Received: 26 April 2023 | Revised: 13 July 2023 | Accepted: 11 August 2023

DOI: 10.1002/elsc.202300204

Engineering in Life Sciences

RESEARCH ARTICLE

Investigation and evaluation of a 3D-printed optical modified cultivation vessel for improved scattered light measurement of biotechnologically relevant organisms

Johanna S. Rehfeld[1] | **Louis M. Kuhnke**[1] | **Christian Ude**[2] | **Gernot T. John**[2] | **Sascha Beutel**[1]

[1]Institute of Technical Chemistry, Leibniz University Hannover, Hannover, Germany

[2]PreSens Precision Sensing GmbH, Regensburg, Germany

Correspondence
Sascha Beutel, Institute of Technical Chemistry, Leibniz University Hannover, Callinstr. 3, 30167 Hannover, Germany.
Email: beutel@iftc.uni-hannover.de

Funding information
Ministry for Economic Affairs and Energy (BMWi); ZIM-iniative, Grant/Award Number: Project no. 16KN070927; Leibniz Universität Hannover

Abstract

In the field of bioprocess development miniaturization, parallelization and flexibility play a key role reducing costs and time. To precisely meet these requirements, additive manufacturing (3D-printing) is an ideal technology. 3D-printing enables rapid prototyping and cost-effective fabrication of individually designed devices with complex geometries on demand. For successful bioprocess development, monitoring of process-relevant parameters, such as pH, dissolved oxygen (DO), and biomass, is crucial. Online monitoring is preferred as offline sampling is time-consuming and leads to loss of information. In this study, 3D-printed cultivation vessels with optical prisms are evaluated for the use in upstream processes of different industrially relevant microorganisms and cell lines. It was shown, that the 3D-printed optically modified well (OMW) is of benefit for a wide range of biotechnologically relevant microorganisms and even for mammalian suspension cells. Evaluation tests with *Escherichia coli*, *Bacillus subtilis*, *Saccharomyces cerevisiae*, and Chinese hamster ovary (CHO) cells were performed, providing highly reproducible results. Growth behavior of OMW cultures was comparable to behavior of shake flask (SF) cultivations and the signal to noise ratio in online biomass measurement was shown to be reduced up to 95.8% by using the OMW. Especially the cultivation phases with low turbidity respective optical densities below 1.0 rel.AU could be monitored accurately for the first time. Furthermore, it was demonstrated that the 3D-printed optics are transferable to different well geometries and sizes, enabling efficient biomass monitoring for individual requirements with tailor-made 3D-printed cultivation vessels in small scale.

Abbreviations: CHO, Chinese hamster ovary; DO, dissolved oxygen; k_La, volumetric mass transfer coefficient; μ_{max}, maximum specific growth rate; NSR, noise to signal ratio; OD, optical density; OMW, optically modified well; SF, shake flask; SFR, shake flask reader; VCC, viable cell count.

KEYWORDS
3D-printing, mammalian cell cultivation, microbial cultivation, online monitoring

1 | INTRODUCTION

In the biopharmaceutical industry, various prokaryotic and eukaryotic organisms are used for the production of recombinant proteins, antibiotics, or vaccines [1, 2]. To serve the ever-increasing pressure to reduce manufacturing costs and accelerate the process development, miniaturization becomes progressively important [3, 4]. Miniaturization offers many advantages for bioprocesses such as high level of parallelization by increasing the experimental throughput. The small working volumes are intended to save valuable resources, which provides great potential for reducing process development costs [4–6].

Orbital shaken systems, such as microtiter plates and shake flask (SF), are basic tools for effective process development due to their simplicity, low-costs, and their possibility of parallel experimentations [7]. In the first steps of bioprocess development, the screening of large numbers of mutants, the microtiter plate became more and more popular [8, 9]. Due to their simple handling and possibility of parallelization, SFs are the commonly used shaken cultivation system in academia as well as in industry. They are not only used for process and media optimization, but also inoculum expansion of the preculture for bioreactor cultivations.

Since sampling is difficult and does change the environmental conditions during the process in miniaturized-scale fermentations due to the small working volume, online monitoring of dissolved oxygen (DO), pH, and biomass gets more and more important [10, 11]. For fast and efficient bioprocess development, it is essential to monitor DO and pH at early stages of development to detect oxygen limitations and critical changes in pH early on [12]. Additionally, one of the most essential parameters in bioprocesses is biomass, which provides information about the progress of the process and determines the time point for induction and harvest [12, 13]. In order to overcome the challenges of small working volumes and limited space inside the vessel, non-invasive optical measurement techniques are often used instead of invasive probes which are generally applied in bench-scale bioreactors [14]

By means of recombinant protein technology, expression of protein-based biopharmaceuticals has been achieved by using several organisms such as bacteria, yeast, or mammalian cells [15–17]. However, the different organisms and cell lines have individual requirements toward their cultivation conditions, for example, oxygen demand or shear stress [18, 19]. To meet those different requirements, 3D-printing technology is used to produce customized cultivation vessels. 3D-printing allows rapid prototyping and cost-effective fabrication of individually designed tailor-made devices of almost unlimited complexity on demand and directly on-site in the laboratory [20–22]. The "layer-by-layer"-fabrication process in additive manufacturing facilitates the implementation of complex components and geometries, which cannot be realized by conventional manufacturing [23].

A previous report was already demonstrated a fully 3D-printed single piece cultivation vessel including prisms for improved scattered light measurement [24]. The integration of the prisms leads to a changed light guidance. Instead of vertical scattered light measurement through the bottom of the vessel [25], the measurement takes place in the horizontal plane by 90° light deflection through the prisms. In addition to the increased linearity of the turbidity measurement, especially in the low optical density (OD) range, the sensitivity could be significantly increased and the noise to signal ratio (NSR) was reduced [24].

This paper focuses on the biotechnological upstream application of the optically modified well (OMW) as described in ref. [24]. To investigate the potential applications of the OMW, initial proof-of-concept cultivations of biotechnologically relevant organisms were performed. For this purpose, the cultivation processes were monitored online with a shake flask reader (SFR) vario (PreSens Precision GmbH, Regensburg, Germany). In addition to biomass monitoring, also pH and DO were recorded using glued-in sensor spots. For further validation, cultivations were conducted in the OMW as well as in commonly used SFs to compare the quality of the online biomass monitoring and the growth behavior of the cultivated organisms. To identify possible limitations of the online biomass measurement with the OMW, it was investigated how sensitive the biomass signal reacts to different vessel geometries and sizes, as well as to metabolic changes of the organisms concerning the amount of carbon source.

2 | MATERIALS AND METHODS

2.1 | Measurement device

In this study, the SFR vario from PreSens Precision Sensing GmbH (Regensburg, Germany) was used. The SFR vario is an optical unit for SFs enabling the non-invasive determination of pH, DO, and biomass simultaneously. The

biomass measurement is based on scattered light detection using a red LED with $\lambda = 630$ nm and a photodiode with an integrated amplifier as a detector. The multisensor device is controlled by the associated PreSens Flask Studio PFS software.

2.2 | Design and measurement principle of the OMW

3D-printed cultivation vessels were designed using the CAD software "Autodesk Inventor Professional 2020" (Autodesk, Inc., SanRafael, USA). Vessels were manufactured using the 3D inkjet printer Agilista-3200 W (Keyence Corporation, Osaka, Japan). The transparent material AR-M2 (Keyence Corporation, Osaka, Japan) was used. Figure 1A shows the printed OMW with a 3D-printed threaded cap to close the vessel. This cap included a thread for commercially single-use cultivation tube lids with gas-permeable membranes and sampling ports and a filter for pressure compensation. Seal rings were integrated for making the system airtight.

For measuring biomass, a light guide was integrated into the 3D-printed design of the well [24]. The prism-like light guide is located outside the well and extends through the wall of the vessel (see Figure 1B–D). This light guide uses its reflective surface to redirect the light used for the scattered light measurement by 90° in the horizontal plane inside the bottom of the vessel.

The translucency of the printing material and the internal reflection was improved by smoothing the rough surface of the 45° slope of the light guide caused by the printing process. Moreover, the bottom and slope of the light guide were bonded with a 4 mm round cover glass. The panes of the light guide on the inside were covered with a clear acrylic lacquer (DETAX, Ettlingen, Germany). For DO and pH determination, two sensor spots (PreSens Precision Sensing GmbH, Regensburg, Germany) containing reversible fluorescent dyes indicating changes in DO and pH were integrated into the cultivation vessel (see Figure 1B,C).

The three holes in the base were used for screws to attach the device directly on the commercial sensor platform.

2.3 | Microorganisms and media

Standard microbial expressions systems were used to evaluate the 3D-printed cultivation vessel. All media were prepared with deionized water (Arium 661 Ultrapure water system, Sartorius Stedim Biotech S. A., Göttingen, Germany). *Escherichia coli* BL21 (DE3) as prominent example for gram-negative bacteria were grown from 20% v/v glycerol stocks stored at −80°C in lysogeny broth (LB) media: 5 g L^{-1} yeast extract (Carl Roth), 10 g L^{-1} peptone from casein (Sigma–Aldrich), 10 g L^{-1} NaCl (Carl Roth) pH 7.1. Pre-cultures were carried out in 250 mL SF with baffles at 30°C at 150 rpm overnight. Main-cultures were conducted at 37°C. As a gram-positive model microorganism, *Bacillus subtilis* (DSM 168) was used. Cells were stored as glycerol stock with 20% v/v glycerol at −80°C. Pre-cultures and main-cultures were conducted in the same way as the *E. coli* cultures. *Saccharomyces cerevisiae* NCYC 1024 a yeast was selected as simple model organism for eucaryotes. For cultivations Yeast-Peptone-Dextrose (YPD) media were used: 10 g L^{-1} yeast extract, 20 g L^{-1} peptone from casein, and a variant amount of glucose (Carl Roth), pH 6.0. Pre-cultures were performed in 250 mL SFs with 20% filling volume at 30°C at 150 rpm overnight.

PRACTICAL APPLICATION

The development of a robust, reproducible and efficient bioprocess requires online monitoring of essential process parameters, such as pH, dissolved oxygen (DO), and biomass. In this study, a 3D-printed cultivation vessel was investigated for the cultivation of various biotechnologically relevant organisms. By geometrical adaptation to fit a commercial sensor platform, online monitoring and recording of process data was enabled, which data was then used for evaluation. The additively fabricated optical modification for improved scattered light-based biomass measurement enables, for the first time, recording of reliable growth curves in 3D-printed culture vessels. After successful cultivation of bacteria and yeast, the use in mammalian cell culture was also shown. Here, it could be shown that a culture of CHO cells with low optical density (OD) properties ($OD_{600} < 1.0$ rel.AU) in shaken culture could be online-monitored accurately. With its high flexibility, 3D-printing made it possible to transfer this optical modification to individual vessel geometries. This allows fast and simple adjustments to the wide-ranging requirements of bioprocesses.

2.4 | Microbial cultivations in 3D-printed OMW

3D-printed cultivation vessels were sterilized by autoclaving (20 min, 121°C). For online monitoring of pH, DO, and biomass, the OMW was set up on the SFR vario. Cultivations of *E. coli*, *S. cerevisiae*, and *B. subtilis* were carried out

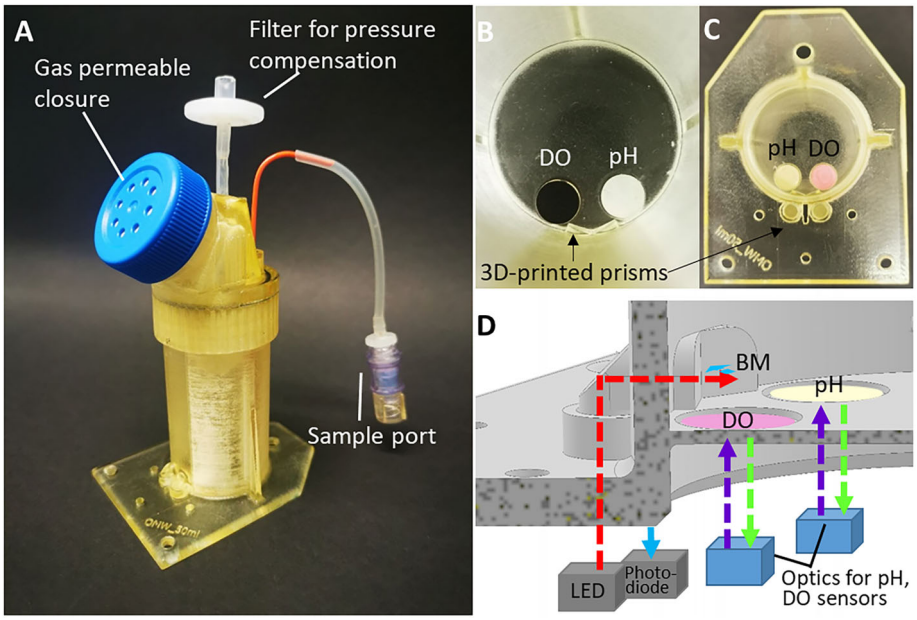

FIGURE 1 3D-printed optically modified well (OMW). (A) Complete OMW with a total volume of 50 mL and a lid with a closure of a commercial 50 mL single-use cultivation tube. (B) Close-up of the vessel bottom from the inside with glued sensor spots for online measuring of pH and dissolved oxygen (DO), and the two prisms used for scattered light-based biomass determination; (C) View of the underside through the transparent bottom of the OMW. (D) Schematic illustration of the beam paths for biomass measurement (excitation light path in red, detection light path in blue) and DO and pH measurement (excitation light path in purple, emission light path in green).

with a filling volume of 12.5 mL on an orbital shaker with 25 mm shaking diameter (Solaris2000, ThermoFisher Scientific, Waltham, USA) and a bench top incubator (CERTOMAT HK, B.Braun Biotech International, Melsungen, Germany). The shaking frequency was set to 345 rpm with a trigger angle of 180° and an illumination time of 68.9 ms.

2.5 | Microbial cultivations in shake flasks

SFs cultivations of *E. coli*, *B. subtilis*, and *S. cerevisiae* were conducted in a commercial 500 mL SF with baffles with sensor spots (SFS 500 WB, PreSens Precision Sensing GmbH, Regensburg, Germany) and a filling volume of 100 mL in the respective media and temperature. The SFR vario with the SF was installed in an incubator with a shaking diameter of 50 mm. Shaking frequency was set to 150 rpm. Trigger angle was set individually for each organism: *E. coli* (310°), *B. subtilis* (0°), and *S. cerevisiae* (20°).

2.6 | CHO K1 cell cultivation

As model organisms for complex eukaryotic cells the mammalian suspension cell Chinese hamster ovary (CHO) K1 were chosen. CHO cells were routinely cultivated in 125 mL SFs in chemically defined media TC-42 w/o L-Glutamine (Sartorius Xell GmbH, Schloß Holte-Stukenbrock, Germany) supplemented with 8 mM L-Glutamine (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) in a 5% CO_2, humidified atmosphere at 37°C at a shaking rate of 120 rpm with an orbital diameter of 19 mm. For cultivation of CHO K1 cells in the 3D-printed OMW, an extensive post-processing after printing was necessary to remove cell toxic leachables and extractables [26, 27]. Therefore, the OMW was first autoclaved then incubated in 70% v/v ethanol at 30°C for 24 h and afterwards autoclaved for a second time. Moreover, no DETAX in case of CHO cell cultivation were used. OMW-cultures were inoculated with 0.3×10^6 cells mL^{-1} and were carried out with a shaking frequency of 150 rpm, a trigger angle of 310° and an illumination time of 68.9 ms. Samples were taken every 24 h and the viable cell count (VCC) and viability of the culture were analyzed using a trypan blue assay-based Cedex cell counter (Cedex HiRes, Roche Diagnostics GmbH, Mannheim, Germany). For SF cultivation, a 125 mL flask with baffles and sensor spots were used with a filling volume of 25 mL and a shaking frequency of 120 rpm. Trigger angle was set to 0°.

2.7 | Biomass calibration

For the correlation of scattered light signal and OD dilution series of stationary phase cells suspensions were

used. Therefore, each cell line was cultivated in the corresponding media until they reached the stationary phase. *E. coli* and *B. subtilis* were cultivated overnight, *S. cerevisiae* for 24 h and CHO cells for 3 days. The cells were harvested by centrifugation and the resulted cell pellet was resuspended. For the microorganisms 0.9% w/v NaCl-solution and for the CHO cells phosphate-buffered saline (PBS) were used. For the calibration procedures sequential dilutions were produced.

The reference measurement of OD was carried out at 600 nm in a $d = 1.00$ cm cuvette (Sarstedt AG & Co.KG, Nümbrecht, Germany) with a photometer (Libra S80, Biochrom GmbH, Cambridge, UK). Calibration procedures itself were performed as described in ref. [24].

3 | RESULTS AND DISCUSSION

3.1 | Correlation of scattered light signal and OD_{600} for different organisms

To estimate the progress of a cultivation, the quantification of cell growth is the most evident parameter. The most established method for offline-biomass quantification is the measurement of OD at 600 nm. This method is simple and fast to perform, but it is only available by offline measurement. The SFR vario enables continuous online measurement of biomass by scattered light.

In our previous work, we already demonstrated a high correlation between the scattered light signal of the SFR vario and offline determined OD_{600} using the turbidity standard formazine and a cell suspension of *S. cerevisiae* in the OMW over the total OD range, especially in the low OD-range [24]. Due to the different morphologies of various organisms and the resulting influence on the scattered light signal [25], calibration procedures with different model organisms were investigated. Therefore, relevant cell lines, used in academia as well as industry for biotechnological application, were chosen. To exclude the influence of the used media on the scattered light signal [28], cultures were harvested once the stationary phase was reached, and the resulting pellets were resuspended in saline or PBS, respectively. Calibrations were performed by sequential dilutions of cell suspensions. Figure 2 shows the calibration curves of *E. coli*, *S. cerevisiae*, *B. subtilis*, and CHO cells. The calibration curves of the microorganisms can be divided into two sections. Considering the lower OD_{600} range (Figure 2B), an exponential correlation between the online measured scattered light signal and the offline determined OD_{600} can be observed. The amplitude for all three organisms measured by the SFR vario only varies slightly between OD_{600} of 0.2 and 1 rel.AU. With increasing OD, there is a clear difference between the organisms in the scattered light signal. With the same offline determined OD, *E. coli* shows the highest amplitude values, followed by *B. subtilis* and *S. cerevisiae*. The calibration curve of *S. cerevisiae* differs from those of *E. coli* and *B. subtilis* from an OD of 1 rel.AU onwards. This can be attributed to the different cell morphologies – round cell shape of *S. cerevisiae* compared to the rod-shaped morphology of *E. coli* and *B. subtilis* [29]. For OD_{600} values above 5 rel.AU, the correlations can be described by linear fits (Figure 2A). When the OD_{600} values are higher (18.1–19.0 rel.AU), the sensor amplitude of *E. coli* is 1.2-fold higher than of *B. subtilis* and even two times higher than of *S. cerevisiae*. Due to their poor light scattering properties, it was so far difficult to record robust growth curves of CHO cells with the SFR vario without high signal to noise ratio [28, 30]. Here, it was shown for the first time, that using 3D-printed prisms enables accurate results concerning correlation of offline determined OD and sensor amplitude by a linear fit with a regression coefficient of determination of 0.997. These results indicate that meaningful correlations between scattered light signal and offline measured OD were achieved over a wide range for all tested organisms. This provide the possibility to establish calibration curves under cultivation-specific conditions (medium, filling volume, shaking frequency) for a direct correlation between the online biomass signal and the OD during cultivation experiments. The here used calibration method by sequential dilution does not take into account the morphological changes of the cells during an ongoing cultivation. Therefore, it is recommended for later applications to perform dynamic calibrations using running cultures and simultaneous online and offline measurements to achieve higher accuracy of prediction.

3.2 | Proof of concept cultivation

After successful correlation of scattered light and OD_{600}, cultivations of different cell lines in the OMW with online monitoring of the critical process parameters DO, pH, and biomass were conducted to investigate the feasibility of the OMW. Figure 3 shows exemplary datasets of *S. cerevisiae* cultivations in the OMW. The results demonstrate the successful use of the OMW for cultivation purposes of *S. cerevisiae* with high reproducibility. Recorded growth curves clearly depicts typical growth behavior of a yeast culture on complex media. DO and pH were monitored over the entire cultivation time by sensor spots placed at the transparent bottom of the OMW. Moreover, the unique metabolic properties can be clearly detected in the biomass signal as well as in online monitored pH and DO signal. In the first 5 h, the biomass signals increase slowly, followed by a short period of exponential growth. Then, a metabolic

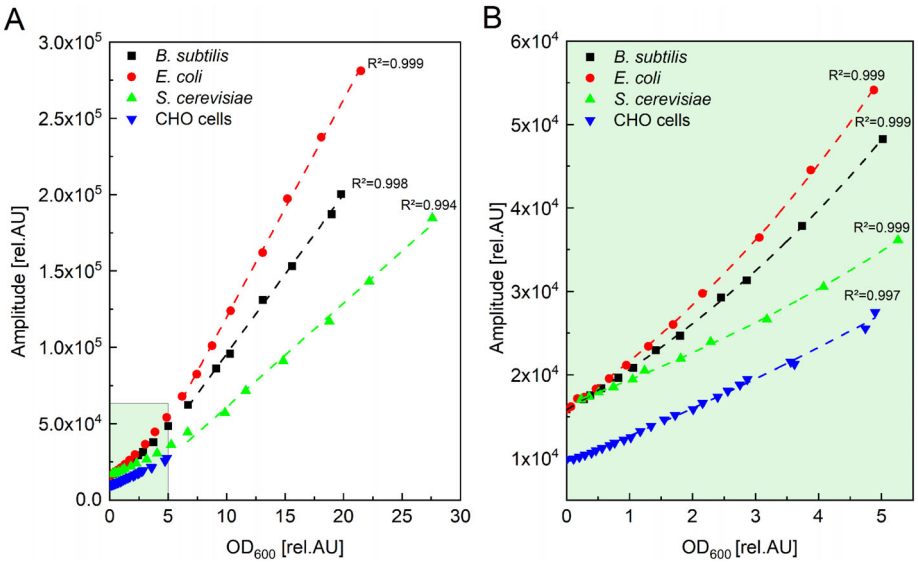

FIGURE 2 Correlation of scattered light measurements and dissolved oxygen $(OD)_{600}$ for different organisms. Comparison of scattered light intensities with OD_{600} values for *B. subtilis*, *E. coli*, *S. cerevisiae*, and Chinese hamster ovary (CHO) with data collected from dilution series of stationary phase cell suspensions. For each data point 10 measurement points were recorded (one measurement point every 30 s). (A) Total calibration range with linear fit between OD_{600} of 5 and 30 rel.AU. (B) Calibration range of lower OD_{600} range with exponential fit.

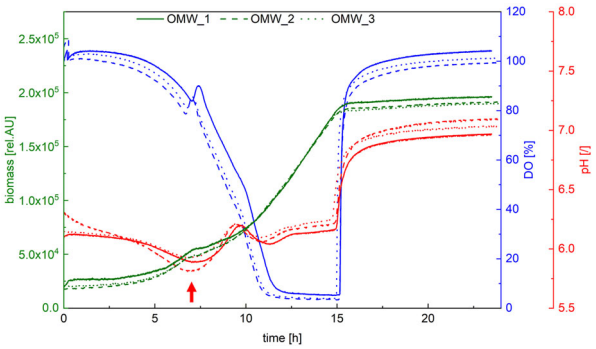

FIGURE 3 Online monitoring of pH, dissolved oxygen (DO) and biomass of *S. cerevisiae* in the optically modified well (OMW) using the shake flask reader (SFR) vario, $n = 3$. *S. cerevisiae* was grown on Yeast-Peptone-Dextrose (YPD) media with 10 g L^{-1} glucose at 30°C, 345 rpm shaking frequency, and 25 mm shaking diameter.

shift is detectable by pH shift, a short increase in the DO signal and a plateau in the biomass signal (indicated by the arrow). At this time point, the primarily carbon source glucose is completely consumed and the cells begin to metabolize the peptone contained in the media as energy source. After 11 h, the cultures reached the phase of maximal growth with maximum specific growth rate (μ_{max}) of between 0.19 and 0.22 h^{-1}. Reaching of the stationary phase is characterized by an abrupt increase in the DO and pH signal, as well as the stable biomass signal. Moreover, further organisms, *E. coli*, *B. subtilis*, and CHO cells, were successfully cultivated in the OMW. Data are shown in Supplemented information (see Figure S1).

3.2.1 | Comparison with commonly used shake flask cultivations

After successful proof of concept cultivations in 3D-printed OMWs, the comparability of growth behavior in the OMW to commonly used SFs was investigated. Therefore, each organism was cultivated in parallel in both cultivation systems. Beyond online monitoring of pH, DO, and biomass, samples were taken for offline determination of OD_{600} using a photometer. Comparing the offline data (see Figure 4), growth was comparable in both systems, regardless of the cell type. Earlier studies showed higher NSRs during calibration procedure for measurement in vertical direction than the horizontal measurement principle due to interfacial reflections [24]. These findings are also reflected in the presented cultivation data (see Figure 4). Biomass signals from all SF cultivations, which are using the vertical measurement principle through the flask bottom, show higher signal noises, especially at the beginning of the experiments, when the OD is low. The signal to noise ratio is also organism dependent. This effect can be contributed to additional reflections of the media surface in the SF. They overlay the reflection signal and are less visible for higher layer thickness, that is, depending on volume and rotation speed and actual OD [25].

To compare the noise characteristics of the biomass signals from OMW to SF, the data of biomass signal from the first hour of cultivation were used. A median filter with a window size of 20 was applied and the average deviation from the original signal was determined (see Table 1). The highest NSR reduction by using the OMW instead of the SF

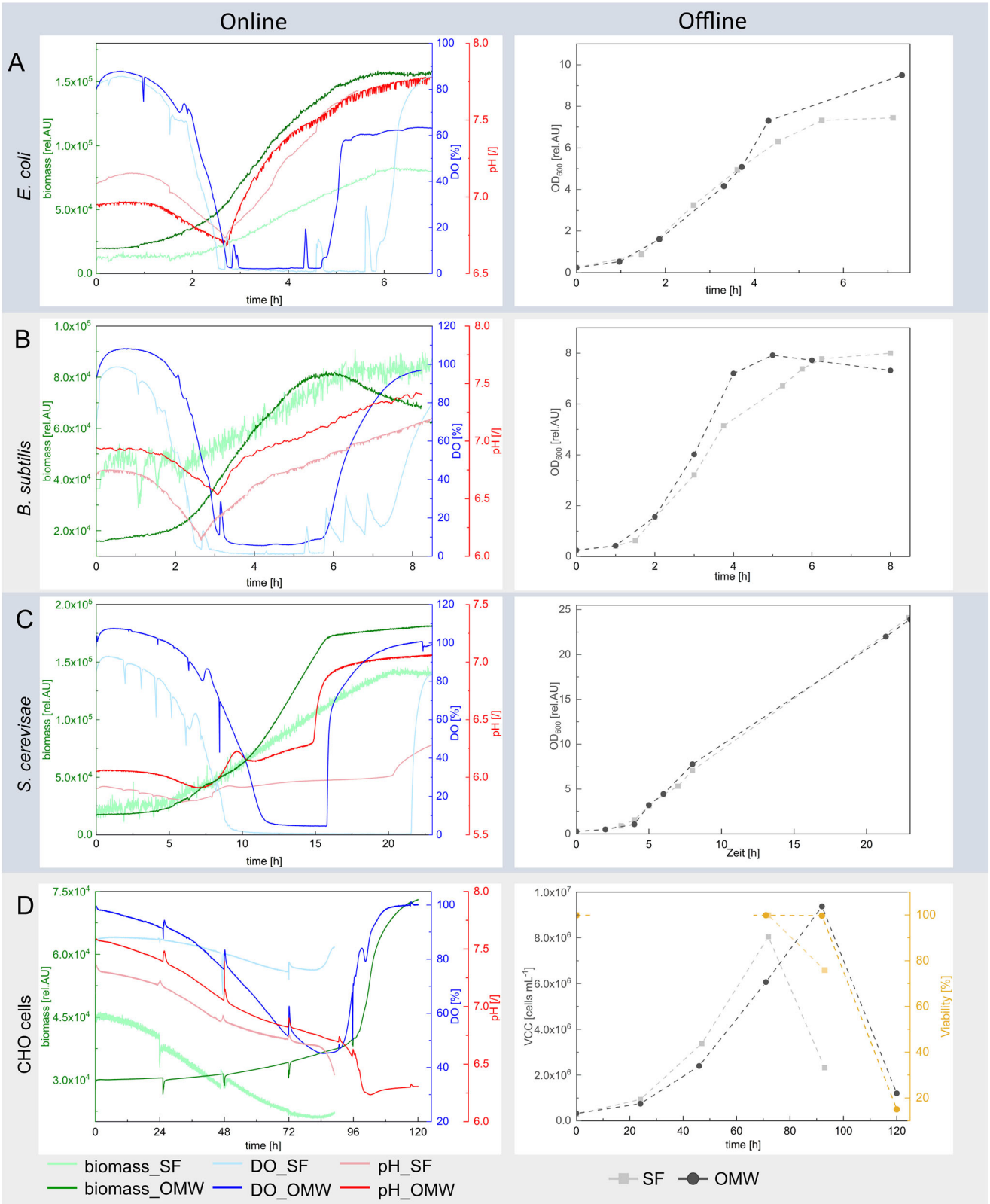

FIGURE 4 Comparison of online monitored biomass, dissolved oxygen (DO) and pH and offline determined growth curves of optically modified well (OMW) and shake flask (SF) cultivations of different model organisms. (A) *E. coli*, (B) *B. subtilis*, (C) *S. cerevisiae*, and (D) Chinese hamster ovary (CHO) cells.

TABLE 1 Comparison of noise to signal ratio of biomass signal from OMW and SF.

Organism	Relative NSR OMW (%)	Relative NSR SF (%)	Relative NSR reduction (%)
E. coli	0.62	7.55	91.78
B. subtilis	0.54	3.87	86.14
S. cerevisiae	0.58	13.82	95.78
CHO cells	0.21	1.266	83.81

CHO, Chinese hamster ovary; NSR, noise to signal ratio; OMW, optically modified well; SF, shake flask.

was achieved with *S. cerevisiae* by more than 95 %. For all tested organisms, an extensive reduction of relative NSR of more than 83% could be observed.

So far, online monitoring of mammalian cell cultures has been difficult due to low optical densities [28, 30]. Data of the CHO cell cultivations highlights the advantage of online biomass measurement with the OMW in comparison to SFs. The online biomass signal of SF cultivation shows a steady decrease, although the VCC increases (Figure 4D). In contrast, the OMW biomass signal shows a steady increase and thereby corresponds to the offline VCC data. Using the improved scattered light measurement by 3D-printed prisms enabled sensitive online monitoring of CHO cells with the SFR vario for the first time. Especially at the beginning of cultivations with low cell numbers and thus lower OD, the online measured OMW growth curves show a small but steady increase in the biomass signal, which much better correlates with the offline data than the growth curves obtained in SF. After 96 h of cultivation, the biomass signal of OMW cultivation increases rapidly, although the offline determined VCC did not increase in a comparable way or even decreased already. Similar observations were made by Maschke et al. It can be assumed that at this time point of cultivation the cells get into death phase which leads to change in cell morphology and cell surface. The increase of the signal could also be confirmed visually by a milky turbidity [30].

Comparing, DO signals with each other, the oxygen limitation in the SF occurs earlier and lasts longer than in the 3D-printed OMW. Both systems were operated with the specific standard conditions. Investigations of the oxygen transfer coefficient (volumetric mass transfer coefficient [$k_L a$]) by the sulfite-oxidation method [31] showed that under these standard conditions, the $k_L a$ value of the SF is 13.88% lower, which means the oxygen supply is worse than in the OMW. If the cultivations of SF and OMW are to be compared not only qualitatively but also quantitatively with each other, the settings should be chosen in such a way that the same $k_L a$ value is achieved in both systems.

In conclusion, the test results highlight the applicability of the 3D-printed cultivation vessel. Not only is the growth in the 3D-printed OMW comparable or even slightly superior to the growth in conventional SFs, but also enables the OMW improves biomass monitoring, which is of great advantage in bioprocesses. By cultivating in the 3D-printed OMW, the NSR could be greatly reduced.

3.2.2 | Variation of the amount of carbon source

The potential of the new scattered light-based online monitoring via 3D-printed prisms was demonstrated by cultivating *S. cerevisiae* in complex YPD-media with various concentrations of glucose. The aim was to investigate limitations of detection of metabolic shifts at low carbon source concentration, what is associated with lower biomass concentration. *S. cerevisiae* was used for the following experiments due to their unique metabolic properties. Figure 5A depicts the recorded growth curves of *S. cerevisiae* on different amounts of glucose with the SFR vario. Dependent on the initial glucose concentration, the cultures attain the stationary phase at different times and the scattered light levels are also carbon source dependent. The more glucose is available, the more biomass can be produced, which is clearly depicted in the biomass signals detected by scattered light-based monitoring.

When the primary carbon source glucose becomes limiting, the cells enter diauxic shift characterized by decreased growth rate, which can be observed as a plateau in the online monitored biomass signal [32]. The online monitored growth curves via 3D-printed prisms enable the detection of bioprocess relevant phenomena as diauxic growth even at low carbon source concentrations (Figure 5B). This gives the possibility to use the online biomass signal for process control, as even small metabolic changes are detectable.

3.2.3 | Influence of the vessel geometry and size

There are three parameters which are mainly adjusted to increase the oxygen transfer rate (OTR) into the cultivation media of a shaken culture. Since increasing the shaking diameter, frequency or reducing the filling volume leads to smaller measuring volume and thus to higher signal noise, another possibility is to modify the vessel geometry and integrate baffles [33]. Funke et al. [10] have systematically investigated the influence of the well geometry of a microtiter plate on the maximum oxygen transfer capacity and identified the well-geometry with a six-petal flower-shape as the optimal geometry in case of maximal OTR by stable liquid height at any filling volumes and shaking frequency. The so-called FlowerPlate is commercially available and compatible for online measuring with the

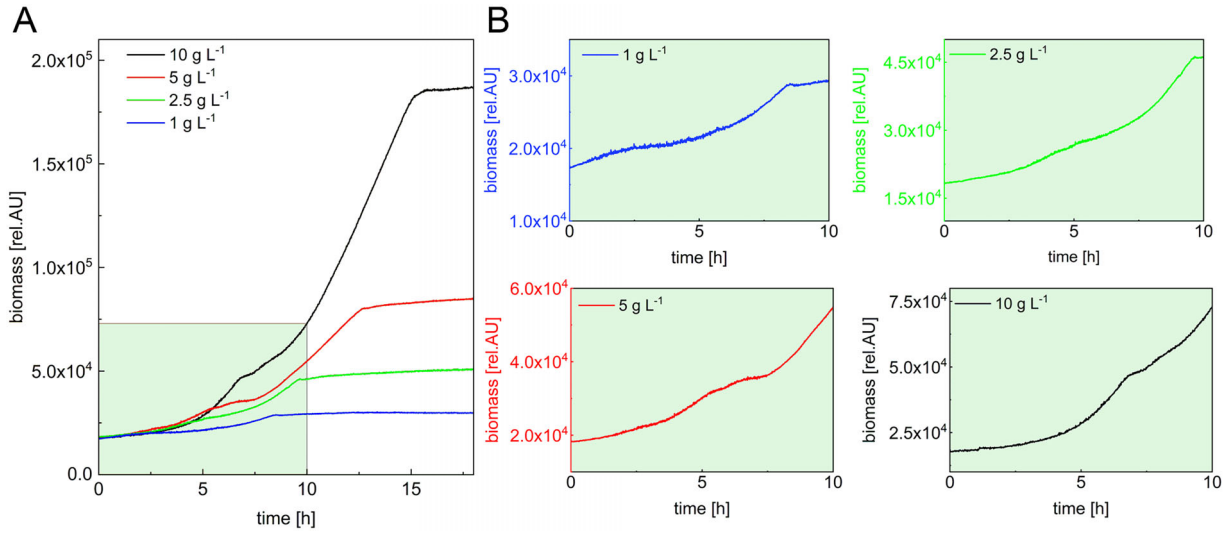

FIGURE 5 Online monitored growth curves and detection of diauxic shift in *S. cerevisiae* cultivations in the optically modified well (OMW) on Yeast-Peptone-Dextrose (YPD)-media with different amounts of glucose (A). Close up to the time point of diauxic shift at each glucose concentration (B).

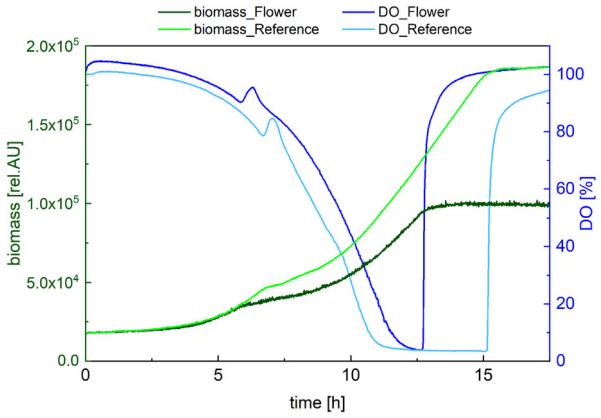

FIGURE 6 Online monitored dissolved oxygen (DO) and biomass signal of *S. cerevisiae* cultures in a standard optically modified well (OMW) (reference) and a flower shaped OMW. *S. cerevisiae* were cultivated in Yeast-Peptone-Dextrose (YPD) media with 10 g L^{-1} glucose at 30°C, 345 rpm shaking frequency, 25 mm shaking diameter.

BioLector (Beckman Coulter GmbH, Aachen, Germany). The high flexibility and straightforwardness of 3D-printing enables the fast prototyping of the OMW in the flower-shape form. Figure 6 shows the results of an *S. cerevisiae* cultivation in the flower-shape OMW in comparison to a non-baffled OMW (reference). Both cultivations show a typical growth curve of *S. cerevisiae*. The online monitored DO signal clearly depicts the higher $k_L a$ value of the flower-shaped OMW. The culture in the non-baffled OMW entered the phase of oxygen limitation earlier and caused longer cultivation times. In the early stage of cultivation, the biomass signals of both systems are quite similar. After 5 h, the NSR of the flower-shape OMW increased. In addition, the amplitude signal increases much more slowly from this point on, reaching only 65% of the maximum value of the reference, although the final measured OD_{600} was even slightly higher. The integration of baffles changes the flow behavior of the fluid [33], which affects the backscattering light after a certain duration of cultivation. These results indicate that the 3D-printed prisms for improved biomass monitoring are transferable to other vessel geometries. However, the here tested flower shape showed lower sensitivity and higher NSR in comparison to the non-baffled OMW.

In some cases, offline analytics are essential for product monitoring or the investigation of potentially produced by-products. For this, a minimum sample volume is required. With a total vessel volume of 50 mL and a working volume of 25% the available sample volume is limited. Above all, a continuous reduction of the cultivation volume would have an influence on the measurement signal [25].

To investigate the effect of the vessel size on scattered light measurement via 3D-printed prisms and growth behavior, two further OMWs with a total volume of 100 and 150 mL were constructed. The height of the vessels and the 3D-printed optic were the same as for the 50 mL OMW. This resulted in an increasing diameter with an increasing vessel volume (see Figure S2).

Figure 7 depicts the online monitored growth curves and DO signals of *S. cerevisiae* cultivations, which were conducted in OMWs with a total volume of 100 mL (OMW_100) and 150 mL (OMW_150). Although, both OMWs were filled with the same culture with an OD_{600} of 0.265 rel.AU, the initial scattered light signal is different. The amplitude value of the OMW_150 is 30.1% higher than of the OMW_100 amplitude value. Both cultures reached

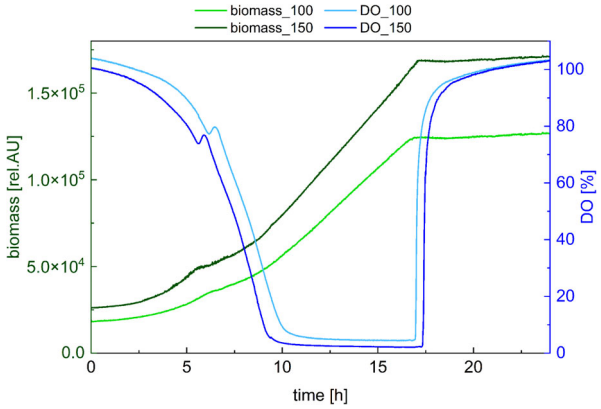

FIGURE 7 Online monitored dissolved oxygen (DO) and biomass signals of *S. cerevisiae* cultures in an optically modified well (OMW) with a total volume of 100 mL or 150 mL. *S. cerevisiae* were cultivated in Yeast-Peptone-Dextrose (YPD) media with 10 g L^{-1} glucose at 30°C, 345 rpm shaking frequency, 25 mm shaking diameter, and a filling volume of 25 mL (OMW_100) and 37.5 mL (OMW_150).

the stationary phase after 17 h of cultivation. The culture in the OMW_100 reached a maximum biomass signal of 127,469.4 rel.AU corresponding to an offline determined OD_{600} of 21.1 rel.AU. Although the offline measured OD_{600} of OMW_150 is with 20.3 rel.AU slightly lower, a higher biomass signal of 171,781.7 rel.AU is obtained, corresponding to the initial relative difference. This could be caused by the increasing surface to volume ratio, which affect the fluid dynamics. Despite the increased diameter, detailed growth curves are obtained, which are comparable to the smaller OMW of 50 mL (see Figure 3).

The change in vessel geometry leads to a change in the light field inside the vessel.

Therefore, new calibrations are required once the vessel geometry or size changes to correlate online determined biomass signal to standard biomass parameters such as OD_{600} or dry/wet cell weight.

4 | CONCLUDING REMARKS

In this study, a 3D-printed cultivation vessel with additive manufactured optical prisms for improved scattered light measurement was tested for biotechnological upstream applications. The potential of the OMW was successfully demonstrated by online monitoring of critical process parameters of bacteria, yeast, and mammalian suspension cells with high reproducibility. A major success was the application of the OMW for suspension CHO cells. The scattered light measurement in the horizontal plane enabled reasonable online biomass monitoring of CHO suspension with low NSR.

The application of the OMW should be extended to further cell types such as microalgae and filamentous organisms to evaluate the 3D-printed system for a wide range of biotechnological relevant organisms. Furthermore, the use should also be evaluated for producing cell lines. Growth behavior of all tested organisms in the OMW was comparable to SF cultivations with the advantage of high-resolution online biomass measurement.

Using the tailor-made OMW in combination with the commercially available SFR vario for online monitoring of shaken cultures makes up a useful tool for accuracy induction or feeding strategies under the guidance of online growth curves. Recorded growth curves enable the identification of bioprocess relevant phenomena such as metabolic shifts, which is mostly hard to detect by manual sampling and offline determination of biomass during bioprocesses.

Generally, the 3D-printed cultivation vessel with improved scattered light measurement provides an excellent tool for shaken cultivations in small scale. Moreover, it was possible to transfer the 3D-printed optics to other vessel geometries and sizes. By that, the concept of the scattered light measurement in the horizontal plane can be applied for individual setups, which is enabled by the high flexibility of 3D-printing. This could be of particular interest for development of customized and highly complex cultivation vessel for a wide range of biotechnological relevant organisms.

ACKNOWLEDGMENTS

The authors thank the Ministry for Economic Affairs and Energy (BMWi) for support within the frame of the ZIM-iniative, Project no. 16KN070927. Furthermore, the authors would like to thank the Open Access fund of Leibniz Universität Hannover for the funding of the publication of this article.

Open access funding enabled and organized by Projekt DEAL.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support findings of this study are available from the corresponding author upon reasonable request.

ORCID

Johanna S. Rehfeld https://orcid.org/0000-0001-9474-0269

Sascha Beutel https://orcid.org/0000-0002-0983-9748

REFERENCES

1. McElwain L, Phair K, Kealey C, Brady D. Current trends in biopharmaceuticals production in Escherichia coli. *Biotechnol Lett*. 2022;44:917-931.

2. Owczarek B, Gerszberg A, Hnatuszko-Konka K. A brief reminder of systems of production and chromatography-based recovery of recombinant protein biopharmaceuticals. *Biomed Res Int*. 2019;2019:4216060.
3. Bareither R, Pollard D. A review of advanced small-scale parallel bioreactor technology for accelerated process development: current state and future need. *Biotechnol Prog*. 2011;27:2-14.
4. Hemmerich J, Noack S, Wiechert W, Oldiges M. Microbioreactor systems for accelerated bioprocess development. *Biotechnol J*. 2018;13:e1700141.
5. Bhambure R, Kumar K, Rathore AS. High-throughput process development for biopharmaceutical drug substances. *Trends Biotechnol*. 2011;29:127-135.
6. Long Q, Liu X, Yang Y, et al. The development and application of high throughput cultivation technology in bioprocess development. *J Biotechnol*. 2014, 192 Pt B:323-338.
7. Klöckner W, Büchs J. Advances in shaking technologies. *Trends Biotechnol*. 2012;30:307-314.
8. Lye GJ, Ayazi-Shamlou P, Baganz F, et al. Accelerated design of bioconversion processes using automated microscale processing techniques. *Trends Biotechnol*. 2003;21:29-37.
9. Duetz WA, Rüedi L, Hermann R, et al. Methods for intense aeration, growth, storage, and replication of bacterial strains in microtiter plates. *Appl Environ Microbiol*. 2000;66:2641-2646.
10. Funke M, Diederichs S, Kensy F, et al. The baffled microtiter plate: increased oxygen transfer and improved online monitoring in small scale fermentations. *Biotechnol Bioeng*. 2009;103:1118-1128.
11. Kensy F, John GT, Hofmann B, Büchs J. Characterisation of operation conditions and online monitoring of physiological culture parameters in shaken 24-well microtiter plates. *Bioprocess Biosyst Eng*. 2005;28:75-81.
12. Harms P, Kostov Y, Rao G. Bioprocess monitoring. *Curr Opin Biotechnol*. 2002;13:124-127.
13. Pörtner R. *Cell Culture Engineering and Technology*. 1st ed. Springer International Publishing; 2021.
14. Lattermann C, Büchs J. Design and operation of microbioreactor systems for screening and process development. In: Mandenius C.-F., ed. *Bioreactors*. Wiley-VCH Verlag GmbH & Co. KGaA; 2016:35-76.
15. Amann T, Schmieder V, Faustrup Kildegaard H, et al. Genetic engineering approaches to improve posttranslational modification of biopharmaceuticals in different production platforms. *Biotechnol Bioeng*. 2019;116:2778-2796.
16. Felberbaum RS. The baculovirus expression vector system: a commercial manufacturing platform for viral vaccines and gene therapy vectors. *Biotechnol J*. 2015;10:702-714.
17. Royle KE, Del Jimenez Val I, Kontoravdi C. Integration of models and experimentation to optimise the production of potential biotherapeutics. *Drug Discov Today*. 2013;18:1250-1255.
18. Frahm B, Brod H, Langer U. Improving bioreactor cultivation conditions for sensitive cell lines by dynamic membrane aeration. *Cytotechnology*. 2009;59:17-30.
19. Betts JI, Baganz F. Miniature bioreactors: current practices and future opportunities. *Microb Cell Fact*. 2006;5:21.
20. Jasiuk I, Abueidda DW, Kozuch C, et al. An overview on additive manufacturing of polymers. *JOM*. 2018;70:275-283.
21. Lerman MJ, Lembong J, Gillen G, Fisher JP. 3D printing in cell culture systems and medical applications. *Appl Phys Rev*. 2018;5:41109.
22. Lücking TH, Sambale F, Beutel S, Scheper T. 3D-printed individual labware in biosciences by rapid prototyping: a proof of principle. *Eng Life Sci*. 2015;15:51-56.
23. Raddatz L, Austerjost J, Beutel S. 3D-Druck: chancen, Möglichkeiten, Risiken. *Chem Unserer Zeit*. 2018;52:42-50.
24. Kuhnke LM, Rehfeld JS, Ude C, Beutel S. Study on the development and integration of 3D-printed optics in small-scale productions of single-use cultivation vessels. *Eng Life Sci*. 2022;22:440-452.
25. Schmidt-Hager J, Ude C, Findeis M, et al. Noninvasive online biomass detector system for cultivation in shake flasks. *Eng Life Sci*. 2014;14:467-476.
26. Anderson JM. *Host Response to Biomaterials: The Impact of Host Response on Biomaterial Selection*. Elsevier/Academic Press; 2019.
27. Siller IG, Enders A, Steinwedel T, et al. Real-time live-cell imaging technology enables high-throughput screening to verify in vitro biocompatibility of 3D printed materials. *Materials*. 2019;12:2125.
28. Ude C, Schmidt-Hager J, Findeis M, et al. Application of an online-biomass sensor in an optical multisensory platform prototype for growth monitoring of biotechnical relevant microorganism and cell lines in single-use shake flasks. *Sensors (Basel, Switzerland)*. 2014;14:17390-17405.
29. Latimer P. Light scattering and absorption as methods of studying cell population parameters. *Annu Rev Biophys Bioeng*. 1982, 11, 129-150.
30. Maschke RW, Pretzner B, John GT, et al. Improved time resolved KPI and strain characterization of multiple hosts in shake flasks using advanced online analytics and data science. *Bioengineering (Basel, Switzerland)*. 2022;9(8):339.
31. Presens Precision GmbH, kLa Determination in Shake Flasks. Accessed July 10, 2023. https://www.presens.de/knowledge/basics/detail/kla-determination-in-shake-flasks-1067
32. Galdieri L, Mehrotra S, Yu S, Vancura A. Transcriptional regulation in yeast during diauxic shift and stationary phase. *OMICS*. 2010;14:629-638.
33. Büchs J. Introduction to advantages and problems of shaken cultures. *Biochem Eng J*. 2001;7:91-98.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Rehfeld JS, Kuhnke LM, Ude C, John GT, Beutel S. Investigation and evaluation of a 3D-printed optical modified cultivation vessel for improved scattered light measurement of biotechnologically relevant organisms. *Eng Life Sci*. 2023;23:e2300204. https://doi.org/10.1002/elsc.202300204