AIChE

Development Of a System for the On-Line Measurement of Carbon Dioxide Production in Microbioreactors: Application to Aerobic Batch Cultivations of *Candida utilis*

Michiel van Leeuwen and Joseph J. Heijnen
Dept. of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands

Han Gardeniers
MESA+ Institute for Nanotechnology, University of Twente, P.O. Box 217,7500 AE Enschede, The Netherlands

Luuk A. M. van der Wielen and Walter M. van Gulik
Dept. of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC Delft, The Netherlands

DOI 10.1021/bp.145
Published online June 8, 2009 in Wiley InterScience (www.interscience.wiley.com).

We developed and applied a conductometric method for the quantitative online measurement of the carbon dioxide (CO_2) production during batch cultivations of Candida utilis on a 100-μL scale. The applied method for the CO_2 measurement consisted of absorption of the produced CO_2 from the exhaust gas of the microbioreactor in an alkali solution, of which the conductivity was measured on-line. The measured conductivity change of the alkali solution showed a linear relation with the total amount of CO_2 absorbed. After calibration of the CO_2 measurement system, it was connected to a well of a 96-well microtiter plate. The mixing in the well was achieved by a magnetic stirrer. Using online measurement of the CO_2 production during the cultivation, we show reproducible exponential batch growth of C. utilis on a 100-μL scale. The CO_2 production measurements obtained from the microcultivation were compared with the CO_2 production measurement in a 4-L bioreactor equipped with a conventional off-gas analyzer. The measurements showed that on-line measurement of the CO_2 production rate in microbioreactors can provide essential data for quantitative physiological studies and provide better understanding of microscale cultivations. © 2009 American Institute of Chemical Engineers *Biotechnol. Prog.*, 25: 892–897, 2009
Keywords: microbioreactor, monitoring, CO_2 production, conductivity, batch culture, Candida utilis

Introduction

High-throughput screening programs can generate large amounts of mutants to find new and highly productive microorganisms for industrial applications or to gain insight in the functioning of microorganisms at a molecular and genetic level. The main challenge lies in the evaluation of this enormous amount of mutants by means of cultivation under well-controlled conditions.[1,2]

Microtiter plates become more and more attractive for high-throughput cell cultivation because of their small working volumes, high degree of parallelization and the available robotics, and liquid handling equipment.[3] Although plate with smaller working volumes and a higher number of wells per plate are available, microtiter plates for high-throughput cultivation of microorganisms typically contain 24 or 96 wells with working volumes of 0.1 to 4 mL.[4]

A major drawback of the use of microtiter plates for screening and testing of microorganisms, e.g., their lack of sensing and control possibilities, is partially addressed by the integration of sensors in the wells. At present 24- and 96-well plates equipped with optical sensors for pH[5] or dissolved oxygen concentration[6,7] are commercially available (e.g., PreSens, Precision sensing GmbH, Regensburg, Germany), as well as systems with measurement and control of temperature, pH, and dissolved oxygen concentration in each of the 24 wells of the plate (μ24, Applikon, Schiedam, The Netherlands).[8]

Because of the small working volume of microreactors, the traditional way of measuring the biomass concentration, i.e., by determination of the amount of biomass in a culture sample, is not feasible. The commonly used technique to quantify the biomass concentration in microtiter plates is online turbidity measurement.[9,10] At high biomass concentrations, however, this technique fails because of its narrow measurement range. Online measurement of the carbon dioxide production rate could fulfill the need for quantification of the rates of biomass growth and/or product formation in microwell size cell cultivation systems by using elemental balancing.[11]

Online measurement of the carbon dioxide production rate in bench-scale bioreactors is state of the art for many years

Correspondence concerning this article should be addressed to W. M. van Gulik at w.m.vangulik@tudelft.nl

 © 2009 American Institute of Chemical Engineers

and provides essential data for quantitative physiological studies. Recently a device for the measurement of the carbon dioxide production rate was developed for shake flaks.[12] To our knowledge, online measurement of the carbon dioxide production rate is not yet reported for microliter-scale cultivation systems.

A way to measure small amounts of produced carbon dioxide is by trapping it in an alkaline solution and subsequently measuring the conductivity change of the solution. The conductometric measurement of carbon dioxide is relatively easy, very sensitive,[13–15] and easy to miniaturize, and thus in principle suitable for the online measurement of the carbon dioxide production rate of microbial cultivations in microtiter plates.

We applied the conductometric measurement technique for the online measurement of the carbon dioxide production rate during batch cultivation of *Candida utilis*. The cultivation was performed in a well of a 96-well plate with a working volume of 100 μL, which served as microbioreactor. Using a carrier gas stream the produced carbon dioxide was transported from the headspace of the well to a scrubber containing and alkali solution in which the CO_2 was absorbed. The change in electrical conductivity of the solution, brought about by the absorption of the carbon dioxide, was measured on-line and from this measurement the carbon dioxide production in the microbioreactor was calculated.

Materials and Methods

Microbioreactor

A well of a 96-well microtiter plate served as microbioreactor. The well was closed with a rubber stopper. The reactor was equipped with a small stainless steel stirrer bar (length: 4.75 mm, width: 1.91mm, height: 1.54 mm, occupied volume 14 μL) (kindly provided by V&P Scientific Inc., San Diego, USA). A 0.6-mm hole was drilled through the stirrer bar for mounting it on a small stainless steel axle. The axle was fixed in the rubber stopper. The axle fixed the stirrer bar in the middle of the microbioreactor at 1 mm above the bottom. A magnetic stirring plate (KMO 2, IKA-Werk Janke & Kunkel KG, Staufen, Germany) was used to drive the stirrer at a speed up to 250 rpm. The complete setup was placed in a temperature-controlled cabinet that was controlled at 30 ± 0.1°C by a temperature controller (6100+ West Instruments, Brighton, UK) and three 100 W light bulbs. A custom made LabVIEW™ (National Instruments, Austin, USA) routine was used to measure and log the temperature via a DAQ card (Advantech-PCI-1710HGL, Milpital, USA). A schematic representation of the microbioreactor setup is shown in Figure 1.

Measurement of carbon dioxide production

A mass flow controller (Brooks Instruments B.V. Veenendaal, The Netherlands) with a flow range of 0–3 mL/min was used to blow a stream of pure O_2 gas (Linde gas, Schiedam, The Netherlands) through the headspace of the well at a flow rate of 600 μL/min. This was accomplished by two syringe needles that were pierced through the rubber stoppers that closed the well and served as gas inlets and outlets. Before the gas entered the wells, all traces of CO_2 were removed via absorption in a 4 M KOH solution. Subsequently the gas was humidified in a separate water bottle to minimize water evaporation from the microreactor and sterilized via a disk filter with a pore size of 0.2 μm (Whatman, 's-Hertogenbosch, The Netherlands).

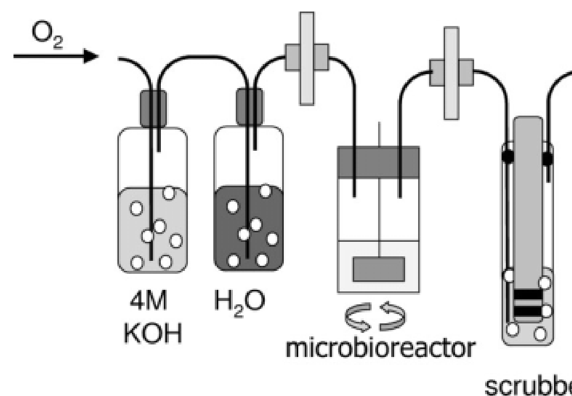

Figure 1. Schematic representation of the microbioreactor setup.

The exhaust gas containing the CO_2 that was produced in the microbioreactor was led through a separate scrubber, i.e., a test tube containing 3 mL of a 80 mM NaOH solution. A Teflon® coated stirring dowel (7 × 2 mm) in the scrubber, which was driven by a magnetic stirring plate, ensured liquid mixing. Herein the CO_2 was stripped from the exhaust gas. A conductivity electrode (Consort SK20b; range 0.1 μS/cm to 100 mS/cm) that was mounted in the scrubber was used to measure the conductivity change of the NaOH solution. The net reaction taking place in the scrubber is

$$CO_2(g) + 2OH^-(aq) \rightarrow CO_3^{2-}(aq) + H_2O \qquad (1)$$

The alkaline environment in the scrubber forces this acid/base equilibrium towards carbonate acid. The measured conductivity change was used as a measure for the amount of CO_2 that was produced in the microbioreactor. A custom made LabVIEW™ routine was written to log the conductivity signal via an RS232 interface. Gas impermeable Tygon® tubing (Masterflex, Cole-Parmer Instrument Company, Chicago, Illinois, USA) was used for all gas connections to prevent CO_2 entering or leaving the system before the scrubber.

Calibration of the CO_2 measurement system

For the calibration method proposed here it was assumed that the conductivity change of the solution is brought about by the change of the sum of the individual ion concentrations that are involved in the reaction. Therefore, calibration lines were made for NaOH (0–100 mM) and Na_2CO_3 (0–50 mM). From these calibration lines the conductivity change of the net reaction (see Eq. 1) was determined by linear regression.

Micro organism and pre-culture conditions

Candida utilis CBS621 was obtained from the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands. *C. utilis* CBS621 was transferred from a glycerol stock into either a 100 mL shake flask filled with 20 mL mineral medium[16] or in a 1,000 mL shake flask filled with 400 mL mineral medium[16] and cultivated in an incubator (Certomat® BS-1 Sartorius BBI Systems GmbH, Melsungen, Germany) at 30°C and 200 rpm. The medium was supplemented with 15 g/L glucose. 1 H_2O, and the ammonium was replaced by urea (2.3 g/L) to reduce acidification of the medium during

growth. The initial pH of the medium was 6. The 1,000 mL shake flask cultures were grown overnight and were used as inoculum for the bench-scale bioreactor. The 100 mL shake flask cultures were grown until the end of the exponential growth phase and were used as precultures for the microbioreactor cultivations.

Batch cultivation in the microbioreactor

The microbioreactor was sterilized with 70% ethanol and afterward washed thoroughly with sterile demi water. Batch cultivation was started by mixing 10 μL of the preculture with 90 μL of fresh medium and subsequently pipetting this mixture into the microbioreactor. This resulted in an initial biomass concentration of around 0.7 g/L.

Batch cultivation in 4 L bioreactor

Bench-scale cultivations of *C. utilis* CBS621 were carried out in a 7 L bioreactor (Applikon, Schiedam, The Netherlands) with a working volume of 4 L. The bioreactor was equipped with sensors for pH (Inpro 3030/120, Mettler-Toledo Tiel, The Netherlands), temperature (PT-100), and dissolved oxygen tension (DOT, Mettler-Toledo GmbH, Greifensee, Switzerland). All sensors were connected to a process control system (DCU3, Sartorius AG, Melsungen, Germany). The temperature of the bioreactor was maintained at 30°C. This was done with a thermo circulator (ADI 1018 Applikon, Schiedam, The Netherlands) which was controlled by the DCU3. The pH and the dissolved oxygen tension (DO) were measured but not controlled. Two Rushton turbine stirrers were used to mix the broth with a speed of 450 rpm. Air was sparged through the reactor with a flow rate of 3 L/min by means of a mass flow controller (Brooks Instruments B.V. Veenendaal, The Netherlands). The bioreactor was operated at an overpressure of 0.27 bar. The bioreactors off-gas was cooled in a condenser that was connected to a cryostat at 2 °C to minimize evaporation of volatile compounds. The CO_2 and O_2 concentrations in the off-gas were analyzed using a combined paramagnetic/infrared gas analyzer (NGA 2000 MLT 1, Fischer-Rosemount GmbH & Co Hasselroth, Germany). Commercial data acquisition software (MFCS, Sartorius AG, Melsungen, Germany) was used for on-line data logging. The obtained biomass dry weight of the bioreactor cultivation was measured as described previously.[17]

Microscopy

Micrographs of yeast culture samples were made with a phase contrast microscope (Axiostar plus, Carl Zeiss, Göttingen, Germany) equipped with a digital camera (Canon Powershot A620, Canon Inc., Tokyo, Japan). No staining was applied.

Results and Discussion

Characterization of the CO_2 measurement system

Pure oxygen gas was blown over the headspace of the microbioreactor to provide the oxygen for metabolic activity and served at the same time as carrier gas to transport an important product of metabolic activity, i.e., carbon dioxide, to a scrubber. In the scrubber, the carbon dioxide was trapped in and reacted with an alkaline solution. The reaction results

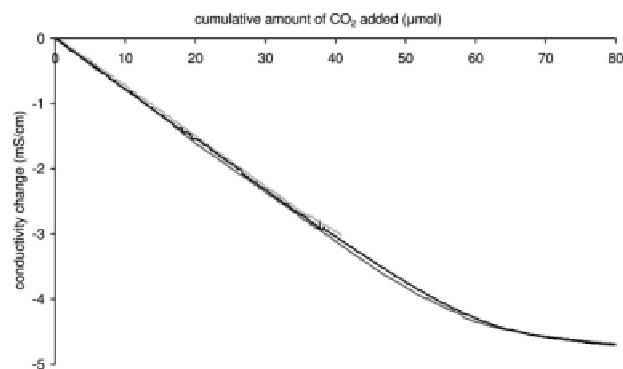

Figure 2. Calibration of the CO_2 measurement system with a calibration gas containing 2% CO_2.

The calibration was performed in triplicate.

in a change of electrical conductivity of the solution. Conductometric measurement of the solution in the scrubber yields the cumulative amount of carbon dioxide (M_{CO_2}), assuming that no other volatile metabolic reaction product interferes with the measurement. For the quantitative determination of the M_{CO_2}, the CO_2 measurement system was calibrated. The conductivity change of the net reaction (see Eq. 1) was determined to be -240 mS cm^{-1} L mol^{-1}. This result compares reasonably well with a previous finding of -235 mS cm^{-1} L mol^{-1} by Rodella and Saboya,[15] taking into account that the concentrations of the stock solutions that these authors used for calibration were almost 5 times as high as in our case.

To verify the obtained calibration, a calibration gas containing 2.0 % CO_2 was blown through the scrubber, corresponding with a CO_2 supply rate of 29 μmol/h and the conductivity change was measured in time. Figure 2 shows the results of three independent experiments. From these results it can be observed that the measurement system is highly reproducible and that the conductivity of the solution in the scrubber changed linearly with the amount of CO_2 that was fed to the measurement system up to at least 50 μmol of CO_2. The average conductivity change for the reaction derived from the linear part in these triplicate experiments was -230 ± 3 mS cm^{-1} L mol^{-1} (average $\pm$ standard deviation). This is very close to the result obtained by calibration with the NaOH and Na_2CO_3 stock solutions (results not shown).

From the results shown in Figure 2 it can be inferred that the capacity of the CO_2 measurement system is 50–60 μmol for the applied conditions. The addition of more than 50–60 μmol of CO_2 resulted in a nonlinear decrease in the conductivity. The nonlinear behavior can be explained by the fact that the pH in the scrubber was decreased to the point that the acid/base equilibrium for carbonate shifted toward bicarbonate, changing the sensitivity of the CO_2 measurement system. The maximal capacity of 50–60 μmol CO_2 for the CO_2 measurement system was calculated to be more than sufficient for the batch cultivation of *C. utilis* on a 100 μL-scale with an initial glucose concentration of 14 g/L in the medium. Under these conditions, the final biomass concentration is expected to be approximately 7 g/L, and the amount of CO_2 produced is approximately 21 μmol. It should be noted that larger amounts of CO_2 can be measured by increasing the NaOH concentration in the scrubber and/or increasing the volume of the NaOH solution.

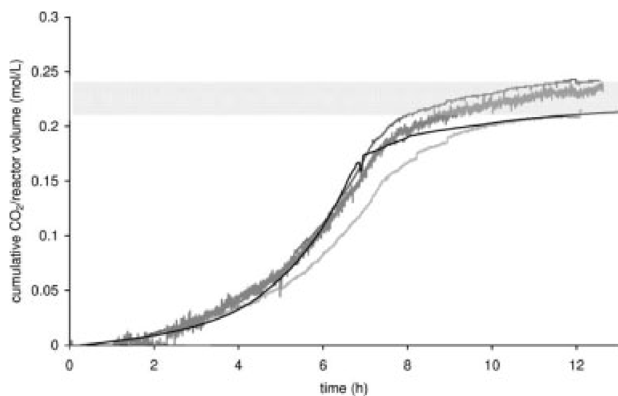

Figure 3. Comparison of cumulative amount of CO_2 (M_{CO_2}) produced in batch cultivations of 100 μL (gray lines) and 4 L volume (black line).

The gray area indicates the cumulative amount of CO_2 that is maximally expected; the expectation is based on the stoichiometry for this conversion and differences in carry over of substrate from the inoculum. For better comparison, the cumulative amount of CO_2 is divided by the reactor volume.

Batch cultivation of C. utilis *on a 100 μL-scale*

C. utilis was cultivated in a batch bioreactor with a working volume of 100 μL. The cumulative molar amount of CO_2 that was produced (M_{CO_2}) in the batch cultivations was measured on-line by means of the conductometric measurement of CO_2 in the off-gas, as described earlier.

The inoculation procedure caused a temperature disturbance of 2 to 3°C in the cabinet in which the setup was placed. It was observed that the disturbance of the temperature in the first hour led to severe disturbances in the CO_2 measurements. It took about 1 h after inoculation before the temperature in the cabinet was restored to its setpoint of 30 ± 0.1°C. It is generally known that conductivity measurements are temperature dependent. Therefore, the data obtained in the first hour after inoculation were not used. It should be noted that relative and not absolute values for electrical conductivity are required for our measurements; therefore, the temperature should be kept constant within an experiment but may, in principle, deviate between experiments.

The results of the cumulative CO_2 measurements obtained in the microbioreactor and in the 4 L lab-scale bioreactor that served as a reference system are presented in Figure 3. To compare the two experiments, of which the cultivation volumes were highly different, the obtained cumulative CO_2 measurements were normalized by dividing them by the reactor volume. All the curves in Figure 3 show the expected batch growth curve: after a short lag phase, an exponential growth phase is observed, as is indicated by an exponential increase in the M_{CO_2}. Seven to eight hours after inoculation, the increase in M_{CO_2} was no longer exponential and M_{CO_2} leveled off. Considering the medium composition, which is such that all components except the carbon source glucose are in excess, this most likely depicted the phase where the glucose concentration became limiting for growth and the growth rate, and thereby the CO_2 production rate, decreased. The possibility of oxygen limitation instead of glucose limitation, at this stage of the batch growth, is discussed later. During this phase some residual CO_2 production remained, probably due to the combustion of storage carbohydrates.

The cumulative carbon dioxide production curves obtained from the 100 μL batch cultivation were comparable with the curve obtained from the 4 L reference cultivation (Figure 3) However, there was some variation in the length of the exponential growth phase. Furthermore, in case of the 4 L cultivation the transition between the exponential growth phase and the stationary phase was more abrupt than for the 100 μL cultivations.

In the 4 L reference cultivation, the maximum amount of CO_2 produced per liter of culture volume, M_{CO_2}/V, was 0.21 mol/L at 11 h after inoculation. The total amount of CO_2 produced during the batch cultivation of *C. utilis* can be predicted by carbon-elemental balancing.[18] Given an initial glucose concentration of 76 mM, an observed yield of biomass on substrate in the 4 L cultivation of 0.48 g biomass per gram glucose and a biomass carbon content of 1 mol per 25.6 g biomass[19] it can be calculated from the stoichiometry of the conversion that the amount of CO_2 produced is equal to 0.20 mol/L. In this calculation, the formation of carbon containing by-products, in addition to biomass and CO_2, are not taken into account. However, due to the consumption of the nitrogen source urea an additional amount of CO_2 is produced. Assuming a nitrogen content of the biomass of 1 mol per 143 g biomass[19] it can be calculated that the additional amount of CO_2 produced due to the consumption of urea is equal to 0.023 mol/L, yielding a total amount of produced CO_2 of 0.22 mol/L. This implies that the total amount of CO_2 produced during the 4 L bioreactor cultivation is slightly lower (5%) than the stoichiometric amount which might have been caused by the formation of small amounts of by-products (e.g., organic acids). The average, M_{CO_2}/V, for the micro reactor cultivations was 0.226 ± 0.015 mol/L (average ± standard deviation) at 11 h after inoculation for the triplicate 100 μL-scale experiments, which is comparable with the calculated amount. However, the average M_{CO_2}/V of the 100 μL cultivations appeared 10% higher than for the 4 L cultivation. Carry over of glucose during inoculation was the most likely cause for the observed variation in final M_{CO_2}/V between the 100 μL experiments as well as for the higher average M_{CO_2}/V of the 100 μL compared with the 4 L cultivation. The experimental setup for the 100 μL cultivation was prone to carry over of substrate, whereas during inoculation of the 4 L bioreactor carry over of substrate from the inoculum was almost absent. The reason for this was that the microbioreactor had to be inoculated with an exponentially growing culture, because otherwise no growth occurred, whereas the 4 L bioreactor was inoculated from an overnight culture, in which no residual glucose was present. Difficulties with the start-up of the 100 μL cultivations were most likely caused by a lack of CO_2 in the ingoing gas stream, which was removed by leading it through a 4 M KOH solution. When the 100 μL reactor was inoculated with an exponentially growing culture, the CO_2 necessary for anabolism was most likely provided by the CO_2 production brought about by the catabolism of the growing cells.

For the 4 L cultivation, air was used for the oxygen supply. The dissolved oxygen concentration in the 4 L experiment was measured and did not drop below 90 μM, indicating that fully aerobic conditions were maintained during the entire batch cultivation. In the microbioreactor experiments, pure oxygen was blown over the headspace to suffice the oxygen need.

Based on the M_{CO_2} measurement profiles of the microbioreactor cultivations, the good agreement in M_{CO_2}/V between the two cultivation scales as well as the good agreement between experimental and stoichiometric M_{CO_2}/V, it is very

likely that also in the microbioreactor experiments the oxygen supply was sufficient to maintain aerobic conditions. It is well known that *C. utilis* is a Crabtree-negative facultative fermentative microorganism. This implies that under aerobic conditions this organism does not produce ethanol at glucose excess conditions. Only under conditions of low oxygen supply it will perform ethanolic fermentation of sugars, thus converting part of the glucose to ethanol.[20] Insufficient oxygen supply during the batch cultivation would therefore have been visible in the cumulative carbon dioxide production measurements. Reutilization of the produced ethanol after the depletion of glucose, i.e., diauxic growth, would be visible in the CO_2 measurement if oxygen limiting conditions occurred. Also, ethanolic fermentation would result in a decrease of the CO_2-production, which was not observed.

The maximum growth rate (μ_{max}) for each of the cultivations was calculated from the exponential growth phase, as is depicted in Figure 4. From this figure it can be inferred that the growth rate was constant for at least 4 h.

The maximum growth rate for the 4 L cultivation was calculated to be 0.599 $\pm$ 0.001 h^{-1}. This is in good agreement with the maximum growth rate of 0.59 h^{-1} that was reported for this strain in literature.[21] The maximum growth rate that was calculated for the three 100 μL cultivations was 0.46 $\pm$ 0.039 h^{-1} (average $\pm$ standard deviation). This implies that the growth rate in the microbioreactor was significantly lower compared with the large scale cultivation.

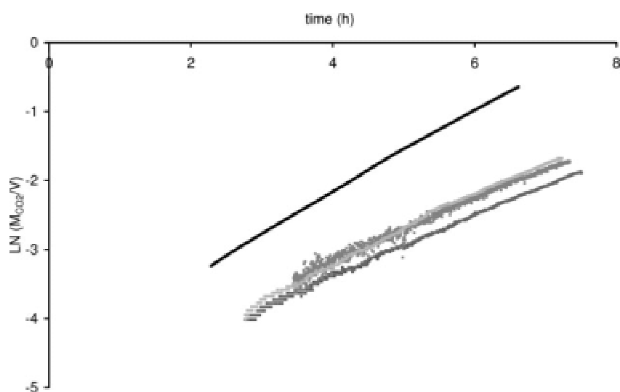

Figure 4. M_{CO_2}/V vs. time on a logarithmic scale for the determination of the maximal specific growth rate for the cultivation performed in the 4 L (black line), and the triplicate 100 μL experiments (gray lines).

Samples that were taken at the end of the microbioreactor experiments, i.e., at 13–16 h after inoculation were inspected under a phase contrast microscope. These samples showed significant amounts of cell debris (Figure 5, middle micrograph) compared with samples taken from stationary phase shake flask cultures or from the 4 L bioreactor (microscopic observation only, no micrographs available).

The most likely cause of the observed presence of cell debris is cell rupture during batch cultivation caused by the mechanical stirring. Therefore, rupture of cells could have caused the lower growth rate observed in the microbioreactors compared with the 4 L bioreactor. In this case, the observed growth rate is lower than the maximum specific growth rate because it is the result of biomass growth and biomass decay.

If indeed cell destruction occurred, due to mechanical stress, leading to a lower observed growth rate, this effect would be more pronounced at higher stirrer speeds. Therefore, duplicate batch cultivations were performed at increased stirrer speeds of 800 rpm. In these cultures, the observed growth rate was 0.36 $\pm$ 0.040 h^{-1} (average $\pm$ standard deviation), thus supporting this hypothesis. Microscopic observation of the cells at the end of the cultivations (Figure 5, right micrograph) indeed showed severe cell destruction. Although further investigations on the effect of stirring in microbioreactors are necessary, these experimental observations indicated that stirring influenced the observed growth rate of *C. utilis* in the microbioreactor in a negative way.

Negative effects of stirring in microbioreactors have not been reported before. This could be inherent to the conventional technique for determining the biomass concentration in stirred microbioreactors, i.e., turbidity measurements.[22,23] We believe that quantification of the growth by measuring the carbon dioxide production has provided more detailed and accurate information on biomass growth in stirred microliter size bioreactors. Detailed comparison between microliter-scale and bench-scale cultivation is very important for the successful application of microliter-scale cultivation systems for high-throughput screening under relevant conditions, i.e., comparable mechanical shear stress in both cultivation systems.

Conclusions

An accurate method for the measurement in 100 μL bioreactors of the production of very small amounts of CO_2,

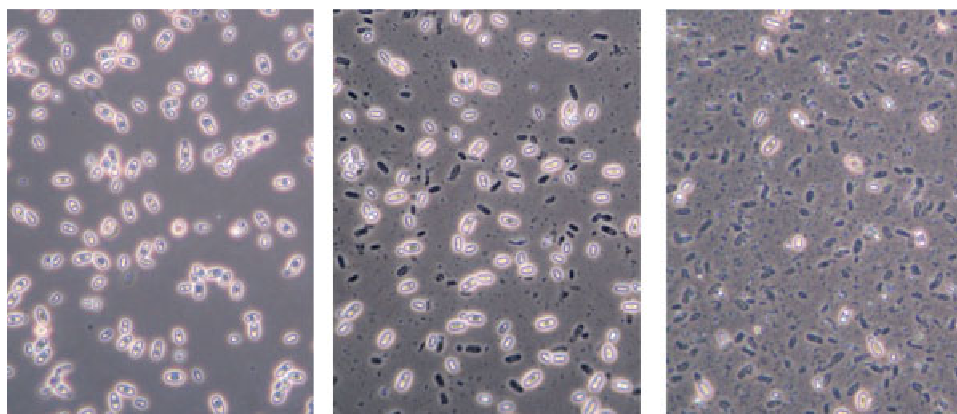

Figure 5. Micrograph of cells after batch growth in shake flask (left) and cells after batch growth in a stirred microbioreactor at 250 rpm (middle) and 800 rpm (right).

has been developed, based on accumulation of the produced CO_2 in an alkaline solution and subsequent measurement of the conductivity change of this solution. Based on this principle, a CO_2 measurement system was built for which it was found that the measured conductivity change was linearly related to the supplied CO_2 up to a total of 50 μmol. The applicability of this system was demonstrated by using it for measurement of the CO_2 production in batch cultivations of *C. utilis* carried out in a 100 μL bioreactor.

It was found that measurement of the CO_2 production with this system was a reliable and reproducible method to quantify growth in a microbioreactor. However, depending on the cultivated microorganism it should be verified whether other volatile compounds, which are also trapped in an alkaline scrubber and could interfere with the conductivity measurement, are produced.

The cumulative CO_2 production curves on 100 μL-scale were comparable with the growth curve obtained in the 4 L reference system and allowed to calculate the maximum growth rate of the cells in the microreactor. It was found that the average maximum growth rate (μ_{max}) in the exponential growth phase calculated from the CO_2 production measurements in the microbioreactor experiments was 0.46 $\pm$ 0.039 h^{-1}, whereas in the reference system the maximal growth rate was 0.599 $\pm$ 0.001 h^{-1}. We hypothesized that the difference in observed growth rate between the bench-scale and the microreactor was caused by mechanical shear of the stirrer in the microreactor. This hypothesis was confirmed by a further decrease of the observed growth rate when the stirrer speed was increased, and from microscopical observation of cell damage in broth samples taken from the microbioreactor after the batch cultivation.

It can be concluded that online measurement of the CO_2 production can provide important insights in the physiological behavior of cells in microliter-scale cultivation systems. For the application of online CO_2 production measurements in high-throughput cultivation applications, the measurement system needs to be parallelized and integrated in a, preferably 96-well format, microbioreactor platform.

Acknowledgments

This work was funded by the Dutch Science Foundation (NWO) through the ACTS program IBOS (Integration of Biosynthesis and organic synthesis), with financial contributions from NWO, the Dutch Ministry of Economic Affairs, DSM Anti-Infectives, Organon, and Applikon BV.

Literature Cited

1. Sauer U. High-throughput phenomics: experimental methods for mapping fluxomes. *Curr Opin Biotechnol*. 2004;15:58–63.
2. Boccazzi P, Zanzotto A, Szita N, Bhattacharya S, Jensen KF, Sinskey AJ. Gene expression analysis of *Escherichia coli* grown in miniaturized bioreactor platforms for high-throughput analysis of growth and genomic data. *Appl Microbiol Biotechnol*. 2005;68:518–532.
3. Büchs J. Biochemical engineering journal—preface. *Biochem Eng J*. 2004;17:153.
4. Duetz WA, Rüedi L, Hermann R, O'Connor K, Büchs J, Witholt B. Methods for intense aeration, growth, storage, and replication of bacterial strains in microtiter plates. *Appl Environ Microbiol*. 2000;66:2641–2646.
5. John GT, Goeling D, Klimant I, Schneider H, Heinzle E. pH-sensing 96-well microtitre plates for the characterization of acid production by dairy starter cultures. *J Dairy Res*. 2003;70:327–333.
6. John GT, Klimant I, Wittmann C, Heinzle E. Integrated optical sensing of dissolved oxygen in microtiter plates: a novel tool for microbial cultivation. *Biotechnol Bioeng*. 2003;81:829–828.
7. Stitt DT, Nagar MS, Haq TA, Timmins MR. Determination of growth rate of microorganisms in broth from oxygen-sensitive fluorescence plate reader measurements. *Biotechniques*. 2002;32: 684–689.
8. Isett K, George H, Herber W, Amanullah A. 24-Well plate miniature bioreactor high-throughput system:assessment for microbial cultivations. *Biotechnol Bioeng*. 2007;98:1017–1028.
9. Warringer J, Blomberg A. Automated screening in environmental arrays allows analysis of quantitative phenotypic profiles in *Saccharomyces cerevisiae*. *Yeast*. 2003;20:53–67.
10. Weiss A, Delproposto J, Giroux CN. High-throughput phenotypic profiling of gene-environment interactions by quantitative growth curve analysis in *Saccharomyces cerevisiae*. *Anal Biochem* 2004;327:23–34.
11. van't Riet K, Tramper J. *Bioreactor Design*. New York: Marcel Dekker Inc.; 1991.
12. Anderlei T, Zang W, Papaspyrou M, Büchs J. Online respiration activity measurement (OTR, CTR, RQ) in shake flasks. *Biochem Eng J*. 2004;7:187–194.
13. Bayliss LEA. Conductivity method for the determination of carbon dioxide. *Biochem J*. 1927;21:662–664.
14. Wolf JM, Brown AH, Goddard DR. An improved electrical conductivity method for accurately following changes in the respiratory quotient of a single biological sample. *Plant Physiol*. 1952;27:70–80.
15. Rodella AA, Saboya LV. Calibration for conductimetric determination of carbon dioxide. *Soil Biol Biochem*. 1999;31:2059–2060.
16. Verduyn C, Postma E, Scheffers WA, van Dijken JP. Effect of benzoic-acid on metabolic fluxes in yeasts—a continuous-culture study on the regulation of respiration and alcoholic fermentation. *Yeast*. 1992;8:501–517.
17. Visser D, van Zuylen GA, van Dam JC, Eman MR, Proell A, Ras C, Wu L, van Gulik WM, Heijnen JJ. Analysis of in vivo kinetics of glycolysis in aerobic *Saccharomyces cerevisiae* by application of glucose and ethanol pulses. *Biotechnol Bioeng*. 2004;88:157–167.
18. Cooney CL, Wang HY, Wang DIC. Computer-aided material balancing for prediction of fermentation parameters. *Biotechnol Bioeng*. 1977;19:55–67.
19. Verduyn C, Stouthamer AH, Scheffers WA, van Dijken JP. A theoretical evaluation of growth yields of yeasts. *Antonie van Leeuwenhoek*. 1991;59:49–63.
20. van Dijken JP, Scheffers WA. Redox balances in the metabolism of sugars by yeasts. *Fems Microbiol Rev*. 1986;32:199–224.
21. Postma E, Kuiper A, Tomasouw WF, Scheffers WA, van Dijken JP. Competition for glucose between the yeasts *Saccharomyces cerevisiae* and *Candida utilis*. *Appl Environ Microbiol*. 1989;55:3214–3220.
22. Zhang Z, Szita N, Boccazzi P, Sinskey AJ, Jensen KF. A well-mixed, polymer-based microbioreactor with integrated optical measurements. *Biotechnol Bioeng*. 2006;93:286–296.
23. Szita N, Boccazzi P, Zhang Z, Boyle P, Sinskey AJ, Jensen KF. Development of a multiplexed microbioreactor system for high-throughput bioprocessing. *Lab Chip*. 2005;5:819–826.

Manuscript received July 28, 2008, and revision received Sept. 26, 2008.