Novel Orbital Shake Bioreactors for Transient Production of CHO Derived IgGs

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Large-scale transient gene expression in mammalian cells is being developed for the rapid production of recombinant proteins for biochemical and preclinical studies. Here, the scalability of transient production of a recombinant human antibody in Chinese hamster ovary (CHO) cells was demonstrated in orbitally shaken disposable bioreactors at scales from 50 mL to 50 L. First, a small-scale multiparameter approach was developed to optimize the poly(ethylenimine)-mediated transfection in 50 mL shake tubes. This study confirmed the benefit, both in terms of extended cell culture viability and increased product yield, of mild hypothermic cultivation conditions for transient gene expression in CHO cells. Second, the scalability of the process was demonstrated in disposable shake bioreactors having nominal volumes of 5, 20, and 50 L with final antibody yields between 30 and 60 mg L^{-1}. Thus, the combination of transient gene expression with disposable shake bioreactors allows for rapid and cost-effective production of recombinant proteins in CHO cells.

Introduction

Recent trends in bioprocessing demonstrate an increased interest for disposable systems throughout the manufacturing facility. The development of bioprocesses based on disposable materials is aimed at simplifying the technology for the production of biopharmaceuticals, resulting in several benefits. First, disposable systems increase the flexibility of bioprocesses (*1*). Compared to stainless steel equipment, the time required for changeovers between cell lines and batches is reduced, mainly because disposable systems require no cleaning and maintenance. Second, disposable systems reduce costs (*2*). In particular, the initial investment necessary for equipping a research and development laboratory or a pilot plant is less, and capital costs are exchanged by consumable costs, resulting in a more balanced cost distribution over time. Improved cost-effectiveness is particularly important in the context of competition and growing governmental and market price controls (*3*). Accelerating the development process for a new therapeutic protein through increased flexibility and improved cost-effectiveness provides opportunities for achieving a competitive advantage.

Here, the use of orbital shaking technology and disposable cell culture containers is proposed as an alternative to conventional stirred-tank bioreactors at scales up to 50 L. The use of such systems for transient gene expression of a recombinant human antibody in Chinese hamster ovary (CHO) cells was investigated. Shaking technology is routinely applied to microbial cultivation, and appropriate orbital shakers with different rotational diameters are commercially available. However, orbital shaking technology has not been widely used beyond the Erlenmeyer flask scales with mammalian cells. Recently, the possibility of shaking suspension cultures of mammalian cells has been considered as an option for increasing their growth kinetics and recombinant protein productivity (*4*). In 2001, Liu and Hong (*5*) reported on successful cell growth in suspension for volumes higher than 10 L using shake bioreactors. They presented the design of systems adapted for animal cell cultures using a shaker and cylindrical vessels of various sizes. Using a fed-batch cultivation strategy, CHO cells were grown to a density of 6×10^6 cells mL^{-1} in a shake reactor containing 18 L of cell suspension. The possibility of using even larger disposable containers has been proposed since the power consumption per unit volume of a large system (20 L) was the same order of magnitude as that of small shake flasks (less than 2 L) and stirred-tank vessels (*6*).

Transient gene expression in suspension cultures of transfected mammalian cells is a rapid approach for the generation of milligram to gram quantities of recombinant proteins for biochemical and preclinical studies (*7*−*9*). In instrumented stirred-tank bioreactors, recombinant monoclonal antibodies have been produced in both CHO and HEK 293 cells at volumes up to 100 L (*10*−*12*). Transient gene expression in these two cell lines has also been performed in Wave bioreactors up to the 20 L scale (*13*, *14*). The use of orbital shake bioreactor systems for transient gene expression has been shown to be feasible to nominal volumes up to 1 L (*10*). Here, we optimized existing poly(ethylenimine) (PEI)-mediated transfection protocols (*10*, *11*) to obtain up to 60 mg L^{-1} recombinant antibody under reduced temperature conditions at scales up to 30 L. Mild hypothermic treatment of transiently transfected CHO cells has been shown to increase their productivity at small scales (*15*). The present study shows for the first time that transient transfection can be exploited in combination with large disposable shake bioreactors to efficiently and reliably express a recombinant antibody in CHO cells. This demonstrated the highly promising potential of large-capacity shake bioreactor systems in terms of flexibility, cost-effectiveness, ease of use, and reliable performance.

Materials and Methods

Cells. Suspension-adapted CHO cells (dihydrofolate reductase-deficient strain DG44) (*16*) were cultured in serum-free ProCHO5 medium (Lonza Verviers SPRL, Verviers, Belgium) supplemented with 0.68 g L^{-1} hypoxanthine and 0.194 g L^{-1} thymidine (HT) (SAFC Biosciences, St. Louis, MO). The cells were maintained at 37 °C in 0.5 and 1 L square-shaped bottles (*4*) agitated at 110 rpm in a CO_2- and humidity-controlled

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10.1021/bp070219i CCC: $37.00
Published on Web 10/03/2007

Table 1. Shaking Speed and Working Volume for Different Disposable Shake Bioreactor Systems[a]

		shaking speed (rpm)		working volume	
nominal volume	geometry	at time of transfection	at 4 h post-transfection	at time of transfection	at 4 h post-transfection
50 mL	round, conical	140	180	5 mL	10 mL
5 L	square	78	89	1.25 L	2.5 L
20 L	square	70	78	5 L	10 L
50 L	round, conical	45	55	15 L	30 L

[a] The rotational diameter was kept constant (50 mm) for all bioreactor volumes.

incubator with a shaker having a rotational diameter of 50 mm (ISF-4-W, Adolf Kühner AG, Birsfelden, Switzerland). The cells were subcultured every 3−4 days at a seeding density of 2−5 $\times$ 10^5 cells mL^{-1}. Cell density and viability were determined by the Trypan blue exclusion method. Packed cell volume (PCV) was determined using VoluPAC tubes (Sartorius AG, Göttingen, Germany) and expressed as the percent of the total culture volume as described elsewhere (*17*). Cell size was measured using a CASY1 Counter (Schärfe System GmbH, Reutlingen, Germany).

Plasmid DNA Preparation. The construction of the human anti-Rhesus D IgG light- and heavy-chain gene expression vectors pKML and pKMH, respectively, has been described (*11*). Plasmid DNA was extracted using a commercial purification kit (NucleobondAX, Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol and stored at a concentration of 1 mg mL^{-1} in sterile TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.4).

Transient Transfection. Linear 25 kDa PEI (Polysciences, Eppenheim, Germany) was prepared in water at a final concentration of 1 mg mL^{-1} (pH 7.0) and sterilized by filtration. For each milliliter of culture, 2.5 μg of DNA and various amounts of PEI were added separately to 50 μL of 150 mM NaCl. Prior to transfection, the PEI/NaCl solution was added to the DNA/NaCl solution and allowed to stand at room temperature for 10 min before addition to the culture. Transfections were performed with a 1:1 (w/w) mixture of pKML and pKMH unless stated otherwise.

Shake Bioreactor Systems. For transfections in 50 mL disposable bioreactors, the cells were centrifuged and resuspended in ProCHO5 at a cell density of 2 $\times$ 10^6 cells mL^{-1} (a PCV of 0.5%). A 5 mL aliquot of culture was added to each 50 mL tube fitted with a filter cap for passive headspace aeration (CultiFlask 50, Sartorius AG, Göttingen, Germany) (*18*). Following transfection the tubes were agitated at 37 °C at 140 rpm in an ISF-4-W shaker incubator with a rotational diameter of 50 mm (Adolf Kühner AG) in an atmosphere with 5% CO_2 and 85% relative humidity. After 4 h, 5 mL of ProCHO5 was added to each tube, the agitation speed was increased to 180 rpm (Table 1), and the temperature was reduced to 31 °C or maintained at 37 °C. IgG expression was determined by sandwich ELISA, as described elsewhere (*19*).

For transfections at the 5 and 20 L scale, the cells were centrifuged and resuspended in ProCHO5 at a density of 3−4 $\times$ 10^6 cells mL^{-1} on the day before transfection. The next day, the cells were centrifuged and resuspended in ProCHO5 medium at 2 $\times$ 10^6 cells mL^{-1} (a PCV of 0.5%). The appropriate volumes (Table 1) of cell suspension were then transferred into 5 or 20 L square-shaped polycarbonate containers (Biotainer Carboy, Cellon SA, Luxembourg, Belgium; Figure 1A). Prior to transfection, the cultures were maintained at 37 °C and agitated at either 80 or 70 rpm for the 5 and 20 L bioreactors, respectively (ISF-4-W shaker incubator, Adolf Kühner AG). At 4 h posttransfection, the cells were diluted with one volume of ProCHO5 medium with supplements (Table 1). The agitation diameter was kept constant at 50 mm, and the agitation speed

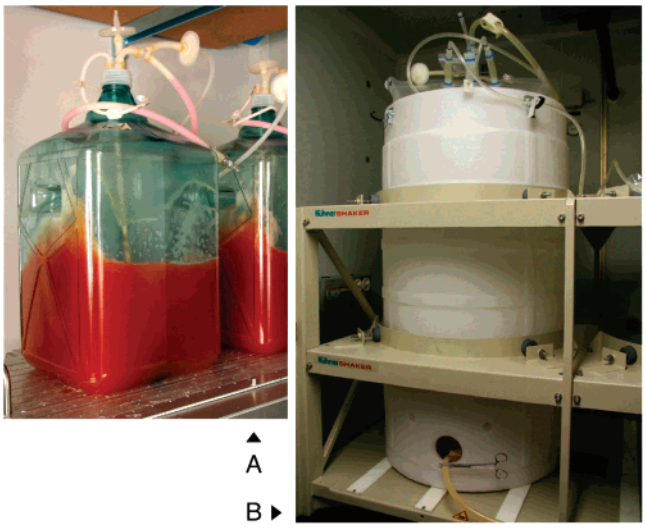

Figure 1. (A) 20 L square-shaped shake bioreactor and (B) 50 L shake bioreactor with large-capacity orbital shaker.

was adjusted to the final working volume (Table 1). The incubator temperature was reduced to 31 °C or maintained at 37 °C. The shake bioreactors were fitted with a three-port cap. One of the ports was connected to a membrane pump, and a constant airflow of 0.5−1.0 L min^{-1} containing an adjustable concentration of CO_2 (1−10%) was pumped into the headspace through a 0.22 μm inlet filter. The level of CO_2 in the inlet air was manually adjusted to maintain a physiological pH. The shake bioreactor gas outlet was fitted with a second sterile filter. Samples were taken directly in the incubator through a sterile sampling port mounted on the cap of the shake bioreactor. The culture was sampled daily for off-line pH measurement (340 pH Meter, Mettler-Toledo, Greifensee, Switzerland) and for analysis of metabolites and antibody quantification. Glucose, lactate, glutamine, and ammonia were measured with a Bio-Profile 200 Analyzer (Nova Biomedical, Waltham, MA).

For transfections at the 50 L scale, disposable bioprocess bags with top and bottom ports and the corresponding container for mounting the bags on the shaker were used (BioProcess Container Systems, HyClone, South Logan, UT; Figure 1B). The outer container, with a cylindrical shape and a conical bottom, was fitted on a modified large-capacity RC-W shaker (Adolf Kühner AG) that was installed in a 15 m^3 warm room. The rotational diameter of the shaker was 50 mm. The volume of ProCHO5 medium required for transfection was pumped into the cell culture bag 1 day prior to transfection to allow the pH and temperature equilibria to be reached. For cell expansion prior to transfection, 5 L shake bioreactors were filled with 2.5 L of CHO cell suspension at a seeding density of 2−5 $\times$ 10^5 cells mL^{-1}. The agitation speed was set at 90 rpm, and 5% CO_2 was added to the inlet airflow (1 L min^{-1}). The medium was exchanged 1 day prior to the transfection and on the day of transfection as described above. The 50 L shake bioreactor was then inoculated with the appropriate volume of cell suspension to reach an initial density of 2 $\times$ 10^6 cells mL^{-1}. Transfection was performed as described above. The temperature was reduced to 31 °C at the time of transfection. At 4 h posttransfection, the cell suspension was diluted with one volume of ProCHO5 medium (Table 1). The airflow rate and CO_2 level were adjusted to keep the pH between 6.8 and 7.0. Due to a low overpressure resulting from the airflow, the bag was completely inflated allowing the bag to adapt its shape to the geometry of the outer container. Samples were taken from the bottom port of the bioprocess bag.

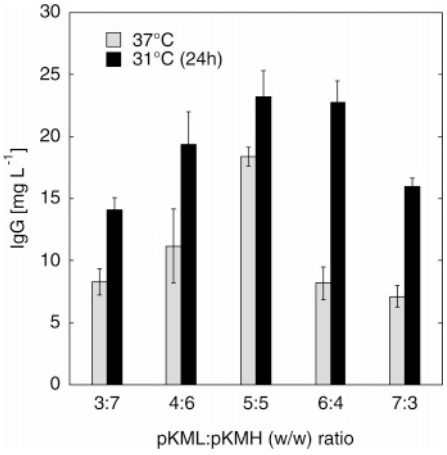

Figure 2. Antibody expression level as a function of various light-chain (pKML) to heavy-chain (pKMH) ratios (w/w) and a constant DNA:PEI ratio of 1:4 (w/w). Total pDNA concentration was kept constant at 2.5 μg mL^{-1} (prior to dilution). Transfections were performed in 50 mL shake bioreactors. At 24 h posttransfection, the transfected cultures were transferred to 31 °C or maintained at 37 °C. Average IgG expression levels at day 6 posttransfection are reported ($n = 3$).

Results

Small-Scale Transient Gene Expression Optimization. Preliminary experiments were performed to determine the optimal ratio of the IgG light and heavy vectors for transient recombinant antibody expression in CHO cells at 31 and 37 °C. The cells were transfected with different mass ratios of pKML and pKMH in 50 mL shake tubes at a DNA:PEI ratio of 1:4 (w/w). At 24 h posttransfection, the tubes were transferred to 31 °C or maintained at 37 °C. Antibody concentration and PCV were assessed on day 6 posttransfection. The highest antibody concentration was obtained at a pKML to pKMH mass ratio of 1:1 at both temperatures (Figure 2). The temperature shift to 31 °C induced an increase in antibody expression over the control at each plasmid ratio tested. The biomass for transfections at 31 °C was reduced as compared to the control transfections at 37 °C with PCV values around 1.0% (corresponding to approximately 4×10^6 cells mL^{-1}) at 31 °C and around 1.5% (6×10^6 cells mL^{-1}) at 37 °C.

To determine the optimal DNA:PEI ratio for transient recombinant antibody production at 31 and 37 °C, various DNA to PEI ratios were tested in 50 mL shake tubes. Keeping the DNA amount at a constant level of 2.5 μg/mL of cell culture (prior to dilution), the amount of PEI was varied from 2.5 to 12.5 μg/mL of cell culture. The pKML to pKMH ratio was maintained at 1:1 (w/w) for all transfections. At 24 h posttransfection, the cells were either shifted to 31 °C or maintained at 37 °C. The optimal DNA to PEI ratio was found to be temperature-dependent. For transfections at 31 °C, the highest antibody yields were obtained with DNA/PEI complexes formed at ratios of 1:3, 1:4, and 1:5 (w/w) (Figure 3A). For the transfected cultures maintained at 37 °C, the highest antibody yields were achieved at DNA:PEI ratios of 1:4 and 1:5 (w/w), as previously reported (*11*). While lower PEI concentrations (ratios of 1:2 to 1:4) resulted in similar PCV values at day 6 posttransfection, an increase in the PEI concentration (DNA:PEI ratio of 1:5) negatively affected cell growth more significantly (Figure 3B). The inhibitory effects with higher amounts of PEI were greater at 31 °C than at the control temperature, resulting in PCV values less than half of those observed at 37 °C (Figure 3B). The comparison of antibody yield and PCV for different DNA:PEI ratios showed that mass ratios of 1:3 and 1:4 were optimal for transfections at 31 and 37 °C, respectively. For extended cultivation periods (more than 9 days), these DNA:PEI ratios were advantageous for preserving the cell culture viability and consequently for yielding higher cumulative antibody levels.

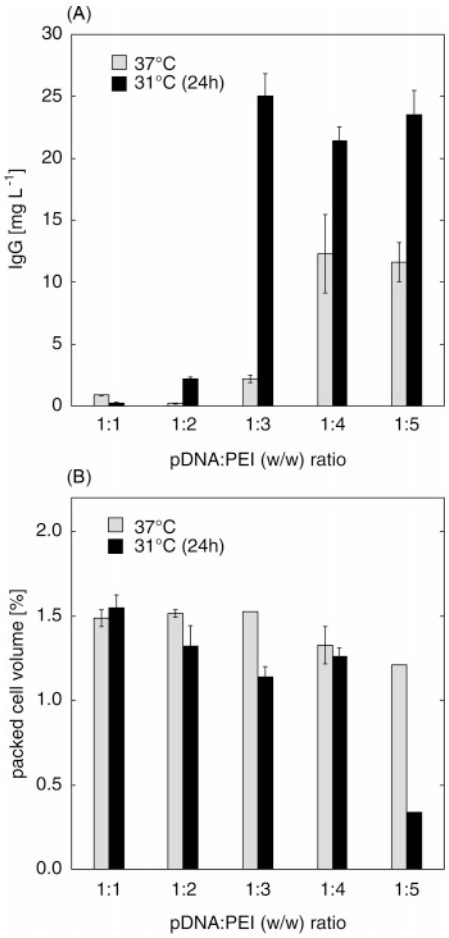

Figure 3. Antibody expression level (A) and packed cell volume (B) as functions of various pDNA to PEI ratios (w/w) and a constant pDNA concentration of 2.5 μg mL^{-1} (prior to dilution). Transfections were performed in 50 mL shake bioreactors. At 24 h posttransfection, the transfected cultures were transferred to 31 °C or maintained at 37 °C. Average IgG expression levels and PCV values of day 6 posttransfection are reported ($n = 3$).

Large-Scale Transient Gene Expression. ***5 L Shake Bioreactor.*** To verify the scalability of shake bioreactors and their suitability for transient protein production, several preliminary tests were made in 5 L square-shaped disposable bottles. First, nontransfected CHO cells were cultivated in ProCHO5 medium at 37 °C, and various combinations of filling volume and agitation speed were tested to optimize cell growth and viability. An airflow rate of 0.5 L min^{-1} was provided continuously through the headspace of the container to ensure that sufficient oxygen would be present at the air−liquid interface. Under these conditions we achieved cell densities of up to $7-8 \times 10^6$ cells mL^{-1} (PCV up to 2%) while maintaining cell viability above 90% for 7−8 days (data not shown). With a working volume of 2.5 L (50% of the nominal volume), agitation speeds between 88 and 90 rpm were found to be ideal in terms of oxygen supply and mixing time.

Subsequently, the impact of three different temperature conditions was assessed for transient recombinant antibody production in CHO cells by transfection with pKML and pKMH at a DNA:PEI ratio of 1:4 (w/w). The temperature was maintained at 37 °C throughout the cultivation period or shifted to 31 °C either at the time of transfection or at 24 h posttransfection. Each of these conditions was tested simultaneously in duplicate. Maximal final antibody yields up to 50−

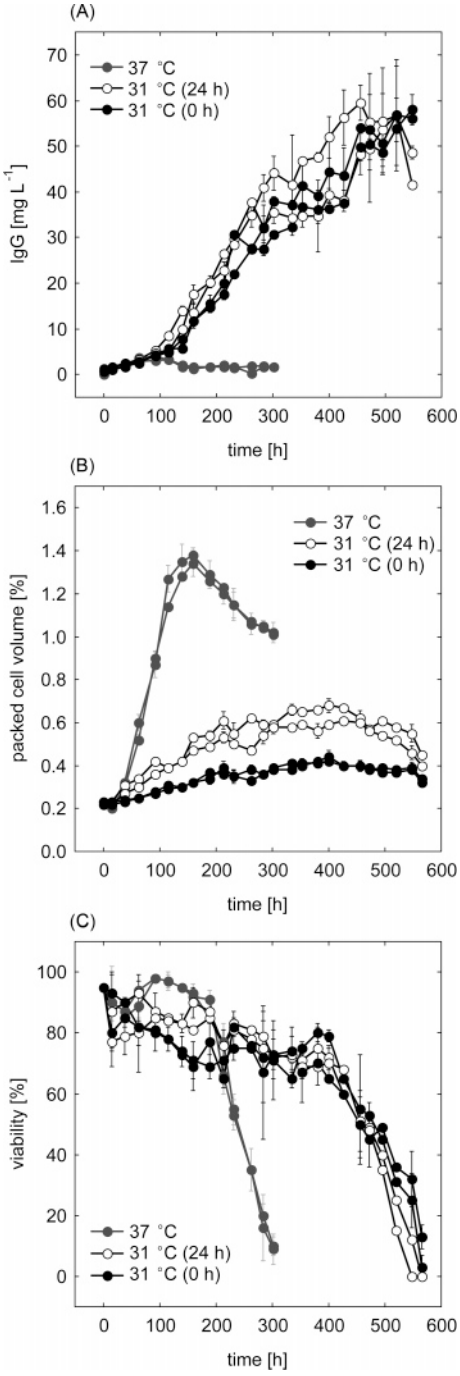

Figure 4. Antibody expression level (A), PCV (B), and viability (C) as functions of time in six 5 L shake bioreactors transfected under identical conditions. The temperature was maintained at 37 °C or shifted to 31 °C either 24 h posttransfection (24 h) or at the time of transfection (0 h). Average IgG ($n = 2$), PCV ($n = 3$), and viability ($n = 2$) values are reported.

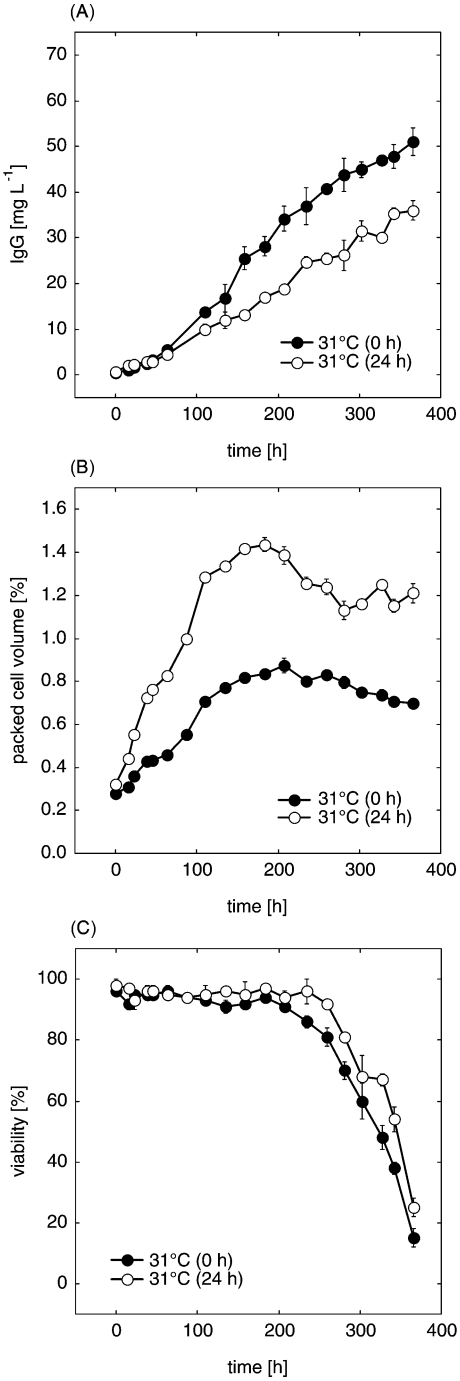

Figure 5. Antibody expression level (A), PCV (B), and viability (C) as functions of time in two 20 L shake bioreactors transfected under identical conditions. The temperature was shifted to 31 °C either 24 h posttransfection (24 h) or at the time of transfection (0 h). Average IgG ($n = 2$), PCV ($n = 3$), and viability ($n = 2$) values are reported.

60 mg L^{-1} were observed for cultures maintained at 31 °C, while the yield was only a few milligrams per liter for the cultures at 37 °C (Figure 4A). The growth kinetics at 37 °C were noticeably different from those at 31 °C (Figure 4B). At 31 °C, the maximal biomass was reached with a delay of about 260 h (11 days) as compared to the cultures at 37 °C (Figure 4B). The maximum PCV for the shake bioreactors shifted to 31 °C at 24 h posttransfection was approximately 1.5-fold higher than the maximum PCV obtained when the temperature was reduced immediately posttransfection (Figure 4B). Typically, the viability of cultures that were transfected and maintained at 31 °C was above 70% for up to 400 h (16−17 days) posttransfection (Figure 4C). Then, the viability decreased. After the viability started to decrease, the antibody expression levels continued to increase (from 40 to 50 to nearly 60 mg L^{-1}) for the transfections at 31 °C (Figure 4A). By comparison, the viability of the cultures at 37 °C was above 70% only for approximately 190 h (8 days).

20 L Shake Bioreactor. Square-shaped polycarbonate containers were used for transient gene expression experiments at the 20 L scale (10 L of working volume) by transfecting cells with pKML and pKMH at a DNA:PEI ratio of 1:4 (w/w). As in experiments at the 5 L scale, an early temperature shift at the time of transfection was compared to a shift at 24 h posttransfection. Here, the earlier temperature shift resulted in an increased antibody yield over time (Figure 5A). After a

cultivation period of more than 340 h (14 days), antibody concentrations of 30 and 50 mg L^{-1} were observed for the cultures shifted to 31 °C at 24 h posttransfection and at the time of transfection, respectively (Figure 5A). These values were similar to transient antibody yields at the 5 L scale. However, the experiments at the 20 L scale were started at a higher initial cell density with PCV values of approximately 0.3% (instead of 0.2% for the 5 L shake bioreactors). Distinct growth kinetics were observed for the two cultures with maximum PCV values of 0.85 and 1.45% for the cultures shifted at the time of transfection and at 24 h posttransfection, respectively (Figure 5B). Cell viabilities over 90% were maintained for more than 200 h of cultivation in both cases (Figure 5C). Residual glucose levels of more than 1 g L^{-1} and glutamine levels between 2 and 3 mmol L^{-1} were assessed in the supernatant toward the end of the production period, indicating that the main nutritional compounds were not rate limiting.

50 L Shake Bioreactor. A further increase in operational scale was achieved using disposable cell culture bags instead of polycarbonate containers. An outer container was used to hold the bag and its contents (Figure 1B). A large-capacity orbital shaker that was modified for this purpose and installed into a warm room provided acceptable shaking power for liquid volumes of up to 50 L. The agitation speed was carefully selected to keep the cells in a homogeneous suspension while avoiding excessive foam formation. CHO cells were transfected with pKML and pKMH as described for the transfections at the 5 and 20 L scales, and the temperature was reduced to 31 °C at the time of transfection. Almost 1 g of human IgG (nonpurified) was produced within 14 days by achieving an antibody concentration of about 30 mg L^{-1} at 340 h posttransfection in a total volume of 30 L (Figure 6A). Similarly to data obtained with the 5 and 20 L shake bioreactors under comparable conditions, the PCV only slightly increased posttransfection (Figure 6B). The PCV reached a maximal value of 0.6% (corresponding to a total cell density of 2.5×10^6 cells mL^{-1}). The cell viability, however, decreased more rapidly than for cultures in the 5 and 20 L containers. Viabilities above 80% were recorded before a rapid decrease occurred approximately 200 h posttransfection (Figure 6B).

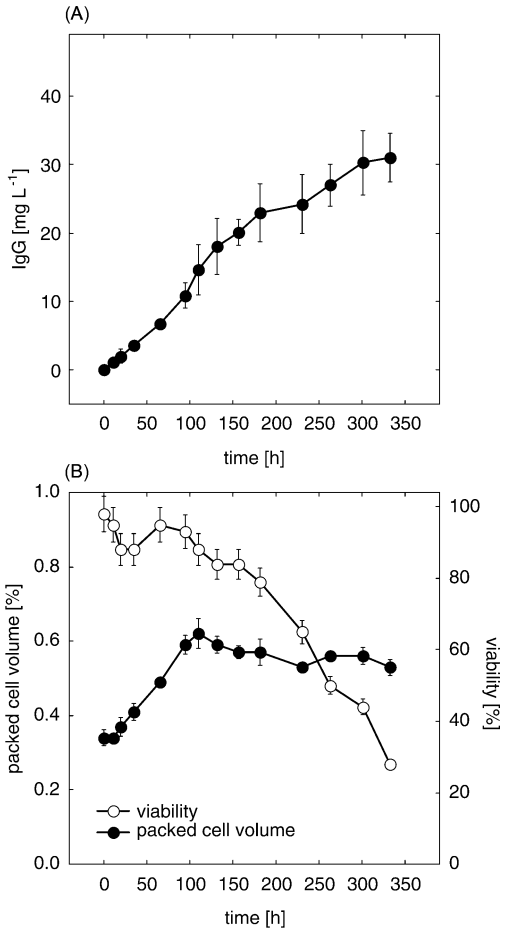

Figure 6. Antibody expression level (A) and PCV and viability (B) as functions of time in a 50 L shake bioreactor with a working volume of 30 L. The temperature was shifted to 31 °C at the time of transfection. Average IgG ($n = 2$), PCV ($n = 3$), and viability ($n = 2$) values are reported.

Discussion

This study demonstrates the potential of shake bioreactor systems for fast and reliable transient production of recombinant proteins in suspension cultures of mammalian cells. Multiparameter experiments with small-scale vessels (50 mL tubes) allowed rapid identification of suitable process parameters with a high degree of confidence. For example, this system was used to show that the optimal DNA:PEI ratio for transfection was temperature-dependent. Afterward, developmental steps were undertaken to verify the scalability of shake bioreactors up to a nominal volume of 50 L. As a consequence, a 1000-fold scale-up for transient recombinant antibody production in CHO cells was accomplished in orbitally shaken disposable containers. Importantly, cell cultivation and transfection in the prototype 50 L shake bioreactor described here relied on the same basic principles as those for the 50 mL shake bioreactors, ensuring smooth process development from laboratory- to pilot-scale. This represents a major advantage over established technologies such as spinner flasks, stirred-tank bioreactors, or wave-type bioreactors because these cultivation systems do not allow operation of multiparameter screening at small scale (less than 25 mL).

Previous studies reported that cultivation of CHO-derived cell lines at reduced temperature enhanced production levels of a variety of recombinant proteins (*20*−*24*). It was shown that despite a decrease in specific growth rate, the improved cell viability and reduced protease activity at low temperature was in part responsible for the higher product yield. Recently, the transient expression of recombinant monoclonal antibodies in CHO cells was found to be up to 8-fold higher at 31−33 °C as compared to 37 °C, demonstrating that similar effects on protein productivity are also applicable to transient gene expression (*15*) (Wulhfard et al., manuscript in preparation). However, these experiments were performed at volumes of less than 100 mL. Now, these findings were confirmed at scales of operation up to 30 L of working volume. The transient recombinant antibody yields (up to 60 mg L^{-1}) observed here were the highest ever reported for the large-scale transfection of CHO cells. Previously, a yield of about 20 mg L^{-1} was observed in a 7 day process in a 150 L stirred-tank bioreactor (80 L working volume) following PEI-mediated transfection (*10*).

As expected, cell growth rates were reduced at 31 °C as compared to 37 °C. This effect was observed at all scales of operation. It is important to note, however, that the increased biomass (PCV) over time for cultures at 31 °C was not correlated to increased cell densities. Instead, the increase in PCV indicated a temperature-dependent cell volume increase under hypothermic culture conditions. CHO cells at 31 °C were found to be up to 1.4-fold more voluminous than the same cells kept under normal cultivation conditions (approximately 2.8 pL at 31 °C and 1.9 pL at 37 °C). Thus, the volume of CHO cells at 31 °C was dramatically expanded. Apparently, the cells adapt their

metabolic activity to a different environment. The fact that hypothermic conditions affect both cell growth and cell division may account for the variability in PCV values observed here for different scales of operation.

The question of oxygen supply in larger shake vessels has not been addressed to date. The oxygen demand (q_{O_2}) of mammalian cells growing in suspension is around $1-3 \times 10^{-10}$ mmol cell^{-1} h^{-1} (*25*−*27*). Using this approximation, the required specific oxygen-transfer coefficient (k_La) is approximately 8 h^{-1} at a cell density of 6×10^6 cells mL^{-1} (which corresponds to a maximal oxygen-transfer rate of 1.8 mmol L^{-1} h^{-1}). In 50 mL shake tubes, maximal k_La values around 45 h^{-1} were measured (Stettler et al., manuscript in preparation). Despite a reduced specific area of the gas−liquid interface for larger systems, we are reasonably confident that sufficiently high oxygen-transfer rates can be obtained in shake bioreactors of scales beyond 100 L under certain conditions. Research to address this question is ongoing.

The shear sensitivity of animal cells in shake bioreactors is another important issue, though there is only limited knowledge of the hydromechanical stress conditions in such systems. Recently, it was shown that, at the same volumetric power consumption, the maximum energy dissipation rate in shake flask systems was 10 times lower than in stirred-tank bioreactors (*28*). Also, this study showed that the same hydromechanical stress conditions (dispersing intensity) can be achieved in unbaffled and baffled shake flasks simply by adapting the agitation speed. This confirms our own observations with shake systems for mammalian cell cultivation. As an example, both square-shaped and cylindrical 20 L shake bioreactors were tested and resulted in very similar growth kinetics (unpublished observations). However, at the same working volume (10 L), very different agitation speeds were used for the two systems (75−80 and 95−100 rpm, respectively). It must be assumed until directly verified that the two different cultures were exposed to similar shear stress conditions given that cell growth and viability were the same.

The 2-fold decrease in maximum IgG yield (Figure 6A) and the lower accumulation of biomass (Figure 6B) observed as the result of the scale-up from 5 to 50 L may have been due to suboptimal process conditions. For the 50 L scale test shown here, the viability was maintained above 80% for only about 180 h, whereas for the same process in the 5 and 20 L bioreactors the viability was maintained above 80% for 300 and 250 h, respectively (Figures 4C and 5C). The results for the transfection at 50 L may be an indication of inappropriate shear stress, insufficient air supply, or nonoptimized shaking speed. In contrast, the data shown here for the 5 and 20 L scales were the result of a more extensive process optimization. Further investigations at the 50 L scale and beyond will focus on these parameters to try to improve cell viability and product yield.

In summary, large-scale disposable shake bioreactors at nominal volumes of up to 50 L were found to be promising in the context of drug discovery programs where milligram or even gram quantities of recombinant proteins are required within short time frames. This is a significant advance because most commercially available mammalian cell cultivation systems in the 1−50 L volume range are costly and often complicated to operate and maintain. Here, it was shown that it is possible to assemble a high-performing large-scale shake bioreactor with conventional laboratory equipment such as orbital shakers, polycarbonate containers, disposable bags, membrane pumps, and incubators. Thus, the present study represents an important step toward flexible and low-cost disposable technologies in modern bioprocessing.

Acknowledgment

The authors acknowledge Jean Cevey and Claude Alves for their valuable technical assistance, the Swiss Innovation Promotion Agency (KTI) for funding this research work and Adolf Kühner AG (Switzerland) for providing a large-capacity orbital shaker that was specifically designed for this project.

References and Notes

(1) Carson, K. L. Flexibility—The guiding principle for antibody manufacturing. *Nat. Biotechnol.* **2005**, *23*, 1054−8.
(2) Farid, S. S.; Washbrook, J.; Titchener-Hooker, N. J. Decision-support tool for assessing biomanufacturing strategies under uncertainty: Stainless steel versus disposable equipment for clinical trial material preparation. *Biotechnol. Prog.* **2005**, *21*, 486−97.
(3) Novais, J. L.; Titchener-Hooker, N. J.; Hoare, M. Economic comparison between conventional and disposables-based technology for the production of biopharmaceuticals. *Biotechnol. Bioeng.* **2001**, *75*, 143−53.
(4) Muller, N.; Girard, P.; Hacker, D. L.; Jordan, M.; Wurm, F. M. Orbital shaker technology for the cultivation of mammalian cells in suspension. *Biotechnol. Bioeng.* **2005**, *89*, 400−6.
(5) Liu, C.; Hong, L. Development of a shaking bioreactor system for animal cell cultures. *Biochem. Eng. J.* **2001**, *7*, 121−125.
(6) Kato, Y.; Peter, C. P.; Akgün, A.; Büchs, J. Power consumption and heat transfer resistance in large rotary shaking vessels. *Biochem. Eng. J.* **2004**, *21*, 83−91.
(7) Baldi, L.; Hacker, D. L.; Adam, M.; Wurm, F. M. Recombinant protein production by large-scale transient gene expression in mammalian cells: State of the art and future perspectives. *Biotechnol. Lett.* **2007**, *29*, 677−84.
(8) Pham, P. L.; Kamen, A.; Durocher, Y. Large-scale transfection of mammalian cells for the fast production of recombinant protein. *Mol. Biotechnol.* **2006**, *34*, 225−37.
(9) Wurm, F.; Bernard, A. Large-scale transient expression in mammalian cells for recombinant protein production. *Curr. Opin. Biotechnol.* **1999**, *10*, 156−9.
(10) Muller, N.; Derouazi, M.; Van Tilborgh, F.; Wulhfard, S.; Hacker, D. L.; Jordan, M.; Wurm, F. M. Scalable transient gene expression in Chinese hamster ovary cells in instrumented and non-instrumented cultivation systems. *Biotechnol. Lett.* **2007**, *29*, 703−11.
(11) Derouazi, M.; Girard, P.; Van Tilborgh, F.; Iglesias, K.; Muller, N.; Bertschinger, M.; Wurm, F. M. Serum-free large-scale transient transfection of CHO cells. *Biotechnol. Bioeng.* **2004**, *87*, 537−45.
(12) Girard, P.; Derouazi, M.; Baumgartner, G.; Bourgeois, M.; Jordan, M.; Jacko, B.; Wurm, F. M. 100-liter transient transfection. *Cytotechnology* **2002**, *38*, 15−21.
(13) Geisse, S.; Jordan, M.; Wurm, F. M. Large-scale transient expression of therapeutic proteins in mammalian cells. *Methods Mol. Biol.* **2005**, *308*, 87−98.
(14) Haldankar, R.; Li, D.; Saremi, Z.; Baikalov, C.; Deshpande, R. Serum-free suspension large-scale transient transfection of CHO cells in WAVE bioreactors. *Mol. Biotechnol.* **2006**, *34*, 191−9.
(15) Galbraith, D. J.; Tait, A. S.; Racher, A. J.; Birch, J. R.; James, D. C. Control of culture environment for improved polyethylenimine-mediated transient production of recombinant monoclonal antibodies by CHO cells. *Biotechnol. Prog.* **2006**, *22*, 753−62.
(16) Urlaub, G.; Kas, E.; Carothers, A. M.; Chasin, L. A. Deletion of the diploid dihydrofolate reductase locus from cultured mammalian cells. *Cell* **1983**, *33*, 405−12.
(17) Stettler, M.; Jaccard, N.; Hacker, D.; De Jesus, M.; Wurm, F. M.; Jordan, M. New disposable tubes for rapid and precise biomass assessment for suspension cultures of mammalian cells. *Biotechnol. Bioeng.* **2006**, *95*, 1228−33.
(18) De Jesus, M. J.; Girard, P.; Bourgeois, M.; Baumgartner, G.; Jacko, B.; Amstutz, H.; Wurm, F. M. TubeSpin satellites: a fast track approach for process development with animal cells using shaking technology. *Biochem. Eng. J.* **2004**, *17*, 217−223.
(19) Meissner, P.; Pick, H.; Kulangara, A.; Chatellard, P.; Friedrich, K.; Wurm, F. M. Transient gene expression: recombinant protein

production with suspension-adapted HEK293-EBNA cells. *Biotechnol. Bioeng.* **2001**, *75*, 197−203.
(20) Kaufmann, H.; Mazur, X.; Fussenegger, M.; Bailey, J. E. Influence of low temperature on productivity, proteome and protein phosphorylation of CHO cells. *Biotechnol. Bioeng.* **1999**, *63*, 573−82.
(21) Hendrick, V.; Winnepenninckx, P.; Abdelkafi, C.; Vandeputte, O.; Cherlet, M.; Marique, T.; Renemann, G.; Loa, A.; Kretzmer, G.; Werenne, J. Increased productivity of recombinant tissular plasminogen activator (t-PA) by butyrate and shift of temperature: a cell cycle phase analysis. *Cytotechnology* **2001**, *36*, 71−83.
(22) Yoon, S. K.; Song, J. Y.; Lee, G. M. Effect of low culture temperature on specific productivity, transcription level, and heterogeneity of erythropoietin in Chinese hamster ovary cells. *Biotechnol. Bioeng.* **2003**, *82*, 289−98.
(23) Fogolin, M. B.; Wagner, R.; Etcheverrigaray, M.; Kratje, R. Impact of temperature reduction and expression of yeast pyruvate carboxylase on hGM-CSF-producing CHO cells. *J. Biotechnol.* **2004**, *109*, 179−91.
(24) Shi, M.; Xie, Z.; Yu, M.; Shen, B.; Guo, N. Controlled growth of Chinese hamster ovary cells and high expression of antibody-IL-2 fusion proteins by temperature manipulation. *Biotechnol. Lett.* **2005**, *27*, 1879−84.
(25) Ruffieux, P. A.; von Stockar, U.; Marison, I. W. Measurement of volumetric (OUR) and determination of specific (qO2) oxygen uptake rates in animal cell cultures. *J. Biotechnol.* **1998**, *63*, 85−95.
(26) Jorjani, P.; Ozturk, S. S. Effects of cell density and temperature on oxygen consumption rate for different mammalian cell lines. *Biotechnol. Bioeng.* **1999**, *64*, 349−56.
(27) Deshpande, R. R.; Heinzle, E. On-line oxygen uptake rate and culture viability measurement of animal cell culture using microplates with integrated oxygen sensors. *Biotechnol. Lett.* **2004**, *26*, 763−7.
(28) Peter, C. P.; Suzuki, Y.; Buchs, J. Hydromechanical stress in shake flasks: correlation for the maximum local energy dissipation rate. *Biotechnol. Bioeng.* **2006**, *93*, 1164−76.

Received July 9, 2007. Accepted August 17, 2007.

BP070219I