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Anaerobic *Clostridium beijerinckii* Fermentation in Small-Scale Bioreactor Control Systems

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Abstract

To demonstrate the feasibility of conducting anaerobic fermentation in Eppendorf small scale fermenters, we grew the obligate anaerobe *Clostridium beijerinckii*, a natural producer of butanol and isopropanol, in BioBLU® f Single-Use Vessels controlled by the DASbox® Mini Bioreactor and DASGIP® Parallel Bioreactor Systems. The regeneration of the NAD(P)$^+$ pool in *Clostridium* species is essential for the direction of the electron flow towards the final product butanol. Moreover, the intracellular ratio of the electron donor pair NAD(P)$^+$/NAD(P)H is related to the redox potential. Therefore, it is important to monitor and control redox potential during *Clostridium* fermentation to track and adjust the physiological status of the anaerobes.

The objectives of this study are (1) to demonstrate the feasibility of running anaerobic fermentation under these two control stations for small scale applications; (2) to show the importance of redox potential monitoring and control in *C. beijerinckii* fermentation and how it affects bacterial growth and solvent production; and (3) to provide a scale-up example of anaerobic fermentation using BioBLU f Single-Use Vessels which are capable of strictly holding oxygen-free conditions. We found comparable growth and butanol production in the two scales tested based on the constant tip speed scale-up principle, and the increased butanol and isopropanol production when redox potential was appropriately controlled.

Introduction

The obligate anaerobe *Clostridium* species are natural producers of butanol, an important industrial solvent, and a superior biofuel component compared to bioethanol [1]. In standard protocols, butanol is largely produced by *Clostridium acetobutylicum* through acetone/butanol/ethanol (ABE) fermentation, while *Clostridium beijerinckii* can further convert acetone to isopropanol, another useful organic solvent [2].

Solvent production by *Clostridium* species is composed of two metabolic steps: acidogenesis and solventogenesis. During acidogenesis which occurs in the early culture, acetic acid and butyric acid are largely produced by metabolizing carbohydrates. In the late growth phase when solventogenesis takes place, organic solvents including butanol are the major products generated as reduced substances from acids [3]. The redox potential is a property referring to a compound's tendency to acquire electrons and therefore to be reduced, and it is highly related to the concentration ratio of the electron donor pair NAD(P)$^+$/NAD(P)H. Redox potential plays a significant role in *Clostridium* solvent production since the regeneration of NAD(P)$^+$ pool in *Clostridium* species is essential in directing the electron flow towards the final product butanol [4]. Therefore, it is important to have real-time redox potential monitoring in *Clostridium* fermentation and other anaerobic bioprocesses to track the intracellular physiological status changes of the anaerobes. Dissolved oxygen (DO) measurement, on the other hand, is not able to provide such useful information.

BioBLU f Single-Use Vessels are specifically designed for robust microbial fermentation. The application of single-use technology eliminates labor-intensive cleaning, improves turn-around time, simplifies validation, and reduces the risk of cross contamination. The rigid wall stirred tank design supports effective mixing and mass transfer, thus allowing scalability same as the traditional glass vessels. Unlike thin film and bag based fermentation set-ups, the extra thick solid vessel wall prevents gas exchange through the plastic, allowing oxygen level to be maintained at zero to conduct true anaerobic fermentation.

In this study, we performed anaerobic fermentation of *C. beijerinckii* in the BioBLU 0.3f Single-Use Vessels controlled by the DASbox Mini Bioreactor System, and in the BioBLU 1f Single-Use Vessels controlled by the DASGIP Parallel Bioreactor System. These two BioBLU vessels are pre-sterilized and ready to use. Real time redox potential was collected by the Mettler Toledo® autoclavable redox sensors connected to the DASGIP module featuring parallel monitoring of pH, DO, and redox potential (ORP). We first carried out a *C. beijerinckii* fermentation in both vessels without redox control to show the natural pH and redox trends with bacterial growth and solvent production. Then we applied redox control accordingly in a separate run to indicate how redox potential can affect the performance of *C. beijerinckii*. The objectives of this study are (1) to demonstrate the feasibility of running anaerobic fermentation with Eppendorf small scale bioreactor control systems; (2) to show the importance of redox potential monitoring and control in *C. beijerinckii* fermentation and how it affects the bacterial growth and solvent production; and (3) to provide a scale-up example of anaerobic fermentation using BioBLU f Single-Use Vessels which are capable of strictly holding oxygen-free conditions.

Maintaining anaerobic conditions is the key in growing obligate anaerobes in microbiological research, and it is also widely applied in the production of industrial chemicals such as organic acids, gases, alcohols, and various nutraceuticals with health benefits. This application note highlights the strength of Eppendorf bioreactor control systems in anaerobic fermentation applicable to both academia and industry.

Material and Methods

Bacterial strain and cultivation media

The bacterial strain *Clostridium beijerinckii* (DSM 6423) was purchased from DSMZ-German Collection of Microorganisms and Cell Cultures GmbH. We prepared the inoculum in an anaerobic jar as described previously [5]. The inoculation ratio was kept at 1 % (v/v) to initiate all bioreactor fermentations.

Two types of media [5] were used in this study, one was for inoculum preparation in the Eppendorf conical tubes, and the other one was fermentation P2 medium used in the BioBLU f vessels. The pH of both media was adjusted to 6.5. The P2 medium was further supplemented with 1 % (v/v) sterile nutrient stock solution after autoclave sterilization.

BioBLU 0.3f and 1f Single-Use Vessels and the vessel setup

The BioBLU 0.3f and 1f Single-Use Vessels have a working volume range of 65 - 250 mL and 250 mL - 1.25 L, respectively. They are equipped with two Rushton-type impellers and an open pipe sparger for gas supply, allowing robust microbial applications. The Pg 13.5 ports on the head plate are suitable for pH, redox, and DO sensor installation. Both vessel types arrive pre-sterilized out of the box, therefore only media and sensors need to be sterilized through an autoclave cycle before being applied to the vessel.

We used DASGIP DO sensors for dissolved oxygen measurement. The DO sensors are 4.7 mm in outer diameter and have 162 mm insertion depth for BioBLU 0.3f and 278 mm for BioBLU 1f fermenters, respectively. We inserted the DO sensor to a designated port on the head plate of the vessels. The bottom of the sensor-holding sleeve is equipped with a gas permeable membrane. Since there is no direct contact between the DASGIP DO sensor and the fermentation broth, no pre-sterilization is needed for such DO sensors. For redox and pH sensors, we used Mettler Toledo autoclavable glass sensors which fit the Pg 13.5 ports on the head plate. The sensors are 12 mm in outer diameter, with 120 mm insertion depth for BioBLU 0.3f and 225 mm for BioBLU 1f vessels, respectively. Both redox and pH sensors were packed in sterilization pouches to go through an autoclave cycle before being installed onto the vessel head plate in the biosafety cabinet. We prepared the fermentation P2 medium accordingly, autoclaved, cooled to room temperature, poured into the vessel in the biosafety cabinet, and added 1 % (v/v) sterile nutrient stock solution to the vessel before transferring the vessels to the temperature controlled wells of the small scale bioprocess systems [5].

Process parameters

The fermentations were run under strict anaerobic conditions with DO at 0 %. The process parameters for DASbox Mini Bioreactor System and DASGIP parallel bioreactor system are listed in Table 1 for the non-controlled runs. We determined the agitation based on the constant tip speed scale-up strategy. In addition, since we made a series of subcultures from the original DSMZ cryovial for multiple fermentations in this study, the anaerobes may not be at the same metabolic stage when used as inoculum, thus possibly causing delays in growth during fermentation. If no active growth was seen 2-3 days post inoculation, we re-inoculated

with the same amount of inoculum to enhance the culture establishment.

The detailed setup of the anaerobic fermentation in BioBLU 0.3f controlled by DASbox Mini Bioreactor System is shown in Fig. 1. In Fig. 2, the setup for DASGIP Parallel Bioreactor System controlled fermentation using BioBLU 1f is illustrated. All experimental parameters were tracked and controlled by DASware® control 5.

Table 1. Process parameters applied in this study for non-controlled anaerobic fermentation.

Parameter	BioBLU 0.3f	BioBLU 1f
Control station	DASbox Mini Bioreactor System	DASGIP Parallel Bioreactor System
Working volume	250 mL	1 L
Inoculation density	1 % (v/v), 2.5 mL inoculum used	1 % (v/v), 10 mL inoculum used
Agitation	Magnetic drive, 144 rpm, 0.23 m/s	Magnetic drive, 100 rpm, 0.23 m/s
Gassing	Pure nitrogen at 7.5 sL/h (0.5 vvm) till anaerobic culture fully establishes, then gradually shut off gas supply	Pure nitrogen at 30 sL/h (0.5 vvm) till anaerobic culture fully establishes, then gradually shut off gas supply
Temperature	35 °C, liquid-free heating and cooling integrated into the DASbox	35 °C, heating and cooling integrated in DASGIP Bioblock
Exhaust condensation	Liquid-free (Peltier)	Liquid-free (Peltier)
Redox	Not controlled. Sensor connected to an additional PH4PO4RD4L4 module[1]	Not controlled. Sensor connected to the PH4PO4RD4L4 module
pH	Not controlled. Fresh medium with a pH at ~6.5 before autoclave	Not controlled. Fresh medium with a pH at ~6.5 before autoclave
Dissolved oxygen (DO)	0, measured by a DASGIP DO sensor	0, measured by a DASGIP DO sensor
Impeller	Two Rushton impellers	Two Rushton impellers[2]
Gas supply	Open pipe sparger	Open pipe sparger

[1] An option for the DASbox controller with integrated pH, DO, and redox measurement is available. Besides pH and DO, either redox or level can be selected. Here an additional module is used since the controller in use does not have the integrated redox board.
[2] There is another option for BioBLU 1f with three Rushton impellers.

Sensor calibration

We tested the redox sensor following the 1-point calibration method outside of the vessel before sterilization. A commercial redox buffer 220 mV/pH 7 (Mettler Toledo, material No. 51350060) was used to verify the proper functioning of the redox sensor [6, 7].

Also, pH sensors were calibrated outside of the vessel before sterilization. We followed the 2-point temperature compensated calibration method by setting offset using one buffer at pH = 7 and adjusting the slope using another buffer at pH = 4 [6, 7].

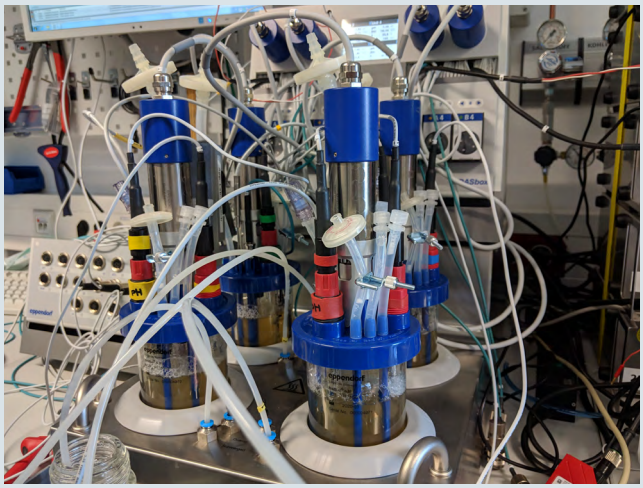

Fig. 1 . Setup of the anaerobic *C. beijerinckii* fermentation in BioBLU 0.3f Single-Use Vessel controlled by DASbox Mini Bioreactor System.

Fig. 2. Set up of the anaerobic *C. beijerinckii* fermentation in BioBLU 1f Single-Use Vessel controlled by DASGIP Parallel Bioreactor System.

We calibrated the DO sensor after autoclaving with the sterile P2 fermentation medium in the vessel. It is recommended that the DO sensor be calibrated under the same condition as during the actual fermentation. Therefore, we set the agitation accordingly based on Table 1, and set the temperature at 35 °C. A 2-point calibration method was applied here [6,7]. We sparged air (21 % oxygen) at 1 vvm until the DO value stabilized to set slope at 100 %; then switched gas supply to pure nitrogen (0 % oxygen) at 1 vvm until the DO value stabilized to set offset at 0 %. Since no DO control was applied in this study, the DO sensors were used to ensure that the cultures were grown under strict anaerobic condition at a 0 % DO.

Redox potential control

Upon completion of the uncontrolled anaerobic fermentation studies in both BioBLU 0.3f and BioBLU 1f bioreactors, we further performed redox potential controlled fermentation in BioBLU 1f vessels. Redox potential was reduced by addition of the membrane filtration sterilized reducing solution of $Na_2S{\cdot}9H_2O$ at 35 g/L. The bottle containing the reducing solution was aseptically connected to the liquid addition port on the vessel head plate through the designated pump head. Pump calibration was performed in advance.

We ran redox potential controlled fermentation using the DASGIP parallel bioreactor system. We divided the four vessels into two groups of duplicates. For the first group, we set the redox potential at -500 mV to keep it at approximately the lowest possible level based on the information collected from the previous uncontrolled fermentation. We integrated a script for automatic feeding as shown in the Appendix. For the second group, based on the simultaneous results collected from automatic liquid addition in the other two vessels, we controlled the redox potential at -420 mV through manual addition of the reducing solution.

Optical density measurement

We collected 1 mL of the fresh medium before inoculation to set the base line for optical density measurement at 600 nm using the Eppendorf BioPhotometer D30. We took samples of the well suspended broth intermittently during each fermentation from all parallel vessels for the optical density measurement. For each run, fermentation concluded at around 72 h after the minimum redox potential was reached, and the final OD_{600} was measured. The culture supernatant was separated from the cells by centrifugation using Eppendorf centrifuge 5702 at 10,000 rpm with standard rotor FA-45-24-11 for 15 min at 4°C. The supernatant was then collected and stored in the freezer at -20 °C for later analysis.

Glucose and solvent analyses

The stored samples were sent to the Microbial Bioprocess Lab of the Forschungszentrum Jülich GmbH, Institute of Bio- and Geosciences, IBG-1: Biotechnology, Jülich, Germany for HPLC analyses. The analytical procedures for glucose, acetic acid, isopropanol, and butanol quantification are described in detail by Pooth et al. [8].

Results

We ran anaerobic *C. beijerinckii* fermentation in the BioBLU 0.3f Single-Use Vessels controlled by the DASbox Mini Bioreactor System, and in the BioBLU 1f Single-Use Vessels controlled by the DASGIP Parallel Bioreactor System. We tracked the real-time pH, DO and redox potential during the uncontrolled *C. beijerinckii* growth phase and used the information to set up the following fermentations with redox control. We took samples to study bacterial growth, glucose consumption, and solvent production. Also, we compared the fermentation results at two different scales to evaluate the scalability of such anaerobic processes.

Anaerobic fermentation in BioBLU 0.3f controlled by DASbox® Mini Bioreactor System

When growing *C. beijerinckii* in BioBLU 0.3f controlled by DASbox Mini Bioreactor System, anaerobic cultures in four bioreactors displayed comparable trends for pH and redox potential (Fig. 3). A re-inoculation at t = 70 h was performed in this run as no obvious growth was observed till then. After re-inoculation, redox decreased from around 30 mV to -500 mV within 24 hours. With the sharp drop of redox potential, pH decreased from 6.2 to around 5.0, showing active

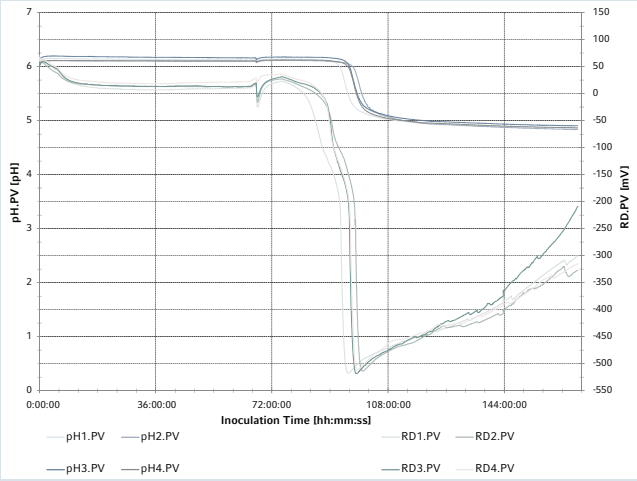

Fig. 3. Anaerobic Fermentation controlled by the DASbox Mini Bioreactor System.
Redox and pH trends of *C. beijerinckii* fermentation without redox control in BioBLU 0.3f at 250 mL working volume.

acidogenesis during which acetic acid and butyric acid were produced in substantial amounts. Redox started to slowly increase in the next 72 hours after reaching the lowest level at -500 mV, and ended at around -300 mV when the fermentation process was stopped. During the redox increase, the pH slighted decreased from 5.0 to 4.8. The broth turned turbid with *C. beijerinckii* growth, and the blue color of the oxygen indicator dye methylene blue disappeared, confirming the establishment of an anaerobic environment in the vessel. Final OD_{600} reached 0.796 with the mean value of 1.836 g/L glucose consumption. The concentrations of butanol and isopropanol were 93 and 62 mg/L, respectively. Foam accumulation was observed especially during the 24 hours with sharp redox decline, and then gradually reduced towards the end of fermentation.

Anaerobic fermentation in BioBLU 1f controlled by DASGIP Parallel Bioreactor System

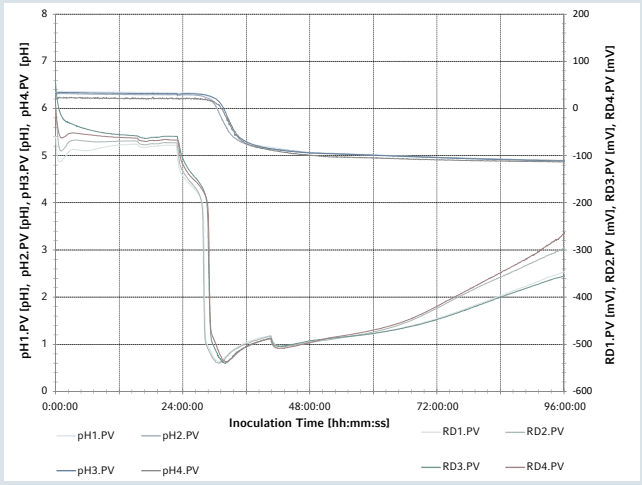

Fig. 4. Anaerobic Fermentation controlled by the DASGIP Bioblock System.
Redox and pH trends of *C. beijerinckii* fermentation without redox control in BioBLU 1f at 1 L working volume.

Based on the constant tip speed scale-up strategy, we reduced the agitation accordingly from 144 to 100 rpm for BioBLU 1f to maintain a constant tip speed of 0.23 m/s. The trends for pH and redox are illustrated in Fig. 4. During this 96-hour fermentation, redox showed drastic decline approximately 24 h after inoculation. After reaching the lowest potential of -540 mV at t = 32 h, redox started to gradually increase to between -350 and -250 mV when fermentation concluded at t = 96 h. Furthermore, the pH dropped from 6.2 to 4.9 during the cultivation. When comparing Fig. 4 with Fig. 3, it is notable that the established *C. beijerinckii* cultures displayed approximately identical trends regarding pH and redox, indicating synchronized metabolic stages between 250 and 1000 mL working volumes. The minor drop of redox potential at t = 40 h was due to the complete shutoff of the nitrogen gas sparging. The final OD_{600} was 0.893 with 2.729 g/L glucose consumption on average, and the concentrations of butanol and isopropanol were 90 and 33 mg/L, respectively.

We illustrate the DO and gas flow rate trends in Fig. 5 to confirm that anaerobic conditions were strictly maintained throughout the fermentation. We started with nitrogen sparging at 30 sL/h (0.5 vvm) and gradually reduced the flow rate till complete shut off at t = 40 h. The DO was well maintained at 0 % even without nitrogen sparging except for a tiny sharp spike to 2-3 % during one of the flow rate adjustments. These observations demonstrate that BioBLU f Single-Use Vessels are capable of strictly holding oxygen-free conditions for anaerobic bioprocess applications.

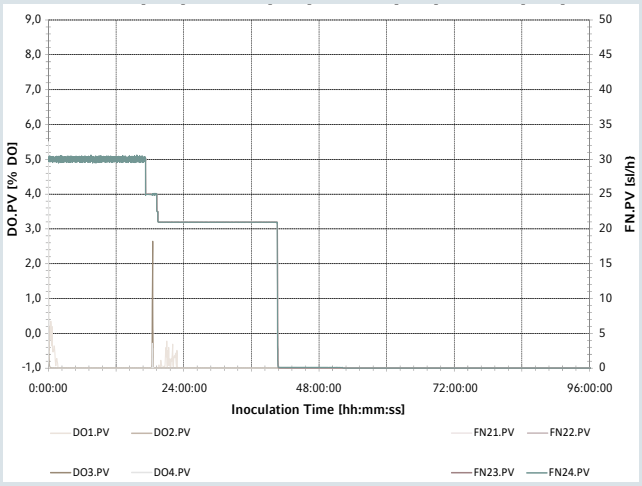

Fig. 5. DO and gas flow trends of *C. beijerinckii* fermentation in BioBLU 1f controlled by DASGIP Parallel Bioreactor System.

Redox controlled anaerobic fermentation

We further implemented redox potential control in *C. beijerinckii* fermentation in BioBLU 1f to measure its effect on growth and solvent production. For the cultures in vessels No. 1 and 2, the addition of 35 g/L $Na_2S{\cdot}9H_2O$ reducing solution was triggered when the redox reached -500 mV for the second time on the rising flank, indicating the start of redox increase after reaching its lowest point. The programming script guided addition started automatically. As observed in Fig. 6, during the 14-hour continuous addition of sodium sulfide solution, the natural increase of redox potential was prevented. Redox was not able to stay low at -500 mV

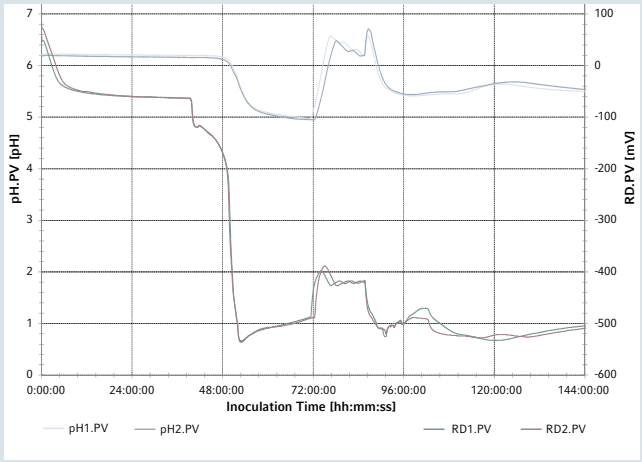

Fig. 6. Redox and pH trends of *C. beijerinckii* fermentation with redox control by script guided feeding of sodium sulfide solution in BioBLU 1f.

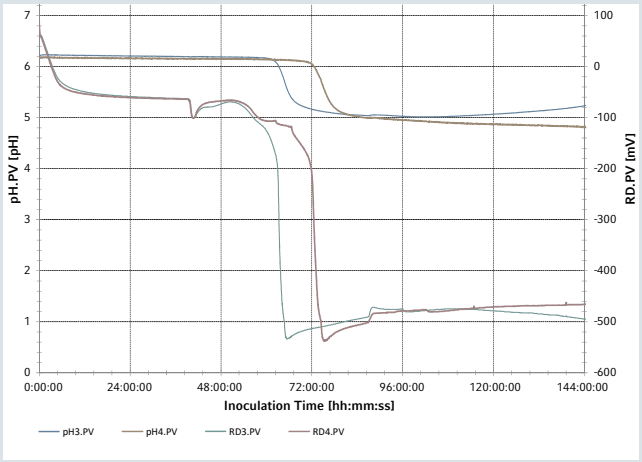

Fig. 7. Redox and pH trends of *C. beijerinckii* fermentation with redox control by manual addition of sodium sulfide solution in BioBLU 1f.

through programmed reducing solution addition, while it was well maintained at -420 mV instead. Simultaneously, since the reducing solution is basic, the addition resulted in an increase of broth pH from 5.0 to 6.5. We stopped the automatic addition of reducing solution at t = 91 h for vessels No. 1 and 2, and manually added 1-3 mL 35 g/L $Na_2S{\cdot}9\ H_2O$ to the other two vessels No. 3 and 4 in order to maintain a low redox potential without causing significant pH changes.

As Fig. 7 shows, the limited amount of sodium sulfide was sufficient to maintain a relatively stable redox potential at -470 mV, while the pH was not affected. Specifically, for vessel No. 3, 30 h after sodium sulfide addition, redox showed a slight decline again to -495 mV, and the pH increased from 5.0 to 5.2. The pH increase reflected effective solventogenesis during which acids generated during acidogenesis were reduced to alcohols as the final solvent products. In this protocol carried out under manual redox control, the final OD_{600} was 0.761, and butanol and isopropanol concentrations were 573 and 458 mg/L, respectively.

Bacterial growth, glucose consumption, and solvent production are summarized in Table 2. For the uncontrolled fermentations at two different working volumes, both anaerobe growth and butanol production were comparable, indicating a feasible scale-up from 250 mL to 1 L. At the 250 mL scale, more isopropanol was produced, and the solvent yield per gram of glucose consumption was higher. When we introduced redox control through the addition of sodium sulfide solution, no growth enhancement was observed. However, butanol production increased more than 6-fold compared to both uncontrolled runs. In addition, isopropanol production was also increased, resulting in a total solvent concentration of 1031 mg/L in the broth upon completion of fermentation. With respect to the solvent yield per gram glucose consumed, the redox controlled run showed a 4.5-fold increase compared to the previously uncontrolled runs at the same working volume. The significant increase in solvent production under redox control demonstrated the enhancement of solventogenesis in *C. beijerinckii* fermentation (Fig. 8).

Table 2. Summary of growth, glucose consumption, and solvent production in all *C. beijerinckii* fermentations (Numbers in brackets are standard deviations of the corresponding means presented).

	OD_{600}	Glucose consumed (g/L)	Butanol concentration (mg/L)	Isopropanol concentration (mg/L)	Total solvent (mg/L)	Total solvent/glucose (mg/g)
Uncontrolled run in BioBLU 0.3f (250 mL)	0.796 (0.020)	1.836 (0.651)	93 (15)	62 (7)	155	84
Uncontrolled run in BioBLU 1f (1 L)	0.893 (0.074)	2.729 (0.861)	90 (20)	33 (18)	123	45
Redox controlled run in BioBLU 1f (1 L)	0.761	5.111	573	458	1031	202

We stress that it is critical to tightly monitor the redox potential in such anaerobic bioprocesses in order to optimize the physiological state of the ongoing microbial culture.

Conclusions

We have demonstrated the feasibility of running anaerobic fermentation using the DASbox Mini Bioreactor System and DASGIP Parallel Bioreactor for small scale applications. With redox monitoring and control, we quantified the changes in *C. beijerinkcii* fermentation.

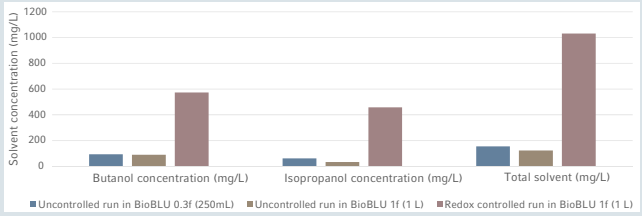

Fig. 8. Comparison of solvent production in *C. beijerinckii* fermentation with and without redox control.

The scale-up strategy from 250 to 1000 ml, based on the constant tip speed principle, resulted in nearly identical redox and pH trends, comparable bacterial growth and butanol production. With an appropriate redox potential control, butanol production increased more than 6-fold, and the solvent production per gram glucose consumed also showed a 4.5-fold increase, indicating the importance of redox potential in regulating the physiological status of the growing anaerobe.

We have demonstrated that BioBLU f Single-Use Vessels are capable of maintaining anaerobic fermentations under oxygen-free conditions for extended periods. Moreover, we verified the anaerobic conditions by applying parallel DO measurements. Therefore, the BioBLU f Single-Use Vessels can be used in a wide array of anaerobic bioprocesses including microbiome related research, and in industrial processes to produce useful organic compounds for pharmaceuticals, fuels, food, and personal care.

Literature

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Appendix

Script to start the Redox-Control directly after a defined threshold is reached.

```
  dim LowRDTrg as double        = -500.0                    ' low Redox trigger level [mV]
  Dim StartDelay_h As Double    = 1/60              ' delay after inoculation [h]


  if p isnot nothing then
    with p
      select case .phase
        case 0 'init
          .phase = .phase + 1
          .LogMessage("Entering phase " & .phase & ": Waiting for InoculationTime > " & format(StartDelay_h, "#0.000") & "
[h]")
        case 1
          if .InoculationTime_H > StartDelay_h then
            .phase = .phase + 1
            .LogMessage("Entering phase " & .phase & ": Waiting for RD.PV < " & LowRDTrg & " [mV]")
          end if
        case 2
          if .RDPV < LowRDTrg then
            .LogMessage("Entering phase " & .phase & ": Activate Redox-Control " )
            .RDActive = true
          end if
      end select
    end with
  end if
```

Ordering information

Description	Order no.
DASGIP® Parallel Bioreactor System	
DASware® control, incl. PC, OS, and licenses, for 4-fold DASGIP® system	76DGCS4
pH Sensor, Hamilton®, for 1 vessel, O.D. 12 mm, L 225 mm	76DGPHHMC220
DASGIP® DO Sensor, for 1 single-use vessel, O.D. 4.7 mm, 220 mm	76DGPODAS220
Redox (ORP) Sensor, Mettler Toledo®, for 1 vessel, O.D. 12 mm, L 220 mm	76DGRDMTI220
Exhaust Condenser, Peltier, for 1 BioBLU® 1f single-use vessel	76DGCONDSU1F
DASGIP® EGC4 Exhaust Condenser Controller, for 4 Peltier actuators, 110 – 240 V/50/60 Hz	76DGEGC4
DASGIP® TC4SC4 Temperature and Agitation Control Module, 110 – 240 V/50/60 Hz, for Bioblock and overhead drives (TC4SC4B), for 4 vessels	76DGTC4SC4B
Overhead Drive RE40, 100 – 1,600 rpm, digitally encoded, for 1 BioBLU® Single-Use Vessel	76DGRE40SU01
DASGIP® Bioblock, 4-position heating/cooling block, max. temp. 99 °C, 110 – 240 V/50/60 Hz, for 4 Vessels	76DGTBLOCK
Accessories for DASGIP® TC4SC4	76DGTCSCUM
DASGIP® PH4PO4RD4L Monitoring Module, for 4 vessels, without sensors, 110 – 240 V/50/60 Hz, pH, DO and redox with level/anti foam option	76DGPH4PO4RDL
Cable, for pH/Redox Sensor, for 1 vessel, AK9 connector	76DGPHRDAK9
Accessories for DASGIP® PHPO	76DGPHPOUM
DASGIP® MX4/4 Gas Mixing Module, Mass Flow Controller, 0.5 – 250 sL/h, 0.5 – 150 sL/h CO2, 110 – 240 V/50/60 Hz, for 4 vessels	76DGMX44H
Accessories for DASGIP® MX4/4(H), MX4/1(H), MF4	76DGMXMFUM
DASGIP® MP8 Multi Peristaltic Pump Module, for 8 feeds, without feed lines and addition bottles, 110 – 240 V/50/60 Hz	76DGMP8
Feed Line Set PTFE, for DASGIP® MP8, 8 tubes incl. addition bottles, L 1.0/0.07/3.0 m, I.D. 0.5/0.8 mm	76DGMP8FL05P13
Accessories for DASGIP® MP4-MP8	76DGMPUM
DASGIP® CWD4 Cooling Water Distribution Unit, incl. connection cable, for 4 condenser-/ and 4 cooling finger ports (CWD4+4)	76DGCWD44
Accessories for DASGIP® CWD4+4, for 4-fold system	76DGCWD44UM
Inline Water Filter, for parallel Bioblock or benchtop systems, incl. accessories for 4-fold or 8-fold systems	76DGIWF
Bracket for Cables/Tubes, for DASGIP® Bioblock and Cooling Water Distribution, 1 long and 1 short pipe	76DGCBLS
Installation material, for a DASGIP® system, for 4 vessels	76DGINMAT4
BioBLU® 1f Single-Use Vessel, fermentation, 2 Rushton-type impellers, sterile, 4 pieces	1386110200
BioBLU® 1f Single-Use Vessel, fermentation, 3 Rushton-type impellers, sterile, 4 pieces	1386110300
Eppendorf BioPhotometer® D30	6133000010
Centrifuge 5702, non-refrigerated, without rotor, rotary knobs, 120 V/60 Hz	022626001

Ordering information - continued

Ordering information

Description	Order no.
DASbox Parallel Bioreactor System	
DASware® control, incl. PC, OS, and licenses, for 4-fold DASbox® system	76DXCS4
pH Sensor, Hamilton®, for 1 DASbox® vessel, O.D. 12 mm, L 120 mm	76DXPHHMC120
DO Sensor, for 1 DASbox® single-use vessel, O.D. 12 mm, L 120 mm	76DXPODAS120
Redox Sensor, for 1 vessel, O.D. 12 mm, L 120 mm	76DXRDMTI120
Exhaust Condenser, Peltier, for 1 BioBLU® 0.3c/f Single-Use Vessels	76DXCONDSU
DASbox® Overhead Drive, 20 – 2,000 rpm, for 1 single-use vessel	76DXOHDSU
DASbox® Feeding and Monitoring Module, without Sensors, 2 Feeds per Vessel, pH, DO and Redox, for 4 vessels (1x MP8-PH4PO4RD4S)	76DXFMR4
Cable, for pH/Redox Sensor, for 1 vessel, AK9 connector	76DGPHRDAK9
Accessories for DASbox PHPO-MP8	76DXPHPOMPUM
DASbox® Base Unit Microbiology, Gassing 0.2 – 25 sL/h, 0.2 – 18 sL/h CO_2, Agitation and Temperature Control, for 4 vessels	76DXBX25
DASbox® Feed Line Set PTFE, for DASGIP® MP8, including addition bottles, L 1.0/0.07/1.0m, I.D. 0.5/0.8 mm	76DXFL05P11
Accessories for DASGIP® MP4-MP8	76DGMPUM
Installation material, for a DASbox® system, for 4 vessels	76DXINMAT4
BioBLU® 0.3f Single-Use Vessel, fermentation, 2 Rushton-type impellers, sterile, 4 pieces	1386100100

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