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E. coli Cultivation in DASbox® Mini Bioreactor System and DASGIP® Parallel Bioreactor Systems

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Abstract

This protocol explains how to prepare and conduct *E. coli* fermentation processes in the DASbox Mini Bioreactor System and the DASGIP Parallel Bioreactor Systems. We would like to give an overview on the steps that have to be taken during the preparation and conduction of a bioprocess run. We also explain how these differ with the use of glass and BioBLU® Single-Use Vessels. We guide the user through all steps of a fermentation process, starting with the preparation of culture medium, to the preparation and operation of the vessels and bioprocess systems, and the analysis of samples.

In this example, we describe the fermentation of *E. coli* K12 in a working volume of 1 L. We explain which process parameters and control strategies can be optimized, depending on factors like the culture volume, the bacterial strain, and the desired end product. The protocol can serve as a starting point for further optimization.

Introduction

E. coli fermentation in controlled, stirred-tank bioreactors can deliver very high cell densities and product yields. The process performance depends on the bacterial strain and the medium composition, as well as the bioprocess control strategies used to keep critical process parameters at setpoint.

The DASGIP Parallel Bioreactor Systems and the DASbox Mini Bioreactor System for microbial applications allow the parallel operation of up to 16 and 24 bioreactors, respectively, and support both the use of conventional glass and BioBLU Single-Use Vessels. Covering a working volume range of 60 mL to 3.7 L altogether, they are valuable tools for advanced process 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 *E. coli* fermentation experiment using glass or BioBLU Single-Use Vessels. We share a protocol that we have thoroughly tested 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.

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Material and Methods

1. Medium preparation

1.1 Complex medium for preculture

To prepare the inoculum, cultivate *E. coli* in Luria-Bertani (LB) medium. This complex medium contains peptone, yeast extract, and sodium chloride. It is the most widely used medium for *E. coli* culture.

LB medium	
Tryptone	10 g/L
Yeast extract	5 g/L
Sodium chloride	10 g/L

Dissolve ingredients in dH_2O and sterilize by autoclaving.

1.2 Chemically defined medium for main culture

In this protocol, we use a chemically defined medium for the main culture. Chemically defined media are favored in industrial bioprocessing, because they contain a defined carbon source. The researcher is thus able to control which carbon source the bacteria metabolize. Furthermore, batch-to-batch variations of complex media components are avoided.

Many recipes for chemically defined media exist. Below we describe the preparation of the medium that we routinely use in our applications lab.

Stock solutions

First, prepare the following stock solutions:

10x PAN medium stock solution	
$CaCl_2 \cdot 2\ H_2O$	0.15 g/L
KH_2PO_4	30 g/L
K_2HPO_4	120 g/L
$(NH_4)_2SO_4$	50 g/L
$FeSO_4 \cdot 7\ H_2O$	0.75 g/L
Tri-sodium-citrate $\cdot 2\ H_2O$	10 g/L

Dissolve in dH_2O. Sterilize by autoclaving.

Magnesium-sulfate stock solution	
$MgSO_4 \cdot 7\ H_2O$	100 g/L

Dissolve in dH_2O. Sterilize by filtration.

10 % (w/v) Anti-foam solution	
Struktol® J-673	50 g
dH_2O	450 mL

Sterilize by autoclaving. Transfer solution to sterile addition bottle.

50 % (w/v) glucose solution	
Glucose $\cdot 1\ H_20$	550 g/L

Dissolve in dH_2O. Sterilize by autoclaving.

Thiamine stock solution	
Thiamin-HCl	5 g/L

Dissolve ingredients in dH_2O. Sterilize by filtration.

PAN trace elements solution	
$Al_2(SO_4)_3 \cdot 18\ H_2O$	2 g/L
$CoSO_4 \cdot 7\ H_2O$	0.8 g/L
$CuSO_4 \cdot 5\ H_2O$	2.5 g/L
H_3BO_4	0.5 g/L
$MnSO_4 \cdot x\ 1\ H_20$	24 g/L
$Na_2MoO_4 \cdot 2\ H_2O$	3 g/L
$NiSO_4 \cdot 6\ H_2O$	31.5 g/L
$ZnSO_4 \cdot 7\ H_2O$	15 g/L
H_2SO_4, 25 %	2.4 mL/L

Dissolve ingredients in dH_2O. Sterilize by filtration.

Culture medium

Prepare 1 L of culture medium from the stock solutions. Below we describe two slightly different procedures, depending on whether a glass or a BioBLU Single-Use Vessel is used.

The volumes of the medium components add up to 950 mL. The final working volume of 1 L is reached with the addition of 50 mL inoculum (5 % of the working volume, see page 7).

1x PAN medium with additions, 1L	
Medium preparation for use of BioBLU Single-Use Vessels	
10x PAN-medium stock solution	100 mL
10 % Struktol J-673	20 mL
dH_2O	745 mL
50 % glucose solution	80 mL
Add the sterile components to the vessel through a Pg 13.5 port. Then, add the following components through a feed tube using a syringe filter.	
Magnesium-sulfate stock solution	3 mL
Thiamine stock solution	1 mL
PAN trace elements solution	1 mL

1x PAN-medium with additions, 1L	
Medium preparation for use of autoclavable glass vessels	
10x PAN-medium stock solution	100 mL
10 % Struktol J-673	20 mL
dH2O	745 mL
Add components to the vessel and sterilize by autoclaving. After cooling, add the following heat-labile components through a feed tube using a syringe filter.	
Magnesium-sulfate stock solution	3 mL
50 % glucose solution	80 ml
Thiamine stock solution	1 mL
PAN trace elements solution	1 mL

1.3 Solutions for pH control

Also, prepare solutions for pH control. They are needed during the process.

We recommend the use of 20 % ammonia and 20 % phosphoric acid to adjust the pH in a working volume of 1 L.

To control the pH in lower working volumes naturally less pH control agent is needed. In that situation, it can be advantageous to use less concentrated pH agents to ensure that they still can be dosed accurately.

Base for pH control, 1 L	
Ammonia (25 %)	800 g (879 mL, ρ = 0.91 g/mL)
Sterile dH_2O	200 g (121 mL, ρ = 1.0 g/mL)
Final concentration	20 % ammonia (w/w)
Transfer solution to sterile addition bottle.	

Acid for pH control, 1 L	
Phosphoric acid (85 %)	235 g (137 mL, ρ = 1.7 g/mL)
Sterile dH_2O	765 g (863 mL, ρ = 1.0 g/mL)
Final concentration	20 % phosphoric acid (w/w)
Transfer solution to sterile addition bottle.	

2. DASbox Mini Bioreactor System and DASGIP Parallel Bioreactor Systems

The protocol describes *E. coli* fermentation 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 0.3f	**DASbox Mini Bioreactor System**	60 – 250 mL
BioBLU 1f	**DASGIP Parallel Bioreactor Systems**	250 mL – 1.25 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 Stirrer Vessels	**DASGIP Parallel Bioreactor System with Bioblock**	200 mL – 1 L 400 mL – 1.5 L 400 mL – 1.8 L
DASGIP Benchtop Bioreactors	**DASGIP Parallel Bioreactor System**	750 mL – 2.7 L 800 mL – 3.7 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, which are needed to control sensors, pumps, agitation, temperature, exhaust cooling, and gassing.

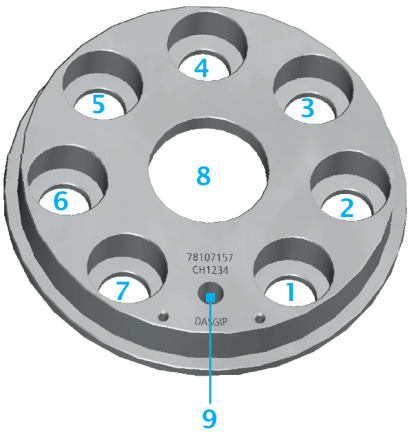
Table 3: Bioprocess system components

Function	Component		
	DASbox	**DASGIP (benchtop)**	**DASGIP (Bioblock)**
pH, DO, and level control	Included in DASbox with MP8-PH4PO4LS option	DASGIP PH4PO4L	DASGIP PH4PO4L
Pump control	Included in DASbox	DASGIP MP8	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 lists the corresponding vessel components. Figures 1 and 2 show typical head plate assignments for glass vessels and BioBLU Single-Use Vessels for 1 L cultures.

Table 4: Vessel components

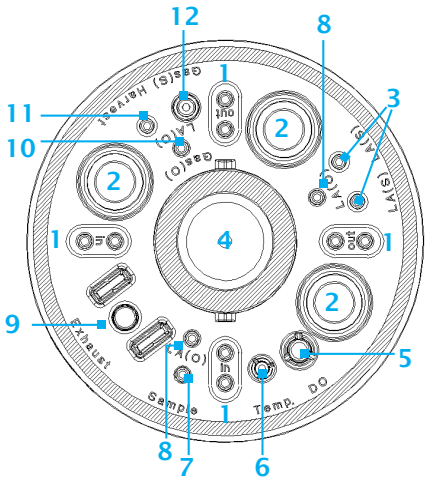
Function	Component
Agitation	Motor: Overhead drive RE40 or DASbox overhead drive; Impeller: Rushton type
Anti foam control	Level sensor
Cooling	DASbox, Bioblock or cooling finger
pH monitoring	pH sensor (polarographic)
DO monitoring	DO sensor (polarographic, Clark sensor)
Temperature monitoring	Platinum RTD Temperature Sensor (Pt100)
Gassing	L-sparger (glass vessels)/open pipe (single-use vessels)
Temperature control	DASbox, Bioblock or heat blanket
Sampling	Sampling tube with valve
Liquid addition	PTFE feed lines, inner diameter 0.8 mm; Bioprene pump head tubings, inner diameter 0.5 mm; Feed line A: Acid, if indicated Feed line B: Base Feed line C: Anti-foam
Options for liquid addition	Short dip tube for anti-foam addition Long dip tubes for pH agent (base) If indicated, long dip tube for pH agent (acid)



	Port	Port accessory	Associated device	Purpose	Connected to
1	Pg 13.5	Compression fitting I.D. 6 mm	L-Sparger with 50 mm silicone tubing and 0.2 µm inlet filter	Gassing	DASGIP MX4/4(H)
2	Pg 13.5	–	DO sensor	Monitoring	DASGIP PH4PO4L
3	Pg 13.5	Triple port position 1 Triple port position 2 Triple port position 3	Short dip tube Long dip tube Short dip tube	Free Base addition Antifoam addition	DASGIP MP8 DASGIP MP8 DASGIP MP8
4	Pg 13.5	Compression fitting I.D. 4 mm	Sample port	Inoculation/ offline sampling	–
5	Pg 13.5	–	pH sensor	Monitoring	DASGIP PH4PO4L
6	Pg 13.5	Compression fitting I.D. 4 mm	Level sensor	Foam monitoring	DASGIP PH4PO4L
7	Pg 13.5	Compression fitting I.D. 12 mm	Condenser with 50 mm silicon tubing and 0.2 µm filter capsule	Exhaust treatment	
8	M30	–	Lipseal stirrer assembly	Agitation	Motor RE40 and DASGIP TC4SC4B
9	M6	Thermowell	Platinum RTD temperature sensor (Pt100)	Temperature monitoring	DASGIP TC4SC4B

I.D.: inner diameter

Fig. 1: Typical head plate assignment for the DASGIP Bioblock Stirrer Vessel with a working volume of 400 mL – 1.5 L (76SR1000ODLS). 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/Label	Port accessory	Associated device	Purpose	Connected to
1	in out	Silicone tubing	Cooling baffles	Bioreactor cooling	DASGIP CWD4+4
2	Pg 13.5	–	pH sensor	Monitoring	DASGIP PH4PO4L
2	Pg 13.5	Triple port position 1 Triple port position 2 Triple port position 3	Level sensor Level sensor Short dip tube	Foam monitoring Foam monitoring –	DASGIP PH4PO4L DASGIP PH4PO4L –
2	Pg 13.5	–		Free to use	
3	LA (s)	70 mm silicone tubing with female Luer lock	Long dip tube Long dip tube	Base addition Second port free to use	DASGIP MP8 DASGIP MP8
4	Mag-netic coupling	–	Magnetic drive motor	Agitation	DASGIP TC4SC4B
5	DO	Long dip tube with silicone membrane	DASGIP DO sensor O.D. 4.7 mm	Monitoring	DASGIP PH4PO4L
6	Temp	Thermowell	Platinum RTD temperature sensor (Pt100)	Monitoring	DASGIP TC4SC4B
7	Sample	70 mm silicone tubing with sample valve	Long dip tube	Inoculation/ Offline sampling	–
8	LA (o)	70 mm silicone tubing with female Luer lock	Short dip tube Short dip tube	Antifoam addition Free to use	DASGIP MP8 DASGIP MP8
9	Exhaust	Silicone tubing with 0.2 µm inlet filter	Condenser	Exhaust treatment	DASGIP EGC4
10	LA (o) Gas (o)	70 mm silicone tubing with female Luer lock	Short dip tube	Additional port for liquids	DASGIP MP8
11	Harvest	70 mm silicone tubing with female Luer lock	Long dip tube	Harvest	DASGIP MP8
12	Gas (s)	Silicone tubing with 0.2 µm inlet filter	Long dip tube	Gassing	DASGIP MX4/4(H)

O.D.: outer diameter

Fig. 2: Head plate assignment for a BioBLU 1f Single-Use Vessel.

3. Preparation of the Bioprocess System

Before the start of the cultivation, the bioprocess system and the vessels have to be prepared. This 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 its connection to the DASbox Mini Bioreactor System and the DASGIP modules, respectively.

Below we describe the preparation steps needed for fermentation runs in glass and BioBLU Single-Use Vessels. Figure 3 gives an overview of the preparation steps.

In the following protocol we refer to the user manuals which 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.

3.1 pH sensor calibration and sterilization

All DASbox Mini Bioreactors, DASGIP bioreactors, and the BioBLU 0.3f and 1f Single-Use Vessels can be operated with standard glass pH sensors.

> Perform a two-point calibration using buffers at pH 4 and 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 head plate and autoclave the vessel (see 3.4). 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.

3.2 Sterilization of the level sensor

> Glass vessel: Insert the level sensor in the vessel head plate and autoclave the vessel (see 3.4).

BioBLU Single-Use Vessel: Two level sensors are required for the BioBLU Single-Use Vessel, due to the design of the vessel head plate. Insert two level sensors in a triple port compression fitting. Equip the remaining port with a short dip tube and close the tube. Autoclave the assembly.

3.3 Pump calibration and clean-in-place of feed lines

> Calibrate the pumps. Please refer to the DASGIP MP8 and MP4 Multi Pump Module operating manual for details on the pump calibration procedure. For an accurate calibration, use the solution which will be added to the bioreactor with this respective pump.

> After calibration, sterilize the PTFE feed lines using a clean-in-place procedure. Please refer to the DASGIP MP8 and MP4 Multi Pump Module operating manual and the DASware control 5 software manual for details.

3.4 Preparation of the bioreactors

For detailed information on the head plate and vessel assembly and connection described below, please refer to the user manuals of the DASbox Mini Biroeactor System, the DASGIP modules, the DASGIP bioreactors, and the BioBLU 0.3c/f Single-Use Bioreactor and BioBLU 1c/f Single-Use Bioreactor instructions for use.

Glass vessels

> Install all necessary equipment in the vessel head plate, including pH, DO, and level sensors, and the stirrer assembly.

> Fill the heat-stable medium components into the vessel.

> Mount the head plate onto the bioreactor.

> Autoclave the bioreactor (20 min, 121 °C).

> After cooling, sterilely add the heat-labile components of the cultivation medium to the bioreactor through a feed tube, using a syringe filter (0.2 µm).

> Connect the vessel components with the DASbox Mini Bioreactor System and the DASGIP modules, respectively, including the sensors, drive, gassing tube, and exhaust cooling.

> Insert the temperature sensor. This sensor does not need to be autoclaved, because it is not in direct contact with the culture medium.

BioBLU Single-Use Vessels

Fill sterile cultivation medium and medium additions into the bioreactor under sterile conditions (see 1.2).

> Equip the bioreactor with the heat-sterilized pH and level sensors (see 3.1 and 3.2). Work under sterile conditions.

> Connect the vessel components with the DASbox Mini Bioreactor System and the DASGIP modules, respectively,

including the sensors, drive, gassing tube, and exhaust cooling.
> Insert the temperature sensor and the DO sensor. These sensors do not need to be sterile, because they are not in direct contact with the culture medium.

Further steps (valid for glass and BioBLU Single-Use Vessels)
> Sterilely transfer the pH agents and anti-foam solution to addition bottles. Connect the feed lines for pH agent and anti-foam with the addition bottles using Luer-lock connectors. Connect the feed lines via the DASGIP MP8 module with tubes for liquid addition on the bioreactor head plate.
> Fill the feed lines in one step.
> Calibrate the DO sensor. Please refer to the DASware control 5 software manual and DASGIP PHPO sensor module operating manual for details.
> The bioreactor is now ready for inoculation.

4. Inoculation

To generate a sufficient amount of biomass for the inoculation of the culture, grow a preculture in shake flasks.

4.1 Preparation of inoculum
Preculture 1:
> Fill 25 mL LB medium into a glass or single-use shake flask without baffles, with a total volume 500 mL.
> Inoculate with *E. coli* K12 from one cryovial (cryopreservation of *E. coli* is described on page 10)
> Incubate the preculture 1 overnight, at 37 °C and 200 rpm (e.g. using an Innova® S44i Shaker)

Preculture 2:
> Fill 100 mL LB medium into a shake flask without baffles, with a total volume of 1 L.
> Inoculate with 5 mL of preculture 1
> Incubate preculture 2 for approximately 7 hours, at 37 °C and 200 rpm (orbit radius 2.54 cm). The optical density at 600 nm (OD_{600}) of preculture 2 should be between 6 and 8.

This volume of preculture is sufficient to inoculate 2 L of main culture. If you plan to inoculate a larger culture volume, use several shake flasks to prepare the needed volume of preculture 2.

4.2 Inoculation of main culture:
> Work inside a laminar airflow cabinet.
> Transfer preculture 2 to a sterile beaker to make it easier to draw up the culture into a syringe (see next step). In case you prepared more than one shake flask, pool the precultures.
> Inoculate main culture to an OD_{600} of 0.3 – 0.4. With a preculture 2 with an OD_{600} of 6 – 8 this corresponds to 5 % of the initial working volume of the main culture. Draw up the required volume in a sterile syringe and inoculate the main culture via the sampling port of the bioreactor.

Preparation of the bioreactor system at a glance

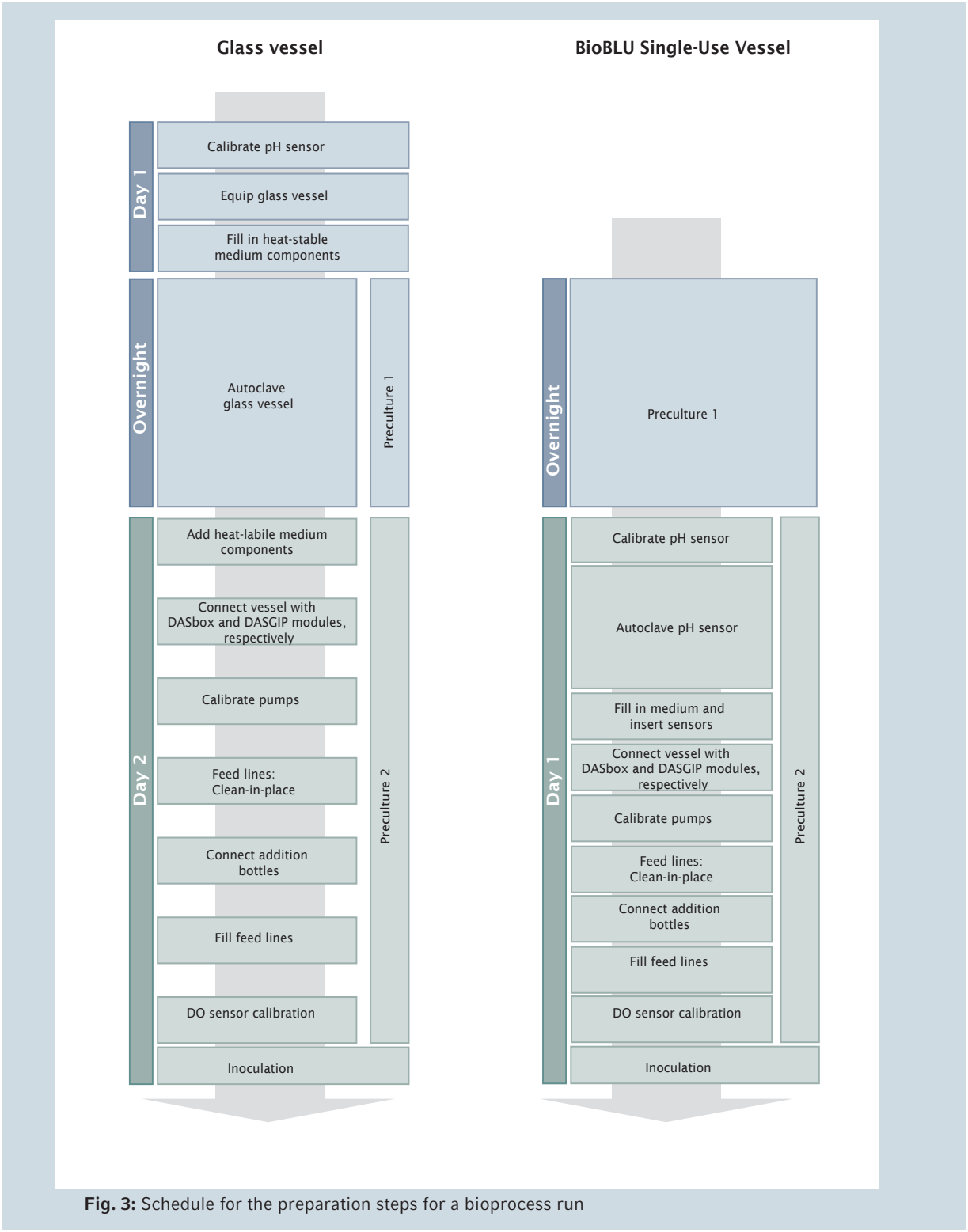


Fig. 3: Schedule for the preparation steps for a bioprocess run

5. Process Monitoring and Control

To maintain optimal bacterial growth conditions in the course of the process, the temperature, the dissolved oxygen concentration (DO), and the pH are controlled online. In addition, take culture samples to monitor bacterial growth offline.

To set up a bioprocess control strategy easily, DASware control 5 software offers templates with predefined setpoints and control strategies. The templates can be changed by the user as required. For aerobic fermentation the user can choose between three different templates. They differ in the pH control strategy and use either only base, only acid or acid and base. The templates contain the following parameters.

5.1 DO regulation

> Control the DO at 30 %. This concentration ensures that the availability of oxygen does not limit *E. coli* growth.

> To control the DO at the setpoint, set up a DO cascade in DASware control 5 software. We suggest the following settings: First, with increasing oxygen demand, the agitation speed is increased to the maximum. If this is not sufficient to maintain DO at setpoint, the O_2 concentration in the gas mix is increased up to 100 %. The gas flow is kept constant at 1 vessel volume per minute (vvm). Usually it is not required to increase the gas flow to meet the oxygen demand of the culture.

> The templates for aerobic fermentation in DASware control 5 software predefine a 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.

5.2 pH regulation

> Set the pH setpoint to 7.0. Please see Tables 5 and 6 for controller settings.

> Usually only base is required to maintain the pH of *E. coli* fermentation broth at setpoint (one-sided control). Choose the respective template in DASware control 5 software.

> Prepare an ammonia solution in dH_2O in a sterile addition bottle. For a working volume of 1 L we recommend a base concentration of 20 %. However, the most suitable concentration of the pH agent might change depending on the culture size (see 1.3).

> Optionally, apply a two-sided pH control with base and acid: In this case, also prepare a phosphoric acid solution in dH_20 in a sterile addition bottle (see 1.3) and choose the respective template for a two-sided pH control.

5.3 Sampling

> To monitor the process offline, for example to analyze bacterial growth or the metabolic profile, take a sample of 2 to 3 mL through the sampling valve.

> Make sure to take a fresh sample from the culture instead of collecting residual liquid from the feed 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 before collecting the sample.

> Enter the obtained offline values into the bioprocess control software. Please refer to the DASware control 5 software manual for details.

6. Ending the Process

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

6.1 Data storage

> Make sure you entered all offline values (e.g. OD_{600} values) into DASware control 5 software.
> Export the data to Microsoft Excel®, if necessary.

6.2 Culture harvest

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

6.3 Disassembly and cleaning of the 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 sensors before autoclaving.
> Carefully clean 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.

7. Preparation of cryoconserved *E. coli* stocks

This protocol describes the processing of a freeze-dried *E. coli* culture. It gives general recommendations, which should be adapted according to the supplier's instructions.

Material

> Sterile LB medium
> Sterile shake flasks without baffles (total volume 500 mL and 1 L)
> Sterile forceps
> Sterile cryovials
> Sterile 70 % glycerol (sterilize by autoclaving)
> Sterile pipet tips (e.g. epT.I.P.S.®)
> Sterile serological pipets (e.g. Eppendorf Serological Pipets)
> Shaker (e.g. Eppendorf Innova® S44i)
> Spectrometer (e.g. Eppendorf BioSpectrometer® basic)
> Freezer (e.g. Eppendorf CryoCube® F740)

Methods

> Open the vessel containing the freeze-dried culture.
> Resuspend the bacteria pellet in 0.5 mL of LB medium and incubate for 30 min.
> In the meantime, fill a shake flask (100 mL total volume) with 10 mL LB medium.
> Inoculate the LB-medium with the bacteria suspension
> Cultivate 37 °C over night, at 200 rpm.
> Transfer the culture to shake flask (1 L total volume) filled with 100 mL LB medium. Cultivate for 5 to 7 hours at 37 °C and 200 rpm.
> Determine the OD_{600} of the culture.
> Prepare *E. coli* glycerol stocks (1 mL per cryovial): For one vial mix 125 µL 70 % glycerol with 875 µL bacteria culture
> Label the vial with information on the bacterial strain, the OD_{600} of the culture, and the date.
> Freeze vials and store them at –85°C.

8. Recommended Controller Settings

The controllers and cascades in DASware control 5 software enable the software to maintain the process parameter values that have been entered as calculation values during the experiment setup or while the experiment is running.

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 controlle output (dependend on setting for AutoResetYi)
> **Safetyband:** Maximum allowed temperature difference between temperature control element and process temperature
> **AutoResetYi:** Resets Ti-value to zero within deadband
> **X1:** Start value controller output
> **Y1:** Start value actuator
> **X2:** End value controller output
> **Y2:** End value actuator

In Table 5 and 6 we suggest controller settings for the following vessels and bioprocess control systems:

> **BioBLU 1f Single-Use Vessel**, controlled with DASGIP Parallel Bioreactor System with Bioblock
> **DASGIP Bioblock Stirrer Vessel (76SR1000ODLS)**, controlled with DASGIP Parallel Bioreactor System with Bioblock
> **BioBLU 0.3f Single-Use Vessel**, controlled with DASbox Mini Bioreactor System
> **DASbox Mini Bioreactor**, controlled with DASbox Mini Bioreactor System

In Table 5 we suggest controller settings for a process with a one-sided pH control and in Table 6 for a process using a two-sided pH-control.

Please note, that the suggested parameters can serve as starting points, but may require further optimization by the end user.

Table 5: Setpoints and regulator parameters. A one-sided pH control is applied.

Parameter			Bioprocess System and Vessel			
			DASGIP Parallel Bioreactor System with Bioblock		DASbox Mini Bioreactor System	
			BioBLU 1f	**Bioblock Stirrer Vessel 76SR1000ODSL**	**BioBLU 0.3f**	**DASbox Mini Bioreactor**
Working volume			1 L	1 L	200 mL	200 mL
Temperature setpoint		Temperature setpoint	37 °C	37 °C	37 °C	37 °C
		Preset (%)	0	0	0	0
		P	30	30	10	10
		Ti (s)	6020	6020	240	240
		Deadband	0.02	0.02	default DASbox	default DASbox
		Safetyband (K)	23	40	default DASbox	default DASbox
pH setpoint (one-sided pH control)		pH setpoint	7.0	7.0	7.0	7.0
		Preset (mL/h)	0	0	0	0
		P	50	50	50	50
		Ti	300	300	300	300
		Deadband	0	0	0	0
		AutoResetYi	true	true	true	true
		Min (mL/h)	0	0	0	0
		Max (mL/h)	40	40	10	10
pH regulation (one-sided pH control)		Pump B (base) (mL/h)	40	40	10	10
		Base	20 % ammonia	20 % ammonia	10 % ammonia	10 % ammonia
		Dosage base	submersed	headspace	submersed	submersed
DO setpoint		DO setpoint	30	30	30	30
		Preset (%)	0	0	0	0
		P	0.2	0.2	0.2	0.2
		Ti	75	75	75	75
		Min	0	0	0	0
		Max	100	100	100	100
DO cascade	**Agitation**	X1 (%)	0	0	0	0
		Y1 (rpm)	600	600	800	800
		X2 (%)	60	60	60	60
		Y2 (rpm)	1,500	1,600	2,000	2,500
		Direction	clockwise	clockwise	clockwise	clockwise
	Gas flow	X1 (%)	0	0	60	60
		Y1 (vvm)	1	1	0.5	0.5
		X2 (%)	100	100	80	80
		Y2 (vvm)	1	1	1	1
	O_2 concentration in gas mix	X1 (%)	60	60	80	80
		Y1 (%)	21	21	21	21
		X2 (%)	100	100	100	100
		Y2 (%)	100	100	100	100
Anti foam regulation		Inactive until: Input level signal (µs)	35	35	35	35
		Output pump C (mL/h)	0	0	0	0
		Active from: Input level signal (µs)	35.01	35.01	35.01	35.01
		Output pump C (mL/h)	40	40	40	40
		Dosage anti foam	headspace	headspace	headspace	headspace

Table 6: Setpoints and regulator parameters. A two-sided pH control is applied.

Parameter			Bioprocess System and Vessel			
			DASGIP Parallel Bioreactor System with Bioblock		DASbox Mini Bioreactor System	
			BioBLU 1f	**Bioblock Stirrer Vessel SR1000ODSL**	**BioBLU 0.3f**	**DASbox Mini Bioreactor**
Working volume			1 L	1 L	200 mL	200 mL
Temperature setpoint		Temperature setpoint	37 °C	37 °C	37 °C	37 °C
		Preset (%)	0	0	0	0
		P	30	30	10	10
		Ti (s)	6020	6020	240	240
		Deadband	0.02	0.02	default DASbox	default DASbox
		Safetyband	23	40	default DASbox	default DASbox
pH setpoint (two-sided pH control)		pH setpoint	7.0	7.0	7.0	7.0
		Preset (mL/h)	0	0	0	0
		P	30	30	30	30
		Ti	300	300	300	300
		Deadband	0.02	0.02	0.02	0.02
		AutoResetYi	false	false	false	false
		Min (mL/h)	–40	–40	–10	–10
		Max (mL/h)	40	40	10	10
pH regulation (two-sided pH control)		Pump A (acid) (mL/h)	40	40	10	10
		Pump B (base) (mL/h)	40	40	10	10
		Acid	20 % phosphoric acid	20 % phosphoric acid	10 % phosphoric acid	10 % phosphoric acid
		Base	20 % ammonia	20 % ammonia	10 % ammonia	10 % ammonia
		Dosage acid*	submersed	headspace	headspace	headspace
		Dosage base*	submersed	headspace	submersed	submersed
DO setpoint		DO setpoint	30	30	30	30
		Preset (%)	0	0	0	0
		P	0.2	0.2	0.2	0.2
		Ti	75	75	75	75
		Min	0	0	0	0
		Max	100	100	100	100
DO cascade	**Agitation**	X1 (%)	0	0	0	0
		Y1 (rpm)	600	600	800	800
		X2 (%)	60	60	60	60
		Y2 (rpm)	1,500	1,600	2,000	2,500
		Direction	clockwise	clockwise	clockwise	clockwise
	Gas flow	X1 (%)	0	0	60	60
		Y1 (vvm)	1	1	0.5	0.5
		X2 (%)	100	100	80	80
		Y2 (vvm)	1	1	1	1
	O_2 concentration in gas mix	X1 (%)	60	60	80	80
		Y1 (%)	21	21	21	21
		X2 (%)	100	100	100	100
		Y2 (%)	100	100	100	100
Anti foam regulation		Inactive until: Input level signal (µs)	35	35	35	35
		Output pump C (mL/h)	0	0	0	0
		Active from: Input level signal (µs)	35.01	35.01	35.01	35.01
		Output pump C (mL/h)	40	40	40	40
		Dosage	headspace	headspace	headspace	headspace

* Dose acid and base either in the headspace or submersed, dependent on the availability of head plate ports

Results

To test the suitability of the controller settings, we recorded processs values and controller output of pH, temperature and DO in *E. coli* fermentation runs. As examples, we show the values for one parameter for each vessel type covered in the Tables 5 and 6 (Figures 4 – 7).

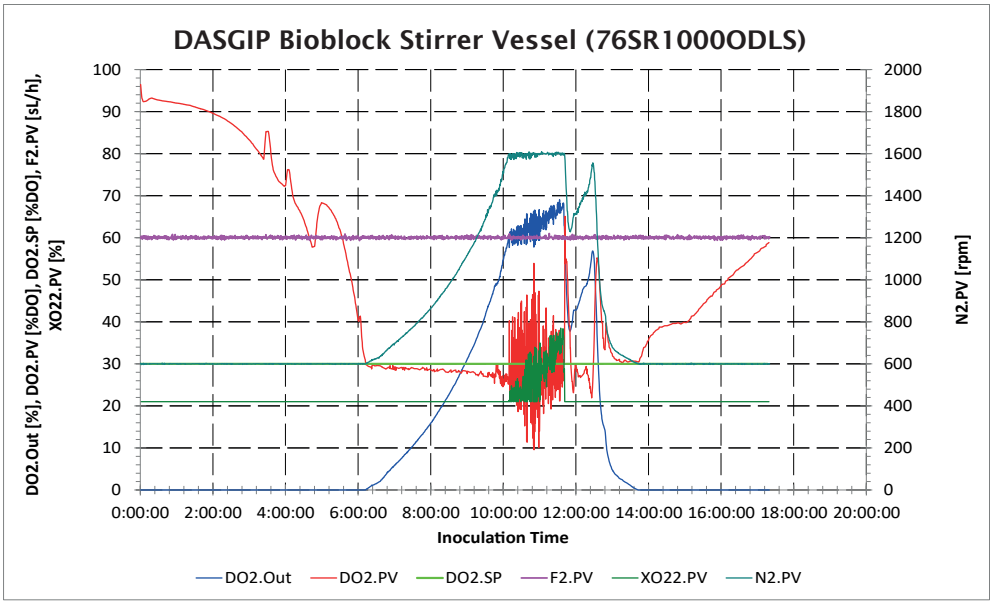


Fig. 4: DO cascade.
To keep the process value (DO2.PV) at setpoint (DO2.SP), the agitation speed (N2.PV) and the oxygen concentration in the gas mix (XO22.PV), and the gas flow (F2.PV) are altered in a cascaded manner. In this example the gas flow rate stayed constant throughout the experiment.
DO2.Out describes the output percentage of the controller.
The controller settings were as described in Table 5.

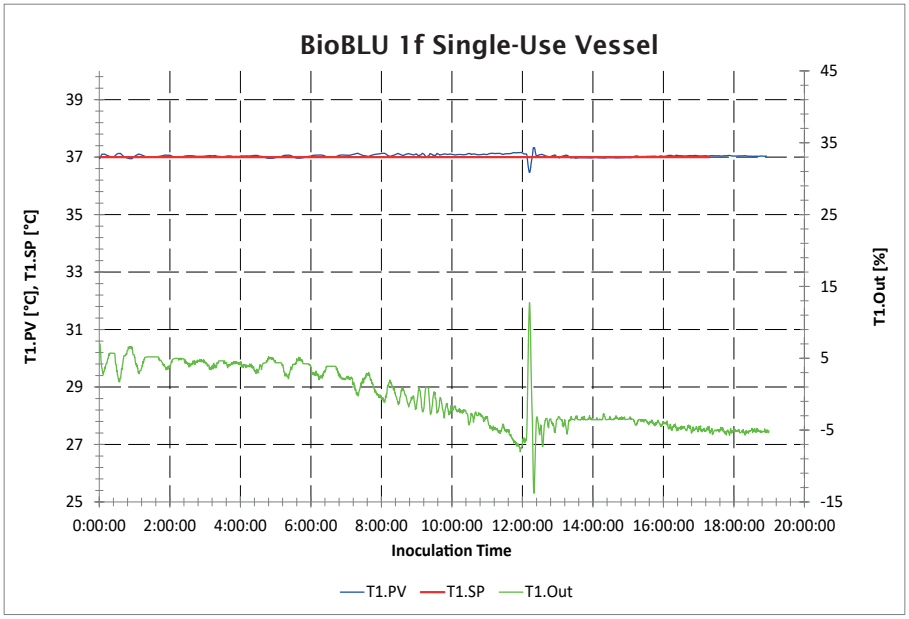


Fig. 5: Temperature control
The temperature setpoint (T1.SP), the process value (T1.PV) and the controller output percentage (T1.Out) are shown.
The controller settings were as described in Table 5.

To monitor bacterial growth, we measured the OD_{600} of the culture offline.

The OD_{600} was typically around 50 at the end of the process.

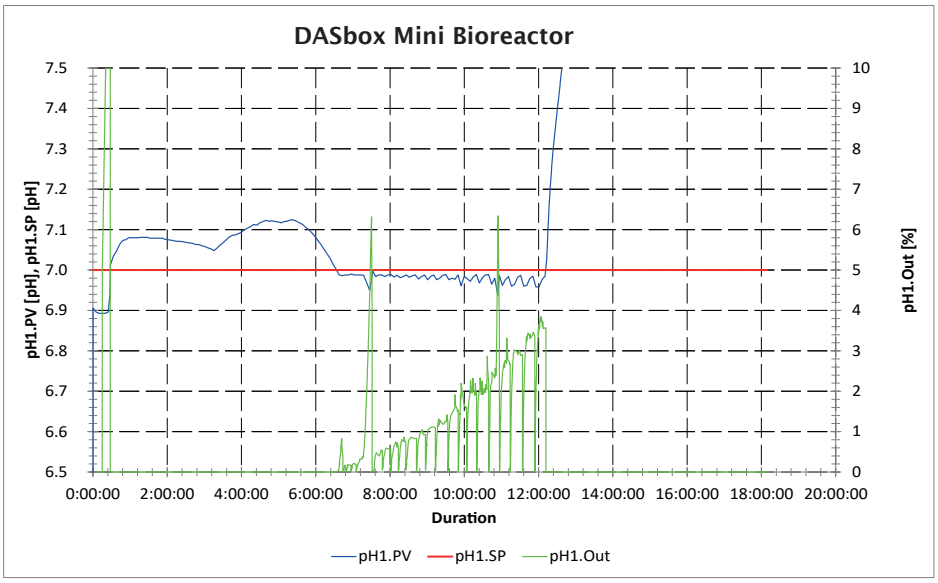


Fig. 6: One-sided pH control.
The pH setpoint (pH1.SP), the process value (pH1.PV) and the controller output percentage (pH1.Out) are shown.
The pH was controlled with 20 % ammonia.
The controller settings were as described in Table 5.

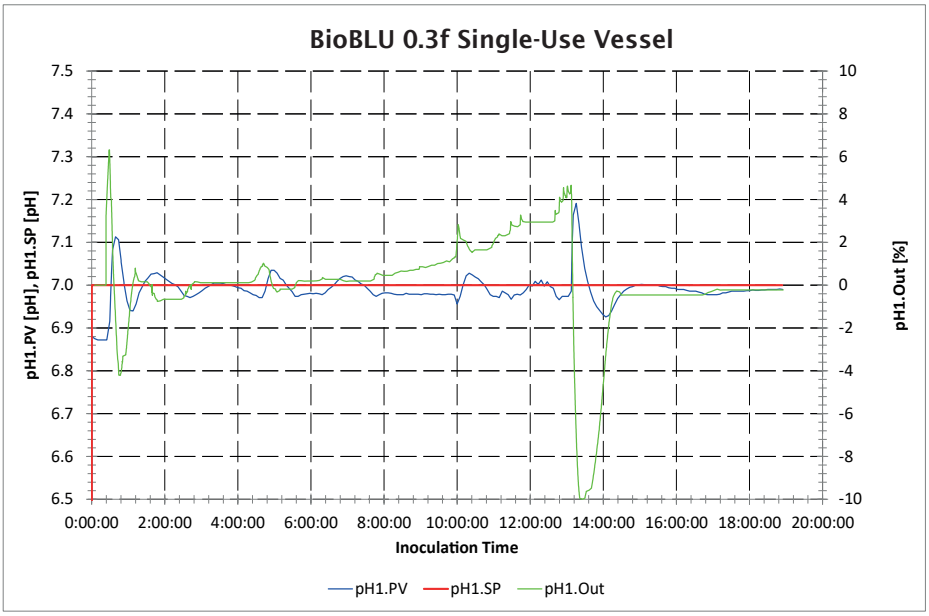


Fig. 7: Two-sided pH control.
The pH setpoint (pH1.SP), the process value (pH1.PV) and the controller output percentage (pH1.Out) are shown.
The pH was controlled with 20 % ammonia and 20 % phosphoric acid.
The controller settings were as described in Table 6.

Ordering information

Description	Order no.
DASbox® Mini Bioreactor System for Microbial Applications, max. 25 sL/h gassing	
4-fold system	76DX04MB
4-fold system for single-use vessels	76DX04MBSU
DASGIP® Parallel Bioreactor System for Microbial Applications, max.250 sL/h gassing	
4-fold system with Bioblock	76DG04MBBB
4-fold system with Bioblock, for single-use vessels	76DG04MBSU
4-fold system, benchtop	76DG04MB
DASGIP® PH4PO4L Monitoring Module, for 4 vessels, without sensors, pH and DO with level/anti foam option	76DGPH4PO4L
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® 0.3f Single-Use Vessel, open pipe, 2 Rushton-type impellers, no pH, sterile, 4 pieces	78903509
BioBLU® 1f Single-Use Vessel, fermentation, open pipe, 2 Rushton-type impellers, no pH, sterile, 4 pieces	1386110200
BioBLU® 1f Single-Use Vessel, fermentation, open pipe, 3 Rushton-type impellers, no pH, sterile, 4 pieces	1386110300
BioBLU® 3f Single-Use Vessel, fermentation, macrosparger, 3 rushton-type impellers, no pH, non-sterile, 4 pieces	1386001000
DASbox® Vessel Type SR0250ODLS, 2x Rushton-type impeller, 60 – 250 mL, overhead drive	76SR0250ODLS
DASGIP® Vessel SR0700ODLS, 200 mL – 1.0 L (2x Rushton-type impeller, L-Sparger, overhead drive, Bioblock)	76SR0700ODLS
DASGIP® Vessel SR1000ODLS, 400 mL – 1.5 L (2x Rushton-type impeller, L-Sparger, overhead drive, Bioblock)	76SR1000ODLS
DASGIP® Vessel SR1500ODLS, 400 mL – 1.8 L (3x Rushton-type impeller, L-Sparger, overhead drive, Bioblock)	76SR1500ODLS
DASGIP® Vessel DR03F, Rushton-type impeller, L-Sparger, 750 mL – 2.7 L, overhead drive	76DR03F
DASGIP® Vessel DR04F, Rushton-type impeller, L-Sparger, 800 mL – 3.7 L, overhead drive	76DR04F

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