lett.* **27:** 1551–1557.
4. Babaeipour, V., S. A. Shojaosadati, S. M. Robatjazi, R. Khalilzadeh, and N. Maghsoudi. 2006. Over-production of human interferon-g by HCDC of recombinant *Escherichia coli*. *Process Biochem*. **42:** 112–117.
5. Cannizzaro, C., R. Gügerli, I. Marison, and U. von Stockar. 2003. On-line biomass monitoring of CHO perfusion culture with scanning dielectric spectroscopy. *Biotechnol. Bioeng*. **84:** 597–610.
6. De Anda, R., A. R. Lara, V. Hernandez, V. Hernandez-Montalvo, G. Gosset, F. Bolivar, and O. T. Ramirez. 2006. Replacement of the glucose phosphotransferase transport system by galactose permease reduces acetate accumulation and improves process performance of *Escherichia coli* for recombinant protein production without impairment of growth rate. *Metab. Eng*. **8:** 281–290.
7. Ferreira, A. P., L. M. Vieira, J. P. Cardoso, and J. C. Menezes. 2005. Evaluation of a new annular capacitance probe for biomass monitoring in industrial pilot-scale fermentations. *J. Biotechnol*. **116:** 403–409.
8. Fyferling, M., J. Uribelarrea, G. Goma, and C. Molina-Jouve. 2008. Oxygen transfer in intensive microbial culture. *Bioprocess Biosyst. Eng*. **31:** 595–604.
9. Gregory, M. E. and C. Tuner. 1993. Open-loop control of specific growth rate in fed-batch cultures of recombinant *E. coli*. *Biotechnol. Techn*. **7:** 889–894.
10. Hoffmann, F., M. Schmidt, and U. Rinas. 2000. Simple technique for simultaneous on-line estimation of biomass and acetate from base consumption and conductivity measurements in high-cell density cultures of *Escherichia coli*. *Biotechnol. Bioeng*. **70:** 358–361.
11. Kaiser, C., T. Pototzki, A. Ellert, and R. Luttmann. 2008. Applications of PAT-process analytical technology in recombinant protein processes with *Escherichia coli*. *Eng. Life Sci*. **8:** 132–138.
12. Knoll, A., S. Bartsch, B. Husemann, P. Engel, K. Schroer, B. Ribeiro, C. Stöckmann, J. Seletzky, and J. Büchs. 2007. High cell density cultivation of recombinant yeasts and bacteria under non-pressurized and pressurized conditions in stirred tank bioreactors. *J. Biotechnol*. **132:** 167–179.
13. Knoll, A., B. Mayer, H. Tscherrig, and J. Büchs. 2005. The oxygen mass transfer, carbon dioxide inhibition, heat removal, and the energy and cost efficiencies of high pressure fermentation. *Adv. Biochem. Eng. Biotechnol*. **92:** 77–99.
14. Lara, A. R., L. Caspeta, G. Gosset, F. Bolivar, and O. T. Ramirez. 2008. Utility of a *Escherichia coli* strain engineered in

the substrate uptake system for improved culture performance at high glucose and cell concentrations: An alternative to fed-batch cultures. *Biotechnol. Bioeng*. **99:** 893–901.
15. Lee, S. Y. 1996. High cell-density culture of *Escherichia coli*. *Trends Biotechnol*. **14:** 98–105.
16. Maier, B., C. Dietrich, and J. Büchs. 2001. Correct application of the sulphite oxidation methology of measuring the volumetric mass transfer coefficient k_La under non-pressurized and pressurized conditions. *Food Bioprod. Process*. **79:** 107–113.
17. Maier, U., M. Loosen, and J. Büchs. 2004. Advances in understanding and modeling the gas–liquid mass transfer in shake flasks. *Biochem. Eng. J.* **17:** 155–167.
18. Markx, G. H. and C. L. Davey. 1999. The dielectric properties of biological cells at radiofrequencies: Applications in biotechnology. *Enz. Microb. Technol*. **25:** 161–171.
19. Mas, S., F. Ossard, and C. Ghommidh. 2001. On-line determination of flocculating *Saccharomyces cerevisiae* concentration and growth rate using a capacitance probe. *Biotechnol. Lett*. **23:** 1125–1129.
20. Maskow, T., A. Röllich, I. Fetzer, J. Ackermann, and H. Harms. 2008. On-line monitoring of lipid storage in yeasts using impedance spectroscopy. *J. Biotechnol*. **135:** 64–70.
21. Maskow, T., A. Röllich, I. Fetzer, J. Yao, and H. Harms. 2008. Observation of non-linear biomass–capacitance correlations: Reasons and implications for bioprocess control *Biosens. Bioelect*. **24:** 123–128.
22. Neves, A. A., D. A. Pereira, L. M. Vieira, and J. C. Menezes. 2000. Real time monitoring of biomass concentration in *Streptomyces cla*6 *uligerus* cultivations with industrial media using a capacitance probe. *J. Biotechnol*. **84:** 45–52.
23. Noll, T. and M. Biselli. 1998. Dielectric spectroscopy in the cultivation of suspended and immobilized hybridoma cells. *J. Biotechnol*. **63:** 187–198.
24. Sanden, A. M., I. Prytz, I. Tubulekas, C. Förberg, H. Le, A. Hektor, *et al*. 2002. Limiting factors in *Escherichia coli* fed-batch production of recombinant proteins. *Biotechnol. Bioeng*. **81:** 158–166.
25. Schmidt, M., E. Viaplana, F. Hoffmann, S. Marten, A. Villaverde, and U. Rinas. 1999. Secretion-dependent proteolysis of heterologous protein by recombinant *Escherichia coli* is connected to an increased activity of the energy-generating dissimilatory pathway. *Biotechnol. Bioeng*. **66:** 61–67.
26. Shiloach, J. and R. Fass. 2005. Growing *E. coli* to high cell density – A historical perspective on method development. *Biotechnol. Adv.* **23:** 345–357.
27. Xiong, Z. Q., M. J. Guo, Y. X. Guo, J. Chu, Y. P. Zhuang, and S. L. Zhang. 2008. Real-time viable-cell mass monitoring in high-cell-density fed-batch glutathione fermentation by *Saccharomyces cerevisiae* T65 in industrial complex medium. *J. Biosci. Bioeng*. **105:** 409–413.