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SHORT PROTOCOL No. 33

CHO Cell Cultivation in a DASbox® Mini Bioreactor System and DASGIP® Parallel Bioreactor Systems

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Abstract

This protocol explains how to prepare and conduct CHO cell culture processes in the DASbox Mini Bioreactor System and the DASGIP Parallel Bioreactor Systems. We guide the user through all steps of a bioprocess, starting from the preparation of the inoculum to the preparation and operation of the vessels and bioprocess systems, the bioprocess run itself, and the analysis of samples. We explain how these differ with the use of glass and BioBLU® Single-Use Vessels. The protocol can serve as a starting point for further optimization.

Introduction

Cultivation of mammalian cells in controlled, stirred-tank bioreactors is used for the production of biopharmaceuticals like antibodies, hormones, and vaccines, as well as for growing cells for research, drug discovery, and cell-based therapies. In cell culture bioprocesses it is crucial to avoid contamination, especially because mammalian cell cultures grow relatively slowly, and will therefore be overgrown quickly by contaminants. The sterility of the equipment and medium must be ensured at the beginning of the process, but contamination must also be avoided during later manipulations such as inoculation, sampling, and the addition of media and liquids for feeding and pH control.

The DASGIP Parallel Bioreactor Systems and the DASbox Mini Bioreactor System for cell culture applications allow the parallel operation of up to 16 and 24 bioreactors respectively and support the use of both conventional glass and BioBLU Single-Use Vessels. Covering a working volume range of 100 mL to 3.8 L, they are valuable tools for advanced process

Content

development.

In this document, we give an overview of the DASbox Mini Bioreactor System and the DASGIP Parallel Bioreactor Systems. We describe their components and we guide the user through an entire CHO cell bioprocess run using glass or BioBLU Single-Use Vessels. The connections of the bioreactor with pumps and addition bottles are critical for building and maintaining a sterile barrier. In this protocol we present a strategy for the connection of feed lines through weldable connectors.

We have thoroughly tested the following protocol in our applications lab. It allows users to achieve quick and easy initial culture success and can serve as a starting point for further optimization.

Material and Methods

1. Cells and medium

1.1 Culture medium

We cultivated CHO-K1 cells in CD OptiCHO™ medium (Thermo Fisher Scientific®, USA), supplemented with 20.6 mL/L 200 mM L-Alanyl-L-Glutamine (Merck Millipore®, USA) and 10.3 mL/L HT Supplement (sodium hypoxanthine and thymidine, Thermo Fisher Scientific). This medium was used for both the preculture and the main culture.

1.2 Cell line

We used the CHO-K1 cell line (DMSZ no.: ACC-110), which we had adapted to grow in suspension in the culture medium described in section 1.1 as follows:

> We cultivated the cells for a few passages in suspension in Ham's-F12 medium (Merck Millipore), supplemented with 10 % fetal calf serum.
> We exchanged the medium with a mixture of one part of Ham's-F12 medium with 10 % serum and nine parts of serum-free CD OptiCHO medium. As a result the medium mixture contained 1 % serum.
> We cultivated the cells in the medium mixture containing 1 % fetal calf serum for several passages.
> When the cells grew robustly for several passages, we exchangend the medium with CD OptiCHO medium without serum.
> Please refer to the CD OptiCHO medium user guide for more information

1.3. pH control

To adjust the medium pH we used 7.5 % (w/v) sodium bicarbonate. The sodium bicarbonate solution can be purchased as a sterile, ready-to-use solution (e.g. from Thermo Fisher Scientific) and be transferred to a sterile addition bottle. Alternatively the solution can be prepared by dissolving 75 g sodium bicarbonate per liter dH_2O and sterilizing the solution by autoclaving.

2. DASbox Mini Bioreactor System and DASGIP Parallel Bioreactor Systems

The protocol describes a CHO cell culture bioprocess using a DASbox Mini Bioreactor System, a DASGIP Parallel Bioreactor System with benchtop vessels, or a DASGIP Parallel Bioreactor System with Bioblock vessels.

2.1 Vessel types

The controllers can be equipped with various sizes of glass or BioBLU Single-Use Vessels. The following tables list the available vessel types.

Table 1: BioBLU Single-Use Vessels for DASbox Mini Bioreactor System and DASGIP Parallel Bioreactor Systems

Vessel	System	Working volume
BioBLU 0.3c	**DASbox Mini Bioreactor System**	100 – 250 mL
BioBLU 1c	**DASGIP Parallel Bioreactor Systems**	320 mL – 1.25 L
BioBLU 3c	**DASGIP Parallel Bioreactor Systems**	1.25 – 3.75 L

Table 2: Glass vessels for DASbox Mini Bioreactor System and DASGIP Parallel Bioreactor Systems

Vessel	System	Working volume
DASbox Mini Bioreactor	**DASbox Mini Bioreactor System**	60 – 250 mL
DASGIP Bioblock Spinner Vessels	**DASGIP Parallel Bioreactor System with Bioblock**	250 mL – 700 L 350 mL – 1.0 L 350 mL – 1.5 L
DASGIP Benchtop Bioreactors	**DASGIP Parallel Bioreactor System**	750 mL – 2.7 L 850 mL – 3.8 L

2.2 Bioprocess system and vessel components

Table 3 lists the components of the DASbox Mini Bioreactor System and the DASGIP Parallel Bioreactor Systems that are needed to control sensors, pumps, agitation, temperature, exhaust cooling, and gassing. Table 4 lists the corresponding vessel components. Figures 1 and 2 show typical head plate assignments for DASbox Mini Bioreactors and BioBLU 0.3c Single-Use Vessels.

Table 3: Bioprocess system components

Function	Component		
	DASbox	**DASGIP (benchtop)**	**DASGIP (Bioblock)**
pH and DO control	Included in DASbox	DASGIP PH4PO4L	
Level control	Included in DASbox with MP8-PHPO4L option	DASGIP PH4PO4L	
Pump control	Included in DASbox	DASGIP MP8	
Agitation and temperature control	Included in DASbox	DASGIP TC4SC4D	DASGIP TC4SC4B
Control of cooling finger/ cooling baffles	–	DASGIP CWD4+4	
Exhaust cooling (glass vessels)	Included in DASbox	DASGIP CWD4+4	
Exhaust cooling (single-use vessels)	Included in DASbox	DASGIP EGC4 exhaust condensing controller	
Gassing	Included in DASbox	DASGIP MX4/4 (up to 50 sL/h) or DASGIP MX4/4H (up to 250 sL/h)	

Table 4: Vessel components

Function	Component		
	DASbox Mini Bioreactor System	**DASGIP Parallel Bioreactor System with Bioblock**	**DASGIP Parallel Bioreactor System**
Agitation	Motor: DASbox overhead drive Impeller: Marine (glass) or pitched blade (single-use)	Motor: Overhead drive RE30 Impeller: Pitched blade	Overhead drive RE30 Impeller: Pitched blade
Cooling	DASbox	Bioblock	Cooling finger (optional)
Exhaust condensation	Liquid-free (Peltier)	Glass: Water-cooled Single-use: Liquid-free (Peltier) or water-cooled	Glass: Water-cooled Single-use: Liquid-free (Peltier)
pH monitoring	Glass: Gel-filled pH sensor Single-use: Gel-filled or optical pH sensor		
DO monitoring	DO sensor (polarographic, Clark sensor) or optical DO sensor (only BioBLU 3c)		
Temperature monitoring	Platinum RTD Temperature Sensor (Pt100)		
Gas supply	Overlay and/or sparger	Overlay and/or sparger	Overlay and/or sparger
Temperature control	DASbox	Bioblock	Heat blanket
Sampling	Sampling tube with valve		
Liquid addition	C-Flex® feed lines; Bioprene® pump head tubing Feed line A: Medium, inoculum Feed line B: Base		
Options for liquid addition	Long dip tubes for pH agent (base)		
Connectors	Male weldable connector (extension of tubing of bioreactor and addition bottles via Luer lock, Figure 4) Female weldable connector (extension of feed lines via Luer lock, Figure 3) Y-connector (weldable expansion of weldable connectors, Figure 5)		

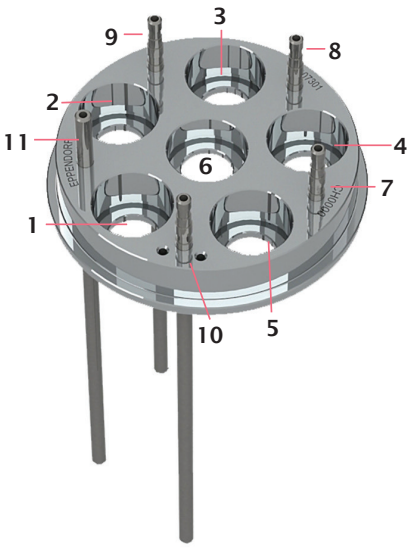


Fig. 1: Typical head plate assignment for the DASbox Mini Bioreactor with a working volume of 100 mL – 250 mL (76DS0250ODSS). The arrangement of the equipment options in the head plate is flexible. Please refer to the DASGIP Bioreactors user manual for more information. Please note that for the other vessel types listed in Table 2 the port accessories may differ.

	Port	Port accessory	Associated device	Purpose	Connected to
1	**Pg 13.5**	Compression fitting I.D. 12 mm	Exhaust condenser with 50 mm silicone tubing and 0.2 µm filter	Exhaust treatment	DASbox base unit
2	**Pg 13.5**	–	pH sensor	Monitoring	DASbox MP8-PHPO module
3	**Pg 13.5**	Compression fitting I.D. 4 mm	Long open pipe with 50 mm silicone tubing with female Luer lock	Welding of Y-connector for pump calibration and base addition	DASbox MP8-PHPO module
4	**Pg 13.5**	Compression fitting I.D. 4 mm	Long open pipe with 50 mm silicone tubing and 0.2 µm filter	Gassing	DASbox base unit
5	**Pg 13.5**	–	DO sensor	Monitoring	DASbox MP8-PHPO module
6	**Pg 13.5**	–	Lipseal stirrer assembly	Agitation	Overhead drive and DASbox base unit
7	**Dip tube short**	50 mm silicone tubing with female Luer lock	–	Welding of Y-connector for addition of medium and inoculum	DASbox MP8-PHPO module
8	**Dip tube short**	50 mm silicone tubing with female Luer lock	–	Not used	–
9	**Dip tube long**	50 mm silicone tubing with sampling valve	–	Sampling	–
10	**Dip tube long**	50 mm silicone tubing with female Luer lock	–	Harvest	–
11	**Ther-mowell**	Thermowell	Platinum RTD temperature sensor (Pt100)	Temperature monitoring	DASbox MP8-PHPO module

I.D. inner diameter

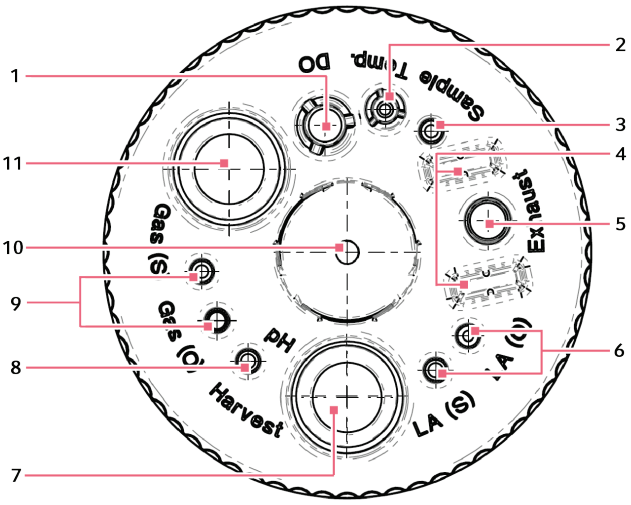


Fig. 2:
Head plate assignment for a BioBLU 0.3c Single-Use Vessel.

	Port/Label	Port accessory	Associated device	Purpose	Connected to
1	**DO**	Long dip tube with silicone membrane	DASGIP DO sensor O.D. 4.7 mm	Monitoring	DASbox MP8-PHPO module
2	**Temp.**	Thermowell	Platinum RTD temperature sensor	Monitoring	DASbox MP8-PHPO module
3	**Sample**	70 mm silicone tubing with sampling valve	Long dip tube	Sampling	–
4	**Mounting of exhaust condenser**	–	–	Exhaust treatment	–
5	**Exhaust**	Silicone tubing with 0.2 µm filter	Exhaust condenser	Exhaust treatment	DASbox base unit
6	**LA(s)** **LA(o)**	70 mm silicone tubing with female Luer lock	Long dip tube Short dip tube	Pump calibration, base addition (submerged) Addition of medium and inoculum (overlay)	DASbox MP8-PHPO module
7	**Pg 13.5**	–	pH sensor	Monitoring	DASbox MP8-PHPO module
8	**Harvest**	70 mm silicone tubing with female Luer lock	Long dip tube	Harvest	DASbox MP8-PHPO module
9	**Gas(s)** **Gas(o)**	Silicone tubing with 0.2 µm filter	Long dip tube Short dip tube	Gassing (submerged) Gassing (overlay)	DASbox base unit
10	**Magnetic coupling**	–	Magnetic drive motor	Agitation	DASbox base unit
11	**Pg 13.5**	–	Blind plug	Not used	–

O.D. outer diameter

Feed lines

To start the bioprocess, culture medium and inoculum have to be added to the bioreactor. During the bioprocess, the base for pH control, and eventually the feed solutions, must also be added. Feed lines are required to add liquids using the system's pumps.

Material and diameter

In this protocol, we use a DASGIP MP8 Multi Pump Module (used with the DASGIP Parallel Bioreactor Systems) or the DASbox MP8-PHPO module (integrated into the DASbox Mini Bioreactor System) to add medium and base to the bioreactor.

DASGIP feed lines consist of three separate parts that are connected to each other: a feed line to connect the addition bottle and pump; in the middle, the pump head tubing, a flexible piece of tubing to insert into the pump head; and another feed line to connect the pump to the bioreactor. While alternative materials are available, in this protocol we use pump head tubing made of Bioprene and feed lines made of C-Flex. As both materials are autoclavable, they are the preferred choice for cell culture applications.

The range of addition rates can be chosen by using pump head tubing with a different inner diameter (please refer to the DASGIP MP8 and MP4 Multi Pump Module operating manual). The recommended inner diameter of the pump head tubing varies depending on the volume to be pumped into the bioreactor. For adding about 200 mL of medium to a DASbox Mini Bioreactor or BioBLU 0.3c Single-Use Vessel, we recommend a pump head tubing with an inner diameter of 2 mm. Using this tubing at maximum pump speed, transfer of 200 mL of medium to the vessel will take approximately 30 min. To add base to the DASbox vessels for pH control, we recommend a pump head tubing with an inner diameter of 0.5 mm. When using bioreactors with larger working volumes, pump head tubing with larger inner diameters may be required.

Eppendorf offers C-Flex feed lines with a length of 1 m and 2 m.

In the following we use the term *feed line* for the assembly of two C-Flex feed lines and pump head tubing.

Connection of feed lines

To add or remove liquids with the system's integrated pumps, feed lines are used to connect the bioreactor via the system's pump to a bottle containing medium, feed solution, pH control solution, or inoculum, or to an empty bottle to collect waste.

As a standard, the parts (feed lines and pump head tubing) are connected with each other and with silicone tubing attached to the bioreactor ports using Luer lock connectors. Alternatively, feed lines can be welded. Welding of feed lines avoids breaking the sterile barrier. Therefore, the welding of feed lines allows the connection of feed lines under sterile conditions, outside a laminar air flow cabinet. This will simplify handling and minimize contamination risk. To be welded, the tubing needs to be made of weldable material like C-Flex and have a inner diameter of 3.2 mm. Tubing made of other materials, such as Bioprene or silicone and/or which has an inner diameter that is not compatible, will first need to be extended with a weldable connector.

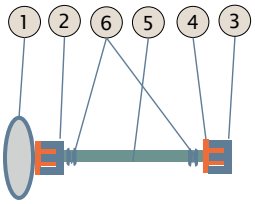
Assembly of weldable connectors

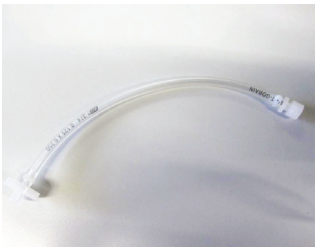
Three types of weldable connectors are required. Female weldable connectors (Figure 3) are used to extend feed lines and male weldable connectors (Figure 4) are used to extend silicone tubing attached to bioreactor ports and addition bottles. The Y-connector (Figure 5) allows for the introduction of a branch.

The assembly of weldable connectors is described below. For use with the DASbox Mini Bioreactor System we recommend connectors made of C-Flex tubing with a length of 20 cm. For larger bioreactors longer tubing might be necessary. The connectors can be autoclaved. To allow gas exchange during autoclaving a syringe filter is connected to one end of the connector; the other end is closed with a Luer lock connector. The syringe filter and Luer lock connectors are displaced during welding. The Luer lock connectors can also be used for the connection of lines without a welding step.

We used the SCD®-IIB Sterile Tubing Welder from Terumo BCT, USA.

Female weldable connector





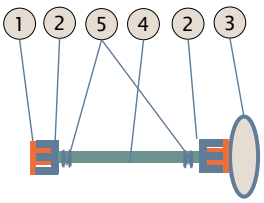
No	Part	Description	Quantity	Material	Possible supplier (order number)
1	Syringe filter (female Luer)	pore size 0.22 µm, non sterile, ø 17 mm	1	PTFE	Carl Roth®, Germany (A075)
2	Luer lock, male	for tubing with I.D. 3.2 mm (1/8'')	1	Kynar®-PVDF	Nordson® Medical, USA (MTLL230-J1A)
3	Luer lock, male cap		1	Kynar-PVDF	Nordson Medical (MTLLP-J1A)
4	Luer lock, female	for tubing with I.D. 3.2 mm (1/8'')	1	Kynar-PVDF	Nordson Medical (FTLL230-J1A)
5	C-Flex tubing	I.D. 3.2 mm, O.D. 6.4 mm, wall thickness 1.6 mm, length 0.2 m	1	C-Flex 374	Saint-Gobain®, France (347-125-2)
6	Mini cable tie	this part is optional	2	Nylon 6.6	Panduit®, USA (BT1M-M39)

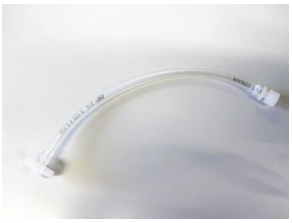
Assembly

> Prepare one piece of C-Flex tubing of 20 cm length.
> Connect a female Luer lock on one side. Optionally, fix the connection using a mini cable tie. Close the Luer lock with a male cap.
> Connect a male Luer lock on the other side. Optionally, fix the connection using a mini cable tie. Connect the Luer lock to a syringe filter that has a female Luer.

Fig. 3: Components and assembly of female weldable connector.

Male weldable connector





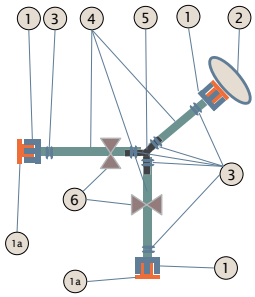
No	Part	Description	Quantity	Material	Possible supplier (order number)
1	Luer lock, female cap		1	Kynar-PVDF	Nordson Medical (FTLLP-J1A)
2	Luer lock, male	for tubing with I.D. 3.2 mm (1/8'')	2	Kynar-PVDF	Nordson Medical (MTLL230-J1A)
3	Syringe filter (female Luer)	pore size 0.22 µm, non sterile, ø 17 mm	1	PTFE	Carl Roth (A075)
4	C-Flex tubing	I.D. 3.2 mm, O.D. 6.4 mm, wall thickness 1.6 mm, length 0.2 m	1	C-Flex 374	Saint-Gobain (347-125-2)
5	Mini cable tie	this part is optional	2	Nylon 6.6	Panduit (BT1M-M39)

Assembly

> Prepare one piece of C-Flex tubing of 20 cm length
> Connect a male Luer lock on one side. Optionally, fix the connection using a mini cable tie. Close the Luer lock with a female cap
> Connect a male Luer lock on the other side. Optionally, fix the connection using a mini cable tie. Connect the Luer lock to a syringe filter that has a female Luer.

Fig. 4: Components and assembly of male weldable connector.

Y-connector



Assembly

> Prepare three pieces of C-Flex tubing of 20 cm length

> Pieces 1 and 2: Connect a male Luer lock on one side. Optionally, fix the connections using a mini cable tie. Close the Luer lock with a female cap. Insert the pieces into a plastic tubing clamp.

> Piece 3: Connect a male Luer lock on one side. Optionally, fix the connection using a mini cable tie. Connect the Luer lock to a syringe filter that has a female Luer.

> Connect the three pieces using a Y-tube fitting.

No	Part	Description	Quantity	Material	Possible supplier (order number)
1	Luer lock, male	for tubing with I.D. 3.2 mm (1/8'')	3	Kynar-PVDF	Nordson Medical (MTLL230-J1A)
1a	Luer lock, female cap		2	Kynar-PVDF	Nordson Medical ('FTLLP-J1A)
2	Syringe filter (female Luer)	pore size 0.22 µm, non sterile, ø 17 mm	1	PTFE	Carl Roth (A075)
3	Mini cable tie	this part is optional	6	Nylon 6.6	Panduit (BT1M-M39)
4	C-Flex tubing	I.D. 3.2 mm, O.D. 6.4 mm, wall thickness 1.6 mm, lenght 0.2 m	3	C-Flex 374	Saint-Gobain (347-125-2)
5	Y-tube fitting	for tubing with I.D. 3.2 mm (1/8'')	1	PVDF	Nordson Medical (Y230-J1A)
6	Plastic tubing clamp	Single-position Mini Clamp	2	Polyester	Bel-Art via VWR (Supplier number: F18227-0000; VWR catalog number: 63022-403)

Fig. 5: Components and assembly of Y-connector.

Extension of feed lines with female weldable connectors

Before autoclaving the feed lines, extend them with female weldable connectors (Figure 6A).

> Connect both sides of the pump head tubing with C-Flex feed lines using Luer lock connectors.

> Extend the feed lines on both ends with female weldable connectors using Luer lock connectors.

3. Overview of equipment that needs to be autoclaved

All parts that will be in direct contact with the culture need to be sterile. The following table lists the equipment required for one bioreactor that needs to be autoclaved before starting the bioprocess run. If you run several bioreactors in parallel the numbers will have to be adjusted accordingly.

Table 5: Equipment that needs to be autoclaved for a single bioreactor for one batch experiment, including pump calibration.

Equipment	Total number needed for one bioreactor		
Male weldable connectors	6	2 to be connected to bioreactor's silicone tubing (Figure 6B) 4 to be connected to addition bottles' silicone tubing (for medium, base, inoculum, waste) (Figure 6B)	
Female weldable connector	4	2 to be connected to feed line A 2 to be connected to feed line B	Connect each feed line with two female weldable connectors before autoclaving (Figure 6A)
Feed line (consisting of pump head tubing and C-Flex feed lines connected via Luer locks)	2	Feed line A and feed line B	
Y-connector	2	1 to extend feed line A (Figure 6C) 1 to extend feed line B (Figure 6C)	
Addition bottles	4	1 for medium (we recommend a 1000 mL bottle) 1 for base (we recommend a 100 mL bottle) 1 for inoculum (we recommend a 100 mL bottle) 1 for waste (we recommend a 500 mL bottle)	
Glass: **Assembled glass vessel including pH and DO sensors**	1		
Single-use: **Glass pH sensor**	1		

4. Preparation of the Bioprocess System

The bioprocess system and vessels must be prepared before the start of cultivation. The preparation comprises the calibration of sensors and pumps; the sterilization of all components that are in direct contact with the culture medium; the assembly of the vessel; and connection of the vessel to the DASbox Mini Bioreactor System or the DASGIP modules.

Below we describe the preparation steps needed for cultivation runs in glass and BioBLU Single-Use Vessels. Figure 7 gives an overview of the preparation steps.

In the following protocol, we refer to the user manuals that have been delivered together with your DASbox Mini Bioreactor System, DASGIP modules, DASGIP bioreactors, BioBLU Single-Use Vessels, and DASware® control software. We recommend having them at hand when preparing a bioprocess run.

4.1 pH sensor calibration and sterilization

In this protocol we use standard glass pH sensors.

> Perform a two-point calibration using buffers at pH 4 and pH 7 outside the bioreactor. The value measured at pH 4 is used to define the slope and the value at pH 7 is measured to set the Zero of the calibration curve. Please refer to the DASware control 5 software manual and DASGIP PHPO sensor modules operating manual for details.

> After calibration sterilize the pH sensor by autoclaving:

>> Glass vessels: Insert the pH sensor into the vessel headplate and autoclave the sensor together with the vessel.

>> BioBLU Single-Use Vessels: Autoclave the pH sensor separately before insertion into the bioreactor head plate.

> To avoid sensor damage, put the sensor in medium or buffer during autoclaving. Do not autoclave the sensor dry and do not autoclave it in deionized water.

> We offer BioBLU c Single-Use Vessels, which can be operated with optical pH sensors. Please contact us for more information.

4.2 Preparation of the bioreactors

Assemble bioreactors

Glass vessels:

> Assemble the bioreactor using the equipment according to Figures 1 and 2, including the DO sensor and the calibrated pH sensor. Please refer to the DASGIP bioreactor user manual for details.

> Extend two of the short dip tubes equipped with 50 mm silicone tubing with Luer lock with male weldable connectors (using Luer lock connectors; Figure 6B).

> Autoclave the bioreactor.

> Place the bioreactor in the DASbox Mini Bioreactor System.

Single-use vessels:

> In a laminar airflow cabinet, insert the pre-sterilized pH sensor.

> In a laminar airflow cabinet, extend the tubes attached to the two LA ports with pre-sterilized male weldable connectors (using Luer lock connectors; Figure 6B).

> Place the bioreactor in the DASbox Mini Bioreactor System.

Extend feed lines

The following steps apply for glass and single-use bioreactors

Feed line A

> The feed line already has been extended with one female weldable connector on each side before autoclaving (Figure 6A).

> Weld one arm of a Y-connector to the sterile feed line A. Use an arm of the Y-connector which has a plastic clamp (Figure 6C)

Feed line B

> The feed line already has been extended with one female weldable connector on each side before autoclaving (Figure 6A).

> Weld the arm of a Y-connector without a plastic clamp to the sterile feed line B (Figure 6C).

Prepare addition bottles

In a laminar airflow cabinet, prepare the sterile addition bottles as follows.

> Medium-addition bottle: Add 180 mL of cell culture medium.

> Base-addition bottle: Add a sufficient volume of base for the pump calibration and for pH control. For a bioprocess run with one to four DASbox Mini Bioreactors, 200 mL of base should be sufficient. If you do not want to measure the medium, it is also possible to pump it in quantitatively via the pump.

> Inoculation bottle: Add the inoculum immediately before

inoculation.
> Extend the silicone tubing of the addition bottles with male weldable connectors (using Luer lock connectors; Figure 6B). Alternatively, the weldable connectors can be connected already before autoclaving the feed bottles.

Connection of bioreactor, addition bottles, and pumps via feed lines

Feed line A

> Weld the medium-addition bottle tubing to feed line A (using the weldable connectors; Figure 6D.1).
> Weld the bioreactor tubing to the arm of the Y-connector that does not have a plastic clamp (using the weldable connector; Figure 6D.1).
> Insert the pump head tubing into the pump head.
> The remaining arm of the Y-connector is welded to the inoculation bottle tubing later (Figure 6D.3).
> Add the metered content of the medium bottle to the bioreactor via the pump.

Feed line B

> Weld the base-addition bottle tubing to feed line B (using the weldable connectors; Figure 6D.2).
> Weld one arm of the Y-connector to the waste bottle tubing (using the weldable connectors; Figure 6D.2).
> Weld the other arm of the Y-connector to the bioreactor tubing (using the weldable connector; Figure 6D.2).
> Insert the pump head tubing into the pump head.
> If no pump calibration is planned, weld feed line B directly to the bioreactor tubing without using the y-connector.

The bioreactor is now ready to start the culture, a sterile test or (optionally) a pump calibration.

Pump calibration (optional)

> Place the waste bottle on a balance to determine the calibration weight.
> Close the tubing to the bioreactor by closing the respective plastic clamp.
> Open the tubing to the waste bottle by opening the respective plastic clamp.
> Calibrate the pumps. Please refer to the DASbox Mini Bioreactor operating manual and the DASGIP MP8 and MP4 multi pump modules user manual for details.
> After the pump calibration, close the tubing to the waste bottle and open the tubing to the bioreactor. In this way, base can be transferred to the bioreactor via feed line B.

Connection of the vessel

> Insert the temperature sensor.
> *Single-use vessel*: Insert the unsterilized DO sensor. The single-use vessel has a non-invasive DO sensor port. The DO sensor is not in direct contact with the culture medium and does not need to be sterile.
> Connect all cables and equipment (motor, sensors, aeration tubing, and the exhaust air cooling tubing, if applicable).

DO sensor calibration

> Calibrate the DO sensor under process conditions (set temperature, agitation speed, gassing rate).
> Perform a slope calibration by saturating the medium with air.
> If you have nitrogen available, perform a zero-point (offset) calibration by saturating the medium with nitrogen. If you do not have nitrogen available, perform a zero-point (offset) calibration by disconnecting the sensor from the amplifier (so-called "electrical zero")
> Please refer to the DASware control 5 software manual and DASGIP PHPO sensor module operating manual for details.

4.3 Sterility test

> To perform a sterility test, turn on control loops for agitation, temperature, gassing, and sensors 12-24 hours before the planned inoculation time point.
> The sterility test is considered successful if the DO and pH values stay stable over the course of the recording and if the medium does not become turbid.
> If the sterility test was successful, proceed with the inoculation.

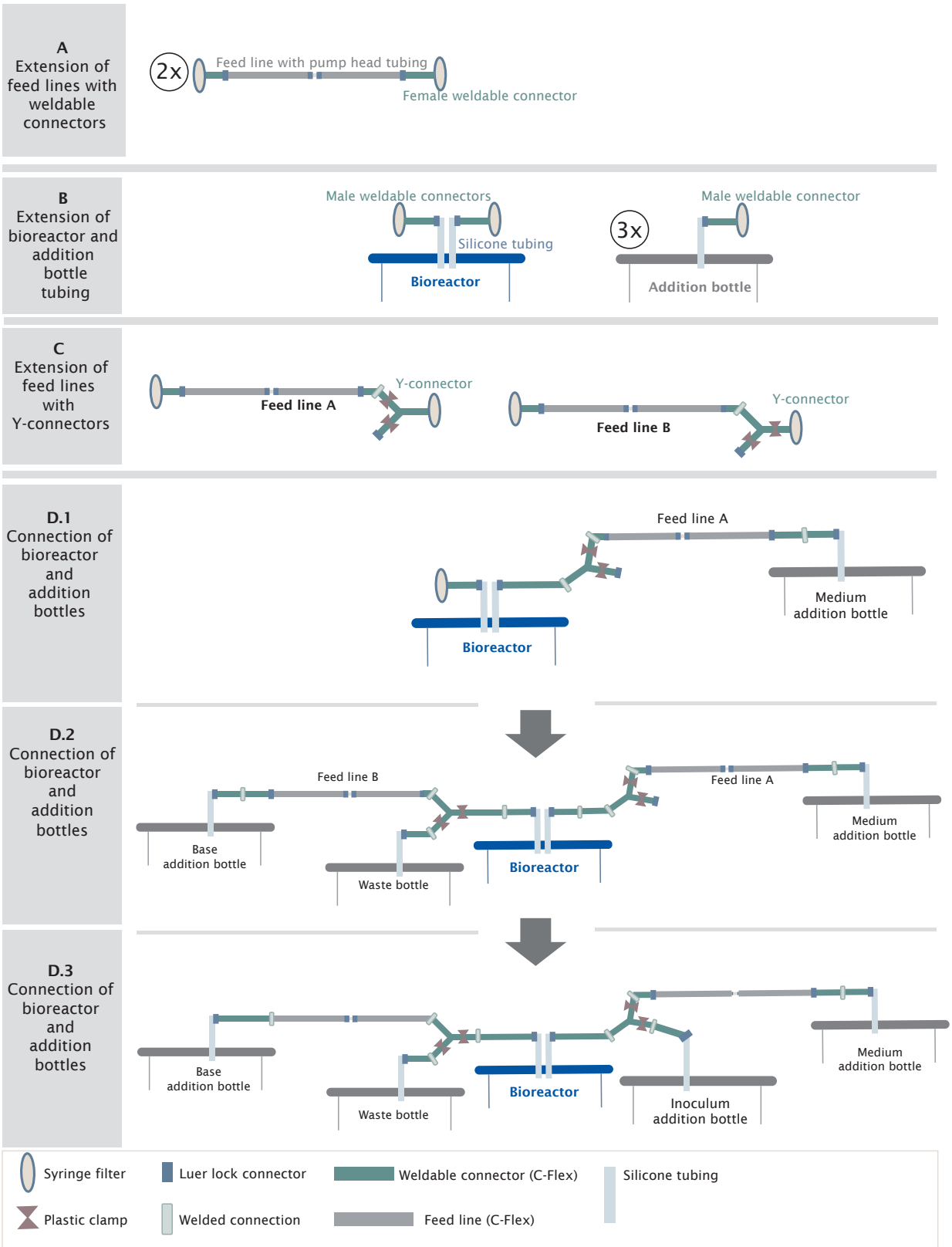


Fig. 6: Welding steps. Please refer to the text for details.

5. Inoculation

5.1 Preparation of the inoculum

> Thaw a cryopreserved CHO culture.
> Cultivate the cells in a shake flask in 15 mL cell culture medium. Incubate the culture at 37 °C, 5 % CO_2, at an agitation speed of 100 rpm (shaker radius of 2.5 cm), for example in the Eppendorf incubator shaker Innova® S44i.
> Incubate the cells for 4-7 days, until a viable cell number of approximately 1-2 x 10^6 cells/mL is reached. The viability should be > 90 %.
> Passage the cells. To do so, dilute the cell suspension to a cell density of 2 x 10^5 cells with fresh cell culture medium.
> After 3-4 days, passage the cells again. Extend the culture volume if necessary. To inoculate a bioreactor with a working volume of 250 mL, approximately 5 x 10^7 cells are required. The cell density in the inoculum should be 2-3 x 10^6 cells/mL and more than 90 % of the cells should be viable.

5.2 Inoculation

Start all control loops in DASware control 5 software. You can inoculate the culture as soon as the system has reached the setpoints.

Inoculate the culture with a maximum of 10 % of the initial working volume. We recommend an initial cell density of 2-3 x 10^5 cells/mL.

> Add the inoculum into the sterile inoculum addition bottle as described above.
> Weld the addition bottle to the free Y-end of the already existing bioreactor connection (Figure 6D.3).
> Transfer the inoculum to the bioreactor by applying pressure via the sterile filter connected to the addition bottle tubing. Use a large disposable syringe. We recommend a syringe volume of 60 mL.

6. Process Monitoring and Control

To maintain optimal growth conditions in the course of the process, control the temperature, the dissolved oxygen concentration, and the pH online. In addition, take culture samples to monitor growth offline. To set up a bioprocess control strategy, DASware control 5 software offers templates with predefined setpoints and control strategies. The templates can be changed by the user as required. Recommended parameter settings are listed in Table 6.

DO control

> Before inoculation, the DO sensor is calibrated under process conditions to 100 % (section 4).
> If you have nitrogen available, set the DO process value to 50 % before inoculation. If you do not have nitrogen available, start the cultivation at a DO process value of 100 %.
> The oxygen consumption of the culture increases with increasing cell density. To keep the DO at setpoint, the increase in oxygen consumption is compensated in the course of the experiment by increasing the percentage of oxygen in the gas mix. If required, the parameter oxygen should increase linearly with the controller output (controller output from 0-100 %) up to pure oxygen supply. The gas flow rate and agitation speed should remain constant over the complete cascade range.
> The templates for cell culture cultivation in DASware control 5 software predefine a suitable DO cascade. For a detailed description of how to newly set up or change a DO cascade, please refer to the DASware control 5 software manual.

pH control

> The cell culture medium is using a bicarbonate/CO_2 buffer system.
> Set the pH setpoint to pH 7.2. It is controlled automatically using the bioprocess control software.
> Control the pH with CO_2 (acid) and sodium bicarbonate solution (base). Preset a concentration of 5 % CO_2 in the gas mix. Limit the percentage of CO_2 to 25 %.
> Recommended control settings are listed in Table 6

Sampling

> To monitor the culture offline take samples of 2-3 mL daily through the bioreactor sampling valve.
> Make sure to take a fresh sample from the culture instead of collecting residual liquid from the sample tube. To obtain a fresh sample, either discard the first 2 mL of liquid you collected or empty the sample tube by pushing sterile
> air through it each time after collecting the sample.
> Determine the cell density and viability, either by using a cell analyzer or manually by cell counting with a counting chamber.
> If required, quantify metabolites and nutrients such as glucose, lactate, glutamine, glutamate or ammonia.

7. Ending the process

Stop the cultivation run when the culture enters the stationary growth phase. At this point the carbon source (glucose) is probably depleted.

Data storage

> Make sure you entered all offline values (e.g. cell density values) into DASware control 5 software.
> Export the data to Microsoft® Excel®, if necessary.

Harvest

> The culture can be harvested either by using the system's integrated pumps or manually for glass vessels, after removing the head plate.
> Further process the culture according to your application.

Disassembly and cleaning of bioreactor

> Disconnect all cables.
> Remove the temperature and the DO sensors.
> Sterilize glass and BioBLU Single-Use Vessels and pH sensors by autoclaving. In case of a BioBLU Single-Use Vessel, remove the sensor before autoclaving.
> Carefully clean the sensors and glass vessel for re-use.
> Clean feed lines. Please refer to the DASGIP MP8 and MP4 multi pump modules user manual for details.

Preparation of the bioreactor system at a glance

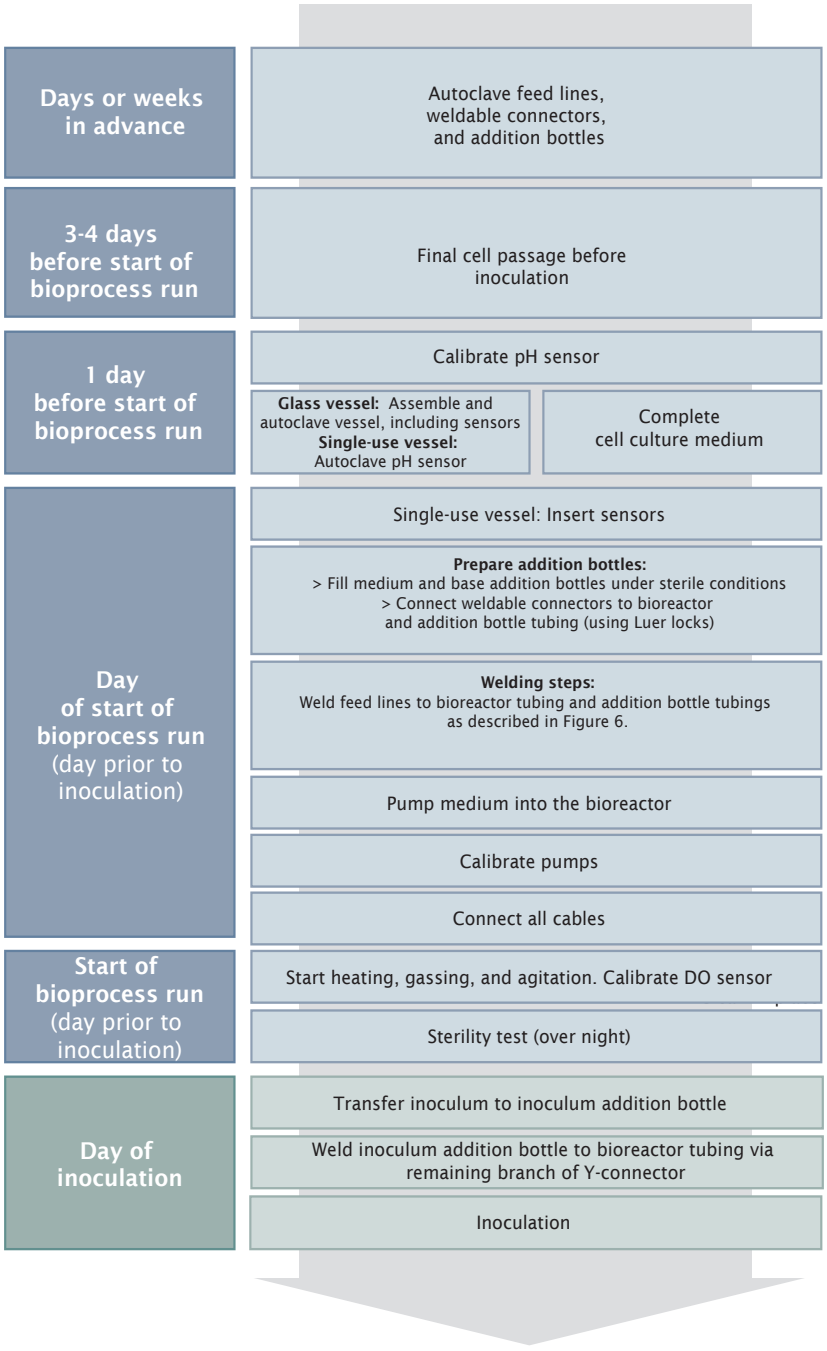


Fig. 7: Schedule for the preparation steps for a bioprocess run

8. Recommended Controller Settings

The controllers and cascades in DASware control 5 software enable the software to maintain the process parameter values.

Controllers in DASware control are PI controllers with proportional and integral parts. The direct proportional controller response depends on the difference between set point and process value. The integral controller response depends on the difference between setpoint and process value over time.

A normal controller output is bound to one active element, the actuator. Cascaded controller reactions, like they are typically applied for DO control, can improve the controller range and control quality. The output of one controller activates multiple sequential actuator outputs.

Controller settings
The controllers in DASware control 5 software can be adjusted, to optimize culture performance. The following variables can be altered.

> **Preset:** Start value of the controller
> **P-value:** Proportional factor
> **Ti-value:** Integral factor
> **Min:** Bottom limit for controller output
> **Max:** Top limit for controller output
> **Deadband:** Area around the setpoint with no control or fixed controller output (dependent on setting for AutoResetYi)
> **Safetyband:** Maximum allowed temperature difference between temperature control element and process temperature
> **AutoResetYi:**
> True: Resets Ti-value to zero within deadband
> False: Controller output is kept constant within deadband
> **X1:** Start value controller output
> **Y1:** Start value actuator
> **X2:** End value controller output
> **Y2:** End value actuator

In Table 6 we suggest controller settings for the following vessels and bioprocess control systems:

> **BioBLU 1c Single-Use Vessel**, controlled with DASGIP Parallel Bioreactor System with Bioblock
> **DASGIP Bioblock Spinner Vessel (76DS1000ODSS)**, controlled with DASGIP Parallel Bioreactor System with Bioblock
> **BioBLU 0.3c Single-Use Vessel**, controlled with DASbox-Mini Bioreactor System
> **DASbox Mini Bioreactor (76DS0250ODSS)**, controlled with DASbox Mini Bioreactor System

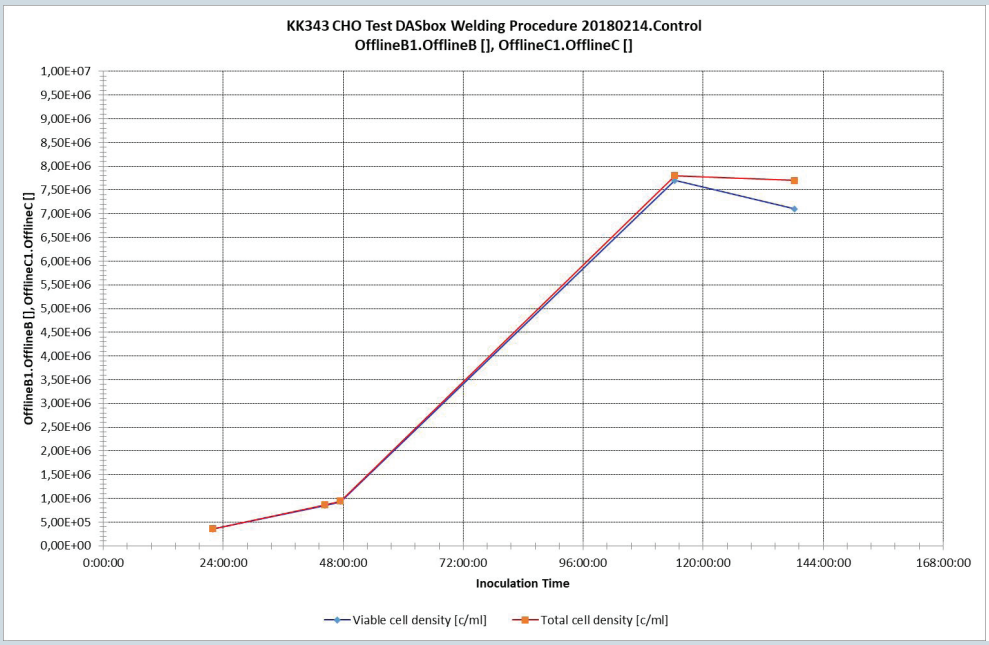
Table 6: Setpoints and control parameters.

Parameter			Bioprocess System and Vessel			
			DASbox Mini Bioreactor System		DASGIP Parallel Bioreactor System with Bioblock	
			BioBLU 0.3c	**DASbox Mini Bioreactor 76DS0250ODSS**	**BioBLU 1c**	**DASGIP Bioblock Spinner Vessel 76DS1000ODSS**
Working volume			200 mL	200 mL	1 L	1 L
Temperature setpoint		Temperature setpoint	37 °C	37 °C	37 °C	37 °C
		P	default	default	6	6
		Ti (s)	default	default	9870	9870
		Deadband	default	default	default	default
		Safetyband (K)	default	default	23	40
pH setpoint		pH setpoint	7.2	7.2	7.2	7.2
		Preset	–5	–5	–5	–5
		P	80	80	80	80
		Ti	1000	1000	1000	1000
		Deadband	0	0	0	0
		AutoResetYi	false	false	false	false
		X.out min (%)	–25	–25	–25	–25
		X.out max (mL/h)	40	40	40	40
two-sided pH control	**Base**	Pump B (base) (mL/h)	40	40	40	40
		Base	7.5 % sodium biocarbonate	7.5 % sodium biocarbonate	7.5 % sodium biocarbonate	7.5 % sodium biocarbonate
		Dosage base, type of addition	submersed	submersed	submersed	submersed
	Acid	Acid	CO_2	CO_2	CO_2	CO_2
		X.CO_2in [%] max	25	25	25	25
DO setpoint		DO setpoint	50	50	50	50
		Preset (%)	21	21	21	21
		P	0.2	0.2	0.2	0.2
		Ti	300	300	300	300
		X.out min	0	0	0	0
		X.out max	100	100	100	100
Agitation		Agitation (rpm)	250	274	167	167
		Direction	counter-clockwise	counter-clockwise	counter-clockwise	counter-clockwise
DO cascade	**Gas flow**	X1 (%)	0	0	0	0
		Y1 (sL/h)	0.6 (0.05 vvm)	0.6 (0.05 vvm)	6 (0.1 vvm)	6 (0.1 vvm)
		X2 (%)	100	100	100	100
		Y2 (sL/h)	0.6 (0.05 vvm)	0.6 (0.05 vvm)	6 (0.1 vvm)	6 (0.1 vvm)
	O_2 concentration in gas mix	X1 (%)	0	0	0	0
		Y1 (%)	0	0	0	0
		X2 (%)	100	100	100	100
		Y2 (%)	100	100	100	100

Results

To test the suitability of the controller settings, we recorded process values and controller output of pH, temperature, and DO in CHO cell culture runs. Additionally, we analyzed total cell density and viable cell density offline. As examples, we show values obtained for CHO cell culture runs in a DASbox Mini Bioreactor System and a DASGIP Parallel Bioreactor System with Bioblock.

A



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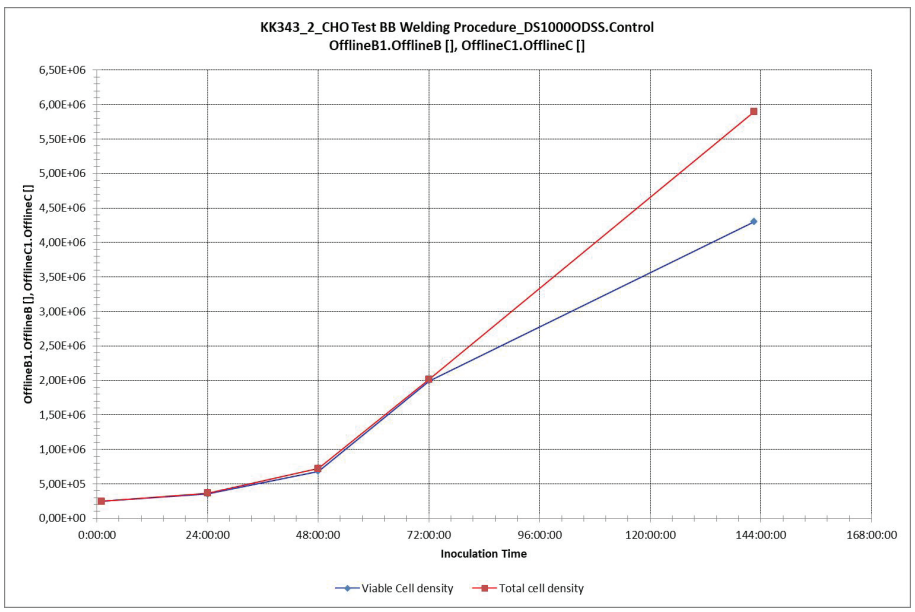
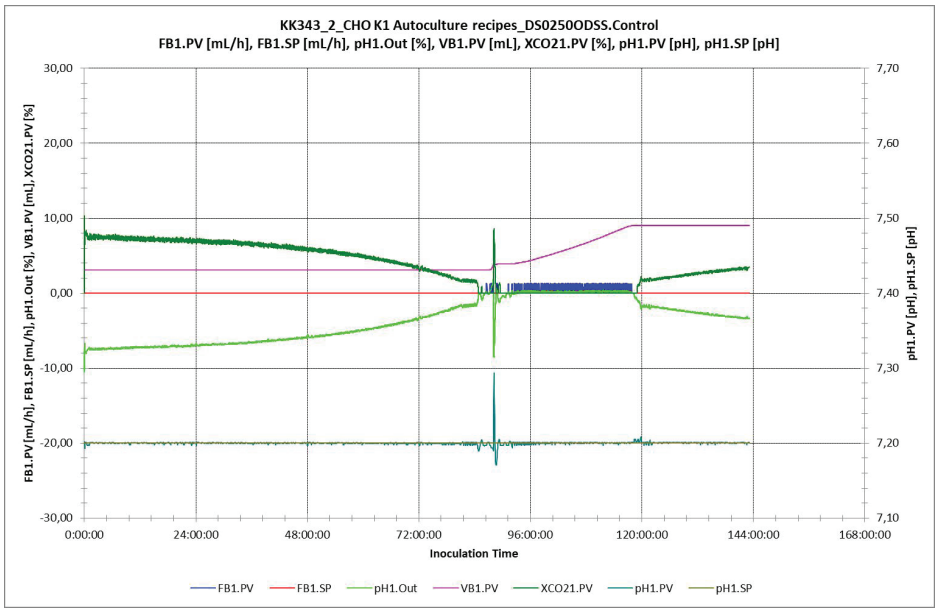


Fig. 8: Cell density
Viable cell density (OfflineB) and total cell density (Offline C) were analyzed offline.
A: CHO cell culture process in DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Vessel
B: CHO cell culture process in DASGIP Parallel Bioreactor System with Bioblock, equipped with DASGIP Bioblock Spinner Vessel (76DS1000ODSS)

A



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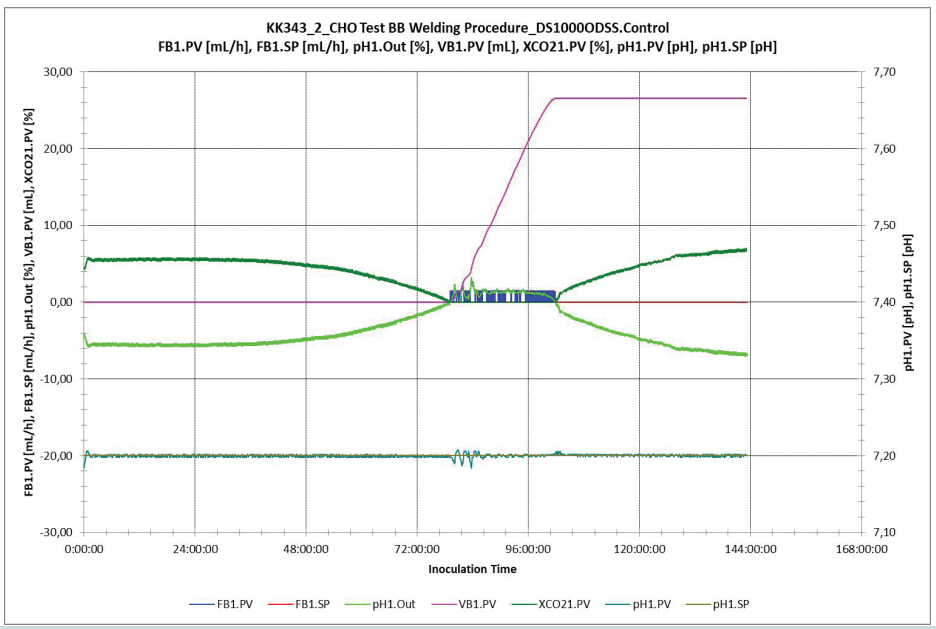
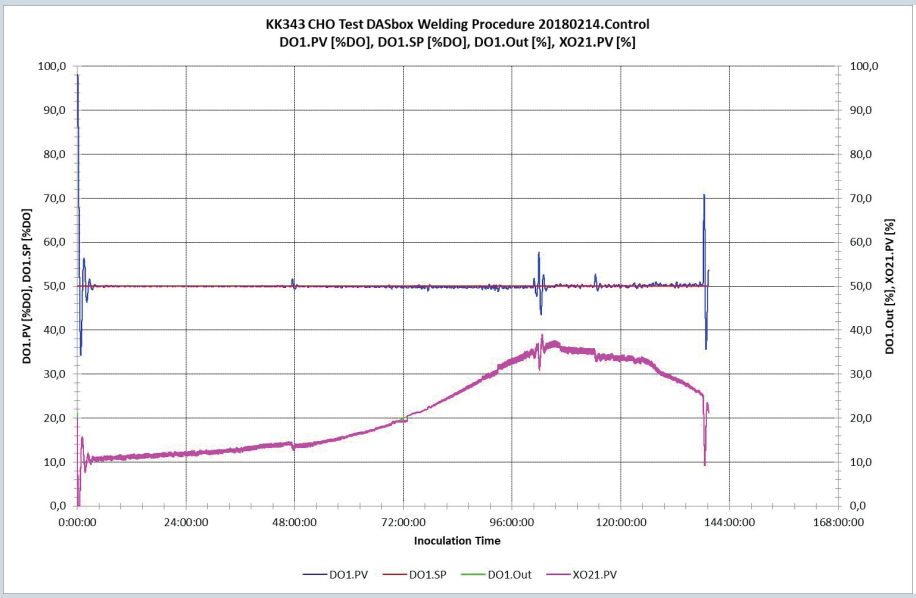


Fig. 9: pH control
The pH setpoint (pH1.SP), the process value (pH1.PV), the controller output percentage (pH1.Out), the percentage CO_2 in the gas mix (X.CO21.PV), the base volume process value (VB1.PV), flow rate setpoint (FB1.SP), and the flow rate process value (FB1.PV) are shown. The pH was controlled with 7.5 % sodium biocarbonate and 5-25 % CO_2.
The controller settings were as described in Table 6.
A: CHO cell culture process in DASbox Mini Bioreactor System equipped with DASbox Mini Bioreactor (76DS0250ODSS)
B: CHO cell culture process in DASGIP Parallel Bioreactor System with Bioblock, equipped with DASGIP Bioblock Spinner Vessel (76DS1000ODSS)

A



B

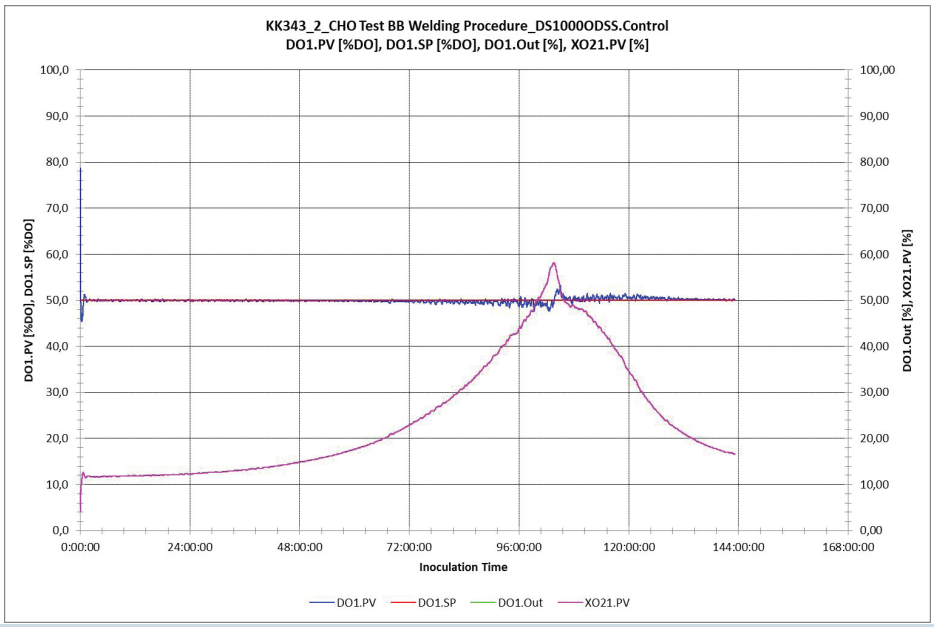
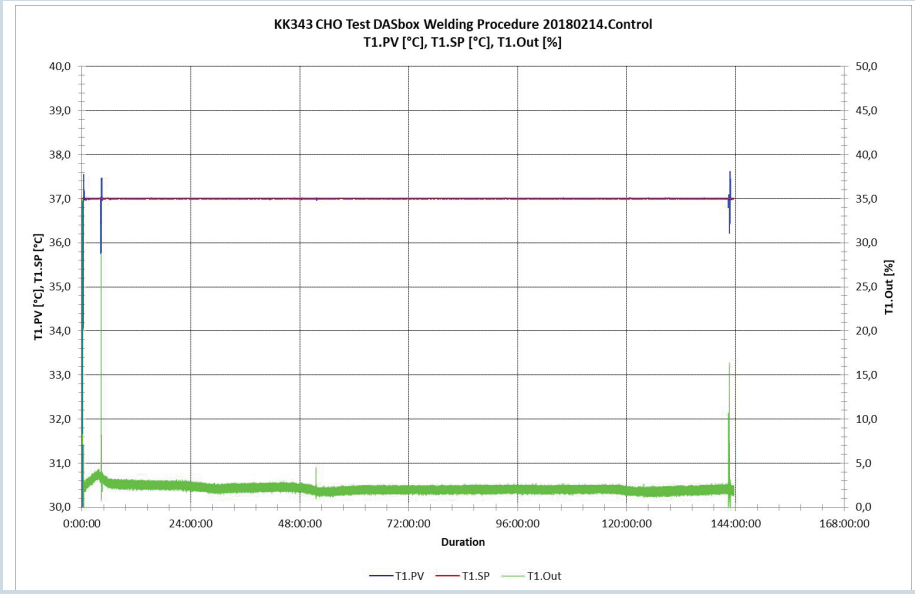


Fig. 10: DO control
To keep the process value (DO1.PV) at setpoint (DO1.SP), the oxygen concentration in the gas mix (XO21.PV) is altered in the course of the process. The gas flow rate and the agitation speed were kept constant throughout the experiment.
The controller settings were as described in Table 6.
A: CHO cell culture process in DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Vessel
B: CHO cell culture process in DASGIP Parallel Bioreactor System with Bioblock, equipped with DASGIP Bioblock Spinner Vessel (76DS1000ODSS)

A



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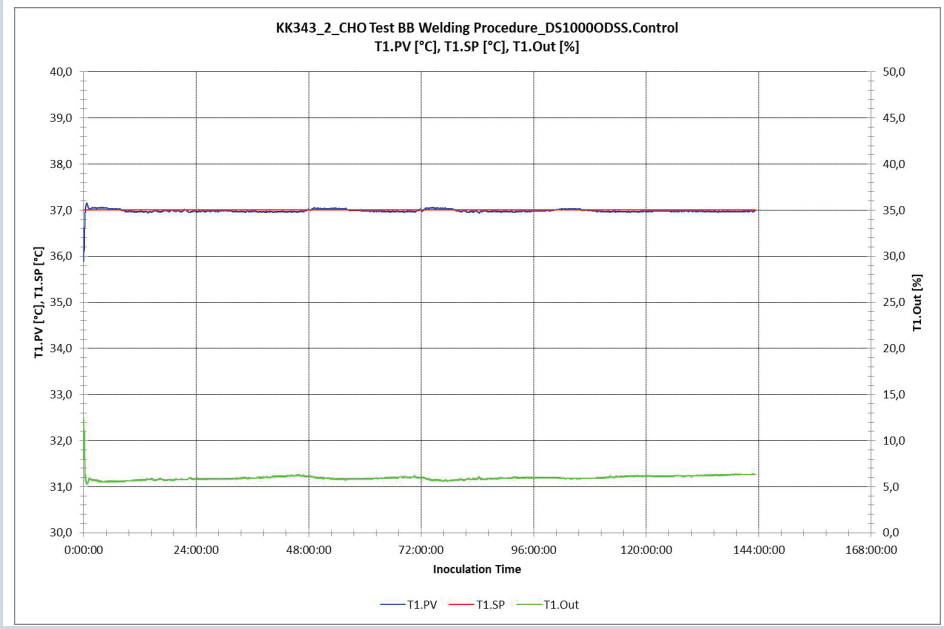

Fig. 11: Temperature control

The temperature setpoint (T1.SP), the process value (T1.PV) and the controller output percentage (T1.Out) are shown.

The controller settings were as described in Table 6.

A: CHO cell culture process in DASbox Mini Bioreactor System equipped with BioBLU 0.3c Single-Use Vessel

B: CHO cell culture process in DASGIP Parallel Bioreactor System with Bioblock, equipped with DASGIP Bioblock Spinner Vessel (76DS1000ODSS)

Ordering information

Description	Order no.
DASbox® Mini Bioreactor System for Cell Culture Applications, max. 5 sL/h gassing	
4-fold system	76DX04CC
4-fold system for single-use vessels	76DX04CCSU
DASGIP® Parallel Bioreactor System for Cell Culture Applications, max.50 sL/h gassing	
4-fold system with Bioblock	76DG04CCBB
4-fold system with Bioblock, for single-use vessels	76DG04CCSU
4-fold system, benchtop	76DG04CC
DASGIP® PH4P04L Monitoring Module, for 4 vessels, without sensors, pH and DO with level/anti foam option	76DGPH4P04L
DASGIP® MP8 Multi Peristaltic Pump Module, for 8 feeds, without feed lines and addition bottles	76DGMP8
DASGIP® TC4SC4 Temperature and Agitation Control Module, without sensors, for heat blankets and overhead drives (TC4SC4D), for 4 vessels	76DGTC4SC4D
DASGIP® TC4SC4 Temperature and Agitation Control Module, without sensors, for Bioblock and overhead drives (TC4SC4B), for 4 vessels	76DGTC4SC4B
DASGIP® MX4/4 Gas Mixing Module, mass flow controller, 0.1 – 50 sL/h, 0.1 – 40 sL/h CO_2, for 4 vessels	76DGMX44
DASGIP® MX4/4 Gas Mixing Module, mass flow controller, 0.5 – 250 sL/h, 0.5 – 150 sL/h CO_2, for 4 vessels	76DGMX44H
DASGIP® EGC4 Exhaust Condenser Controller, for 4 Peltier actuators, 110 – 240 V/50/60 Hz	76DGEGC4
DASGIP® CWD4 Cooling Water Distribution Unit, incl. connection cable, for 4 condenser-/ and 4 cooling finger ports (CWD4+4)	76DGCWD44
BioBLU® 0.3c Single-Use Vessel, cell culture, open pipe, 1 pitched-blade impeller, optical pH, sterile, 4 pieces	78903507
BioBLU® 0.3c Single-Use Vessel, cell culture, open pipe, 1 pitched-blade impeller, no pH, sterile, 4 pieces	78903508
BioBLU® 1c Single-Use Vessel, cell culture, open pipe, 1 pitched-blade impeller, no pH, sterile, 4 pieces	1386110000
BioBLU® 1c Single-Use Vessel, cell culture, open pipe, 2 pitched-blade impeller, no pH, sterile, 4 pieces	1386110100
BioBLU® 1c Single-Use Vessel, cell culture, open pipe, 1 pitched-blade impeller, optical pH, sterile, 4 pieces	1386110400
BioBLU® 1c Single-Use Vessel, cell culture, open pipe, 2 pitched-blade impeller, optical pH, sterile, 4 pieces	1386110500
BioBLU® 3c Single-Use Vessel, cell culture, microsparger, 1 pitched-blade impellers, optical pH, sterile, 1 piece	1386000100
BioBLU® 3c Single-Use Vessel, cell culture, macrosparger, 1 pitched-blade impeller, optical pH, sterile, 1 piece	1386000300
DASbox® Vessel Type DS0250ODSS, marine-type impeller, 60 – 250 mL, overhead drive	76DS0250ODSS
DASGIP® Vessel DS0700ODSS, pitched blade impeller, 250 mL – 700 mL, 2x GL45 side arms, overhead drive, Bioblock	76DS0700ODSS
DASGIP® Vessel DS1000ODSS, 2x pitched blade impeller, 350 mL – 1.0 L, 2x GL45 side arms, overhead drive, Bioblock	76DS1000ODSS
DASGIP® Vessel DS1500ODSS, 2x pitched blade impeller, 350 mL – 1.5 L, 2x GL45 side arms, overhead drive, Bioblock	76DS1500ODSS
DASGIP® Vessel DR03C, pitched blade impeller, dip tube, 750 mL – 2.7 L, overhead drive	76DR03C
DASGIP® Vessel DR04C, pitched blade impeller, dip tube, 800 mL – 3.8 L, overhead drive	76DR04C
Flask, with neck GL 45, transparent, without cap, 1000 mL	78100016
Flask, with neck GL 45, transparent, with cap, 500 mL	78201078
Flask, with neck GL 45, transparent, with cap, 100 mL	78201035
DASGIP® Head Gear, for addition bottles with GL45 neck, C-Flex®, female Luer lock	78510311
Feed Line C-Flex®, with 2x Luer lock fittings, male/male, I.D. 0.8 mm, L 1 m	78510309
Feed Line C-Flex®, with 2x Luer lock fittings, male/male, I.D. 3.2 mm, L 1 m	78510320
Feed Line C-Flex®, with 2x Luer lock fittings, male/male, I.D. 0.8 mm, L 2 m	78510310
Feed Line C-Flex®, with 2x Luer lock fittings, male/male, I.D. 3.2 mm, L 2 m	78510321
Pump Head Tubing, for DASGIP® MP8 pump, Bioprene®, I.D. 0.5/W 1.05 mm, female/female	78510118
Pump Head Tubing, for DASGIP® MP8 pump, Peripren, I.D. 2.0/W 0.8 mm, male/female	78510237

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