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Parallel *Escherichia coli* fermentation in the SciVario® twin, the Flexible Controller for All Your Bioprocess Needs

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Abstract

The range of experimental organisms available for bioprocessing protocols and the complex demands for their cultivation require new options in advanced bioprocess control systems. The SciVario® twin bioprocess controller can be used to regulate both the microbiological parameters and in vitro cell culture needs of these choices, as well as the application of single-use vessels using only a single controller platform.

We performed parallel batch and fed-batch fermentations of *Escherichia coli (E. coli)* with the SciVario twin using Eppendorf 1 L and 3 L glass vessels. These experiments highlighted SciVario twin's capabilities to control complex processes using various vessel sizes.

In this application note, we describe major steps of a typical *E. coli* fermentation, starting from the preparation of the inoculum to the setup of process parameters and control strategies and operation of the vessels. We further describe in detail the preparation of the fermentation medium, the bioprocess run itself and the handling of the SciVario twin controller.

We propose this application note as a starting point for further optimization of our bioprocess control systems.

We used *E. coli* K12 W3110 (DSM 5911) in this study.

The application note can serve as a starting point for further optimization.

Fig. 1: SciVario twin parallel bioprocess control system for glas and single-use bioreactors and fermenter.

Introduction

Stirred-tank bioreactors and fermenters are used to produce biopharmaceuticals including antibodies, hormones, and vaccines, as well as cell production for research, drug discovery, and cell-based therapies. *E. coli* fermentation is the predominant platform for recombinant protein production for these markets.

Microbial fermentation in controlled, stirred-tank fermenters can deliver high cell densities and impressive product yields. The process performance depends on the bacterial strain, the medium composition, as well as the bioprocess control strategies used to keep critical process parameters in the optimal range.

The range of the process window, where growth and/or product formation are maintained at their robust optimum state is usually extremely narrow. Therefore, the ability to control the bioprocess in this window with the least error is critical for effective and efficient process development. However, to meet the varied amounts of process demands, a bioprocess controller needs to cover a variety of activities at different vessel volumes. The SciVario twin bioprocess controller is the new solution from Eppendorf to control two bioreactors individually or in parallel across a wide range of vessel sizes from small- to bench-scale.

It is a dynamic, easy-to-operate system, with the flexibility to adapt to a wide range of experimental configurations.

In this document, we provide a short introduction to the SciVario twin bioreactor control system. We describe the setup of typical *E. coli* fermentation processes, their components, and the design of both batch and fed-batch *E. coli* fermentations using glass vessels. The application note was designed to allow users to achieve rapid and easy initial culture success with the new system. These SciVario twin experiments provide preliminary results addressing feasibility without exploring the range of optimization and thus do not represent the maximum fermentation potential. However, they serve as a starting point for further development of the system.

Materials and Methods

The chemicals and components were purchased from various suppliers; Merck KGaA, Germany through https://www.sigmaaldich.com or https://www.merckmillipore.com; Carl Roth GmbH + Co. KG, Germany (https://www.carlroth.com.) To find the appropriate item from your supplier we provide the CAS-numbers for a fast search.

Microbial strain

E. coli K12 W3110 (DSM 5911) was purchased from the Leibniz Institute - DSMZ-German Collection of Microorganisms and Cell Cultures GmbH.

Complex Medium for preculture

To prepare the inoculum, *E. coli* is cultivated in complex Lysogenic Broth (LB medium, Bertani, 2004), a widely used, classical bacteriological formulation.

Table 1. LB Medium composition

Tryptone	10 g/L
Yeast Extract	5 g/L
Sodium chloride	10 g/L
Dissolve ingredients in dH_2O and sterilize by autoclaving.	

Chemically defined medium for main culture

We used a chemically defined medium for most culturing. Chemically defined media are favored in industrial bioprocessing, because they contain a defined carbon source, which allows tight control of bacterial metabolism. Furthermore, batch-to-batch variations of complex media components are greatly reduced. Among the many recipes for chemically defined media, we use the following formulation.

Table 2. 10 % Antifoam solution

Struktol® J-673	50 g
dH_2O	450 mL
Sterilize by autoclaving. Transfer solution to sterile addition bottle. Struktol® J-673 was purchased from Schill + Seilacher, Hamburg, Germany	

Table 3. 50% Glucose solution

D-Glucose Monohydrate	$C_6H_{12}O_6 \cdot H_2O$	550 g/L
Dissolve in dH_2O and sterilize by autoclaving.		

Stock solutions

First, prepare the following stock solutions:

Table 4. Thiamine stock solution

Thiamine hydrochloride	5 g/L
Dissolve ingredients in dH_2O and sterilize by autoclaving.	

Culture medium

Prepare 1 L of culture medium from the stock solutions. 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, Table 8).

Table 5. 10x PAN medium stock solution

Calcium chloride dihydrate	$CaCl_2 \cdot 2H_2O$	0.15 g/L
Potassium dihydrogen phosphate	KH_2PO_4	30 g/L
Dipotassium hydrogen phosphate	K_2HPO_4	120 g/L
Ammonium sulfate	$(NH_4)_2SO_4$	50 g/L
Iron(II) sulfate heptahydrate	$FeSO_4 \cdot 7H_2O$	0.75 g/L
Trisodium citrate dihydrate	$HOC(COONa)(CH_2COONa)_2 \cdot 2H_2O$	10 g/L
Dissolve in dH_2O and sterilize by autoclaving.		

Table 6. Magnesium-sulfate stock solution

Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	100 g/L
Dissolve in dH_2O and sterilize by autoclaving.		

Table 7. PAN trace elements solution

Aluminum sulfate octadecahydrate	$Al_2(SO_4)_3 \cdot 18H_2O$	2.0 g/L
Cobalt(II) sulfate heptahydrate	$CoSO_4 \cdot 7H_2O$	0.8 g/L
Copper(II) sulfate pentahydrate	$CuSO_4 \cdot 5H_2O$	2.5 g/L
Boric acid	H_3BO_4	0.5 g/L
Manganese sulfate monohydrate	$MnSO_4 \cdot 1H_2O$	24 g/L
Sodium molybdate dihydrate	$Na_2MoO_4 \cdot 2H_2O$	3.0 g/L
Nickel(II) sulfate hexahydrate	$NiSO_4 \cdot 6H_2O$	31.5 g/L
Zinc sulfate heptahydrate	$ZnSO_4 \cdot 7H_2O$	15 g/L
Sulfuric acid, 25 %	H_2SO_4, 25 %	2.4 mL/L
Dissolve in dH_2O and sterilize by autoclaving.		

Table 8. 1x PAN-medium with additions, 1L reference vol.

Medium preparation for use of autoclavable glass vessels	
10x PAN-medium stock solution	100 mL
10 % Struktol J-673	20 mL
dH_2O	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

Feed medium

The feed medium consists of 60 % glucose solution, PAN trace elements, and Thiamine (Table 9).

Table 9. 60 % (w/v) glucose solution

D-Glucose Monohydrate	660 g/L
PAN trace elements solution aseptically added	1 mL/L
Thiamine stock solution aseptically added	1 mL/L

pH control

The SciVario twin bioprocess controller offers the possibility to establish a two-sided pH control. We used 20 % ammonia and 20 % phosphoric acid to adjust the pH for both 1 L and 3 L cultures.

To control the pH in higher working volumes, higher concentration of pH control reagent may be needed. For example, in the 3 L size, 25 % solutions can be used as well.

Measurement of the optical density

To measure the optical density, we used the Eppendorf Biophotometer D30. The samples were taken through the bioreactors' sampling port using a syringe. We diluted the samples to get an absorbance measurement between 0.3 and 0.5 using standard PBS buffer. Measurements were taken at a wavelength of 600 nm (using the method that is already pre-implemented in the biophotometer).

Determination of the cell wet weight

To determine the cell wet weight (cww), we transferred 1 mL of the sample into a 1.5 mL Eppendorf tube. The tare weight of the empty vial was determined previously and noted. We used the Eppendorf centrifuge 5427R together with the rotor type FA-45-24-11 and centrifuged the samples at 10,000 rpm for 15 min. The vials were decanted after the centrifugation while the weight of the Eppendorf vial with the pellet was measured and the final net weight of the biomass was calculated.

Preparation of the SciVario twin Bioreactor Control System

The following describes *E. coli* fermentation using the SciVario twin bioprocess controller together with DASGIP® bench scale or with small scale vessels.

Bioprocess system and vessel components

The general components for configuring the system are listed in table 10.

Table 10. Vessel system components

Function	Components	
Vessel	1 L Vessel SR1000ODLS 0.4 L – 1.5 L	3 L Vessel DR03F 0.75 L – 2.7 L
Temperature Control Cooling	Temperature control well	Cooling Finger
Temperature Control Heating	Temperature control well	Heating Blanket
pH monitoring	pH sensor (polarographic, Analog)	pH sensor (polarographic, ISM)
DO monitoring	DO sensor (Clark sensor, Analog)	DO sensor (Clark sensor, Arc)
Agitation	Overhead drive MD40 (100 – 1600 rpm)	
Impeller	2x Rushton Type Impeller	
Baffles	None	
Temperature monitoring	Platinum RTD Temperature Sensor (Pt100)	
Antifoam control	Level sensor	
Gassing (Small Scale)	Macro-L-sparger	
Sampling	Sampling tube with valve	
Pump head tubing	Bioprene ID 0.8 mm	
Feed lines	PTFE feed lines ID 0.5 mm	
Options for liquid addition	Short dip tube for anti-foam addition Long dip tubes for pH agent (base, acid)	
Exhaust cooling	Liquid condenser	

Recommended Controller Settings

To maintain optimal growth conditions during fermentation, the SciVario twin bioprocess controller provides integrated online control of temperature, dissolved oxygen (DO) concentration, gas flow, agitation, pH, as well as feeding. More sophisticated possibilities for process automation, for example, DO-spike based automatic feeding start, is enabled with the optional integration with the DASware® control software. The SciVario twin bioprocess controller has programmed parameters stored in its system for the standard control loops of Temperature, pH and DO. It is possible to freely change the parameters depending on the process needs (Table 11, Figure 2 and Table 12).

E. coli Batch and Fed-Batch Fermentation

Batch fermentation is a simple fermentation method, in which all nutrients are provided from the beginning and nutrients are not added during cultivation. At time t=0, the sterilized nutrient solution (supplemented with antifoam agent) in the fermenter is inoculated and incubation proceeds at a suitable temperature and gaseous environment for the required period. In the course of the fermentation protocol, nothing is added, except oxygen to drive the aerobic microorganism's metabolic processes, and antifoam agent, and if needed, acid or base to control the pH.

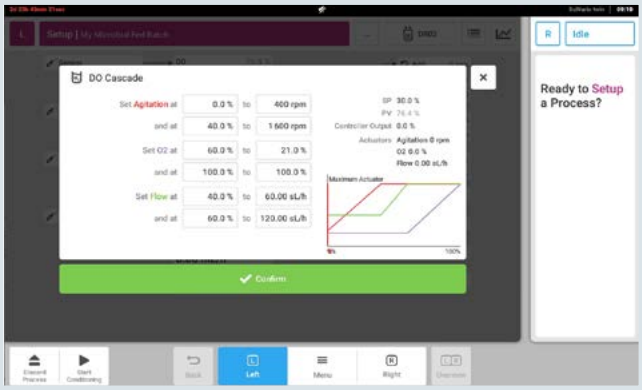

Fig. 2: DO-Cascade screen for the 3 L batch mode.

A fed-batch fermentation starts with a small amount of medium inside the fermenter. An important component is the addition of a defined amount of concentrated medium during the process, starting with a trigger point (e.g., *E. coli* glucose depletion peak as it is defined in this project) or after a certain time point. These substances continue to be added in small doses during the fermentation operation.

Starting culture and Inoculation

To generate enough biomass for the inoculation of the fermenter we prepared the preculture using shake flasks with baffles to which 25 mL of sterile LB medium is added in a total volume of 500 mL. We then inoculated the shake flask with *E. coli* K12 from one cryo-vial and incubated the preculture overnight, at 37 °C and 200 rpm (e.g. using an Innova® S44i Shaker).

For the preparation of the inoculum we filled 100 mL of sterile LB medium into a shake flask with baffles in a total volume of 1 L to expand the preculture of the 25 mL flask. We then inoculated the 1 L flask with 5 mL of the preculture and incubated for 7 hours, at 37 °C and 200 rpm (e.g. orbit radius 2.54 cm). The final optical density at 600 nm (OD_{600}) of the inoculum culture should be between 6 and 8. This volume of inoculum culture is sufficient for 2 L of the final culture.

We subsequently transferred the final inoculum culture to a sterile beaker to make it easier to draw up the culture into a syringe. Just in case, we prepared more than one shake flasks of precultures and pooled them together.

Table 11. Controller parameters and set-points for the fermentation process

Vessel			Unit	SR1000ODLS 0.4-1.5 L	DR03F 0.75-2.7 L
Batch-Volume			[L]	1	2
Fed-Batch-Volume		Process start	[L]	0.5	1
Fed-Batch-Volume		Process end	[L]	1	2
Temperature control		Temperature setpoint	[°C]	37	37
		Proportional-value	[%]	80	80
		Integral-value	[%/s]	0.2	0.2
pH control		pH setpoint	[-]	7.0	7.0
		Proportional-value	[%]	375	375
		Integral-value	[%/s]	7.5	7.5
		Deadband	[-]	without	without
		Controller out min	[%]	-100	-100
		Controller out max	[%]	100	100
Dissolved Oxygen control		DO setpoint	[%]	30	30
		Proportional-value	[%]	0.4	0.4
		Integral-value	[%/s]	0.005	0.005
		Deadband	[%]	0	0
		Controller out min	[%]	0	0
		Controller out max	[%]	100	100
pH control		Acid	Phosphoric Acid 20 %		
		Acid pump	Set max flow [ml/h]	40	40
		Base	Ammonia 20 %		
		Base pump	Set max flow [ml/h]	40	40
Foam control	Inactive from:	Input level signal	[µs]	35	35
		Antifoam pump	[mL/h]	0	0
	Active from:	Input level signal	[µs]	35.01	35.01
		Antifoam pump	[mL/h]	40	40
		Sensing time	[s]	1	1
		Pause time	[s]	2	2
		Dosage anti foam		headspace	headspace

Table 12. DO-Cascade and feeding (fed-batch only) for the fermentation process

		Controller output	Actuator output	Batch		Fed-batch	
				SR1000ODLS	DR03F	SR1000ODLS	DR03F
DO-cascade	Agitation	X1 [%] 0	Y1 [rpm]	400	400	600	600
		X2 [%] 40	Y2 [rpm]	1600	1600	1600	1600
	gassing rate	X1 [%] 40	Y1 [sL/h]	30	60	30	60
		X2 [%] 60	Y2 [sL/h]	60	120	60	120
	$XO_{2,in}$	X1 [%] 60	Y1 [%]	21	21	21	21
		X2 [%] 100	Y2 [%]	100	100	100	100
Feed	Feed control			---	---	Script-controlled after reaching DO hunger peak	
	Feed pum	[mL/h]				10.4	20.8

We inoculate the main culture to an OD_{600} of 0.3 – 0.4 using an inoculum culture with an OD_{600} of 6 – 8, corresponding to 5 % of the initial working volume of the main culture. We drew up the required volume in a sterile syringe and inoculated the main culture via the sampling port of the bioreactor.

Results

To validate the suitability of the controller settings, we recorded process values and controller output of pH, temperature, and DO in microbial runs. Additionally, we analyzed OD_{600} und cell fresh weight (biomass) offline.

Results from *E. coli*-Batch and Fed-Batch fermentation

The batch run using the SciVario twin as a stand-alone bioprocess controller resulted in a final OD_{600} of 56 for the 1 L bioreactor and 53 for the 3 L vessel (data not shown).

For the fed-batch process, the medium was a constant glucose feed with supplementations. The following data (Figure 3 - 7) show a script-based DO-triggered fed-batch run with the SciVario twin bioprocess controller and the DASware control software (version 5.6.0) using a constant feed rate. The DO-triggered feed activation was controlled by a DASware programming control script (see appendix).

The fed-batch run using the SciVario twin as a stand-alone bioprocess controller resulted in a final optical OD_{600} above 100 for the 1 L bioreactor (OD_{600}=107±1) and for the 3 L vessel (OD_{600}=102±1, Figure 3A). These numbers result in a cell fresh weight of cfw = 137.7±21.1 $g \cdot L^{-1}$ for the 1 L bioreactor and cfw=132.0±3.0 $g \cdot L^{-1}$ for the 3 L vessel (Fig. 3B).

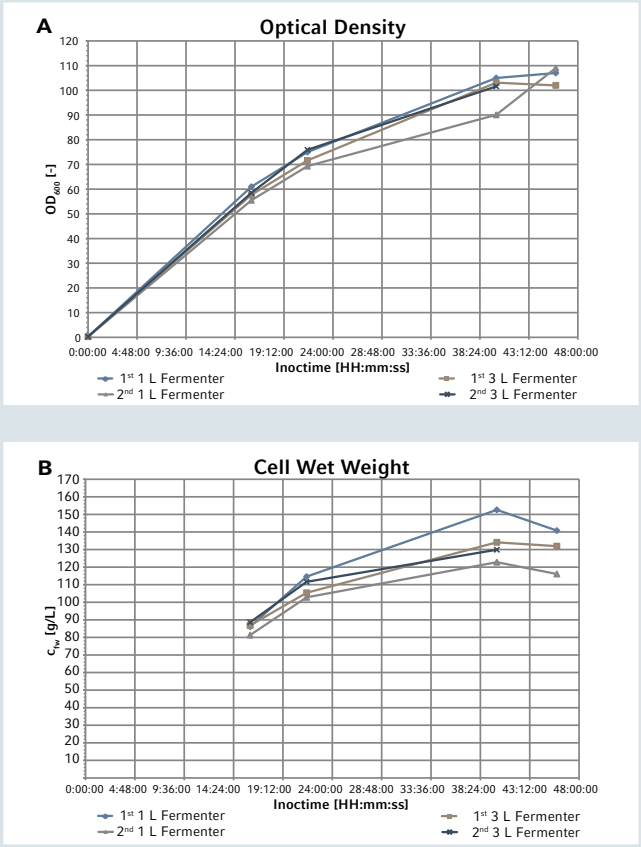

Fig. 3: Optical Density (OD_{600}) **[A]** and cell wet weight (cww) **[B]** measured for the fed-batch process of two 1 L and two 3 L fermenters.

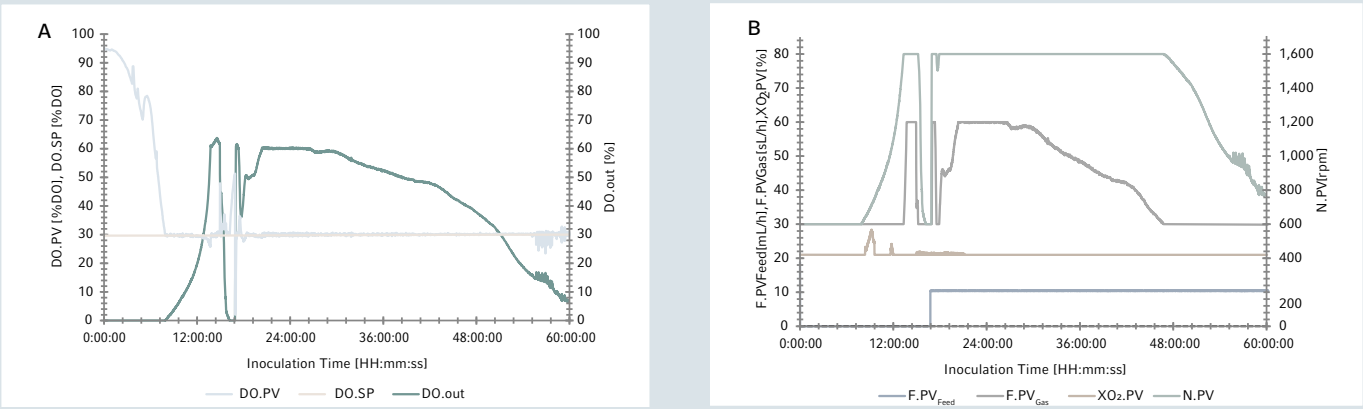

Fig. 4: DO-Control of the *E.coli* Fed-Batch Fermentation controlled by the SciVario twin bioprocess controller in the DASGIP 1L vessel.
A: Dissolved oxygen process value (DO.PV) vs. Dissolved oxygen controller output value (DO.out).
B: Dissolved oxygen controller actuator output – stirring speed (N.PV), oxygen concentration (XO_2.PV), gas flow rate (F.PV).

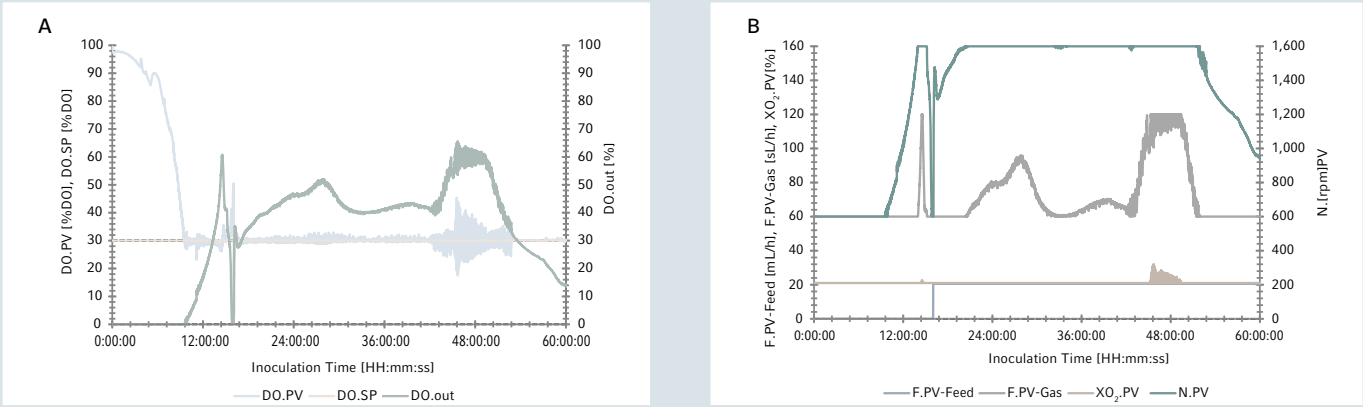

Fig. 5: DO-Control of the E.coli Fed- Batch Fermentation SciVario twin in the DASGIP 3L vessel.
A: Dissolved oxygen process value (DO.PV) vs. Dissolved oxygen controller output value (DO.out).
B: Dissolved oxygen controller actuator output – stirring speed (N.PV), oxygen concentration (XO_2.PV), gas flow rate (F.PV).

We performed the DO control strategy as planned for the fed-batch processes, as shown in Figure 4 (1 L vessel) and Figure 5 (3 L vessel). The growth of the *E. coli* culture was followed by the decrease of the DO till reaching 30 % DO.

At this value the stirring speed increases for adequate dispersion of gas bubbles. After the maximum of the stirring speed was reached, the gas flow of air increased. After the gas flow was maxmized , the concentration of oxygen increased as expected. After the end of the batch phase all actuators decreased, and the DO signal increased. After the DO signal increased to an upper threshold again, here at 50 % DO, the constant feed was initiated, and the output of the actuators increased again, indicating additional growth.

Control of pH during the fermentation process

Typically, the pH of an *E. coli* fermentation protocol producing acetic acid can be controlled via a one-sided pH control using base. As in the batch process we also used a two-sided pH control in the fed-batch fermentation process (Fig. 6). The statistical deviation of the pH control in the 1 L fermentation vessel was around 0.02, whereas in the 3L fermentation vessel the deviation was ca. 0.01. In Figure 6A a higher activity of the pumps was noted around 42 h of process time. This indicates a phase of high growth performance of the *E. coli* culture in the 3 L vessel, also visible in Figure 6B. Such a fluctuation can be reduced by other pH controller settings or base concentration.

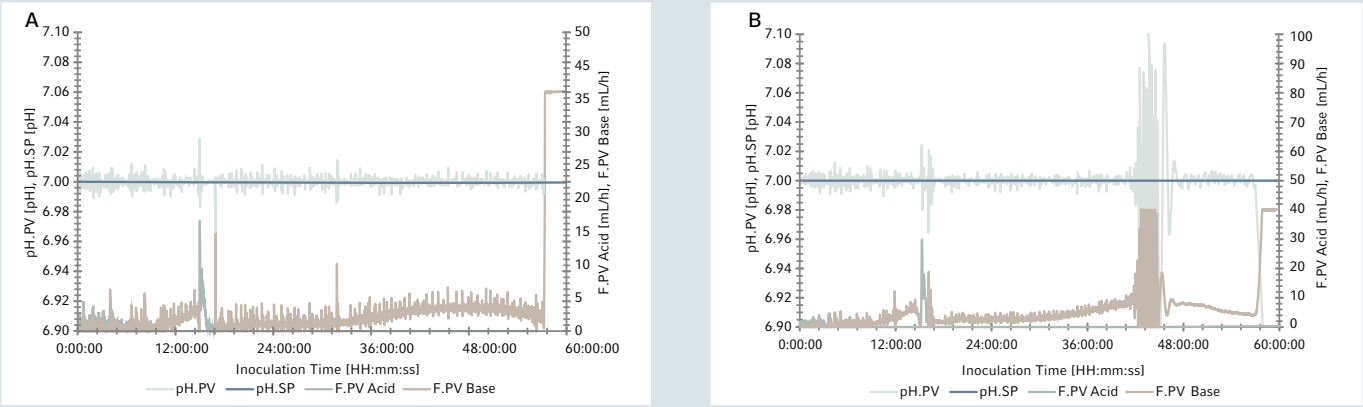

Fig. 6: pH-Control of the *E.coli* fed-batch fermentation.
Actuator outputs are shown in kind pH setpoint and process values (pH.SP, pH,PV) as well as acid and base feed (F.PV).
A: fed-batch fermentation in the DASGIP 1 L vessel and **B**: in the DASGIP 3 L vessel

Temperature control can be managed within an extremely narrow range around the setpoint (Fig. 7). The departure using the temperature control block for the 1 L vessel led to a variation of ca. 0.06 K, while the deviation using the heating blanket and one cooling finger led to a divergence of 0.03 K around the of 37 °C. setpoint

Conclusion

We have described straightforward approaches for conducting batch and fed-batch fermentation using *E. coli* as the reference organism. This includes an overview of the basic setup and instructions on how to use the equipment for a 1 L vessel with a temperature control block and 3 L DASGIP vessel with a heating blanket and cooling finger. We used the predefined temperature control settings from either the temperature control block or heat blankets.

We assert that the controller settings used for pH and DO serve as convenient reference values for process development and optimization. We integrated the DASware control software which enabled a more precise process automation with DO based feeding.

It is noteworthy that protocols developed using Eppendorf's systems can be easily transferred. The SciVario twin can support DASGIP glass and BioBLU® Single-Use Vessels up to 4 L during the initial release phase. Future Agile release train updates will allow users to run vessels from as low as 0.3 L to as high as 4 L glass fermentation vessels and 40 L ready-to-use cell culture vessel on the same controller.

This application note constitutes a set of instructions for optimal utilization of the SciVario twin bioprocess controller package. Future communications will explore in detail further experimental uses of this technology. We welcome questions and reports from users' experiences with these devices.

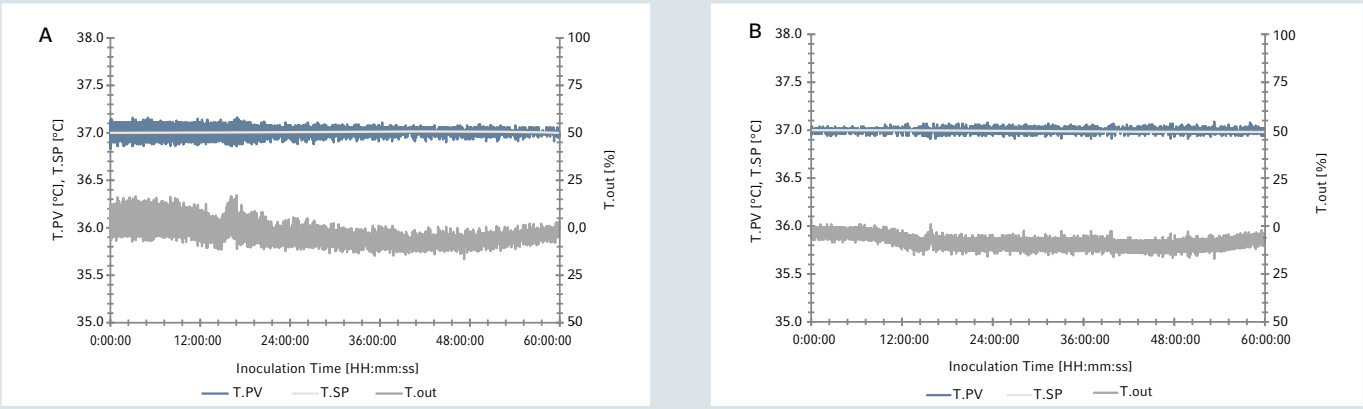

Fig. 7: Temperature- control of the *E.coli* fed-batch fermentation.
A: Temperature control in 1 L DASGIP fermenter; **B:** Temperature control in 3 L DASGIP fermenter

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[2] Keshavarz, T. 2014. FERMENTATION (INDUSTRIAL): Control of Fermentation Conditions (in) Encyclopedia of Food Microbiology (Second Edition).

Appendix

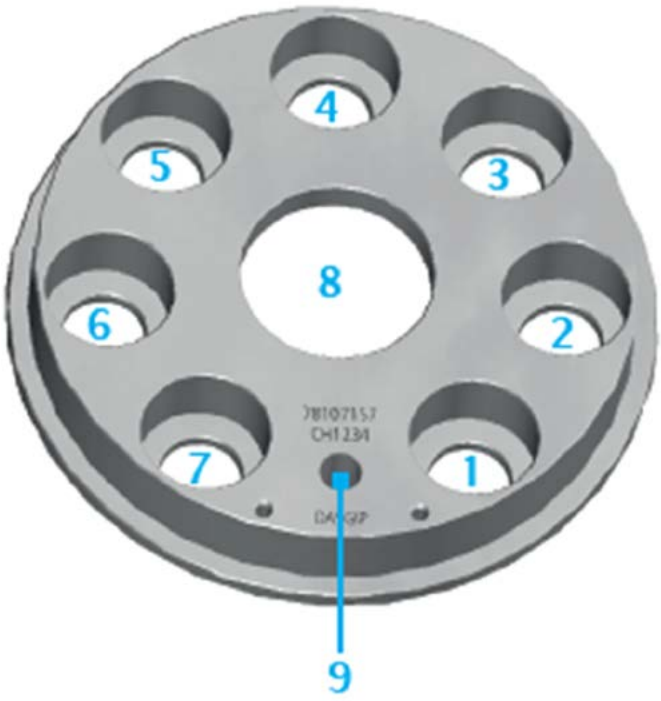
The following visual basic script provides the opportunity to start the feeding based on the DO hunger peak that occurs during the end of the batch phase. Other triggers can be also used to initiate the feed phase. Copy and paste the following script into the DASware control software as vessel script.

```
'Script parameters
Dim StartDelay_H As Double = 1/60 'delay after inoculation [h]
Dim lowDOTrg As Double = 30.0 'DOPV < lowDOTrg start waiting DO peak Trigger
Dim peakDOTrg As Double = 40.0 'DOPV > peakDOTrg DO peak Trigger
Dim FeedFlowRate As Double = 10.4 ' Feed flow rate [ml/h] for SR1000ODLS
'Dim FeedFlowRate As Double = 20.8 ' Feed flow rate [ml/h] for DR03F
Dim FeedDuration_H as Double = 48 ' Feed duration [h]
dim t_h as double

if P isNot Nothing Then
  with P
    select case .phase
    case 0
      .phase = .phase + 1
      .LogMessage("Entering phase " & .phase & ": Waiting for InoculationTime > " & format(StartDelay_H, "#0.00") & " [h]")
      .FCSP = 0.0
    case 1
      if .InoculationTime_H > StartDelay_H then
        .phase = .phase + 1
        .LogMessage("Entering phase " & .phase & ": Waiting for DO < " & lowDOTrg)
      end if
    case 2
      if .DOPV < lowDOTrg then
        .phase = .phase + 1
        .LogMessage("Entering phase " & .phase & ": Waiting for DO > " & peakDOTrg)
      end if
    case 3
      if .DOPV > peakDOTrg then
        .phase = .phase + 1
        .LogMessage("Entering phase " & .phase & ": Start feeding (Duration = " &format(FeedDuration_H, "#0.00") & " [h]")
      end if
    case 4
      'Feed
      .FCSP = FeedFlowRate
      t_h = .InoculationTime_H - .PhaseStart_H
      if t_h > FeedDuration_H then
        .phase = .phase + 1
        .LogMessage("Entering phase " & .phase & ": Stop feeding ")
            .FCSP = 0.0
            .PumpCActive = false
      end if
    case 5
      'Stop
    end select
  end with
end if
```

Appendix Table 1: Vessel configuration of the 1 L microbial bioreactor SR1000ODLS (shown configuration differs from standard configuration)

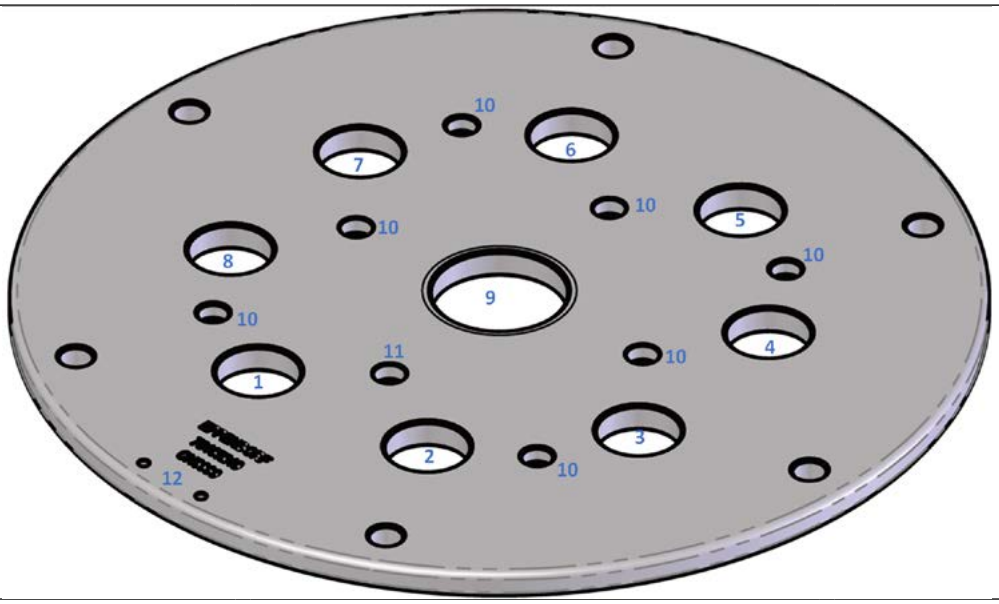
Port	Port Accessory	Associated Equipment	Purpose
1 Pg 13.5	Compression fitting, I.D 6 mm	L-Sparger with 50 mm silicone tubing and 0.2 µm inlet gas filter	Submerged gassing
2 Pg 13.5	-	DO Sensor	pO_2 Monitoring
3 Pg 13.5	Triple port position 1 Triple port position 2 Triple port position 3	Long dip tube Long dip tube Long dip tube	Inoculation/ Sampling Acid addition Base addition
4 Pg 13.5	Compression fitting I.D.12 mm	Condenser with 50 mm silicon tubing and 0.2 µm filter Capsule	Water based exhaust gas cooling
5 Pg 13.5	Triple port position 1 Triple port position 2 Triple port position 3	Short dip tube Short dip tube Short dip tube	Antifoam addition Feed addition Free
6 Pg 13.5	-	pH Sensor	pH Monitoring
7 Pg 13.5	Compression fitting I.D. 4 mm	Level Sensor	Foam monitoring
8 M30	Lip seal stirrer Assembly	Motor MD30 or MD40	Agitation
9 M6	Thermowell	Platinum RTD temperature sensor (Pt100)	Temperature monitoring



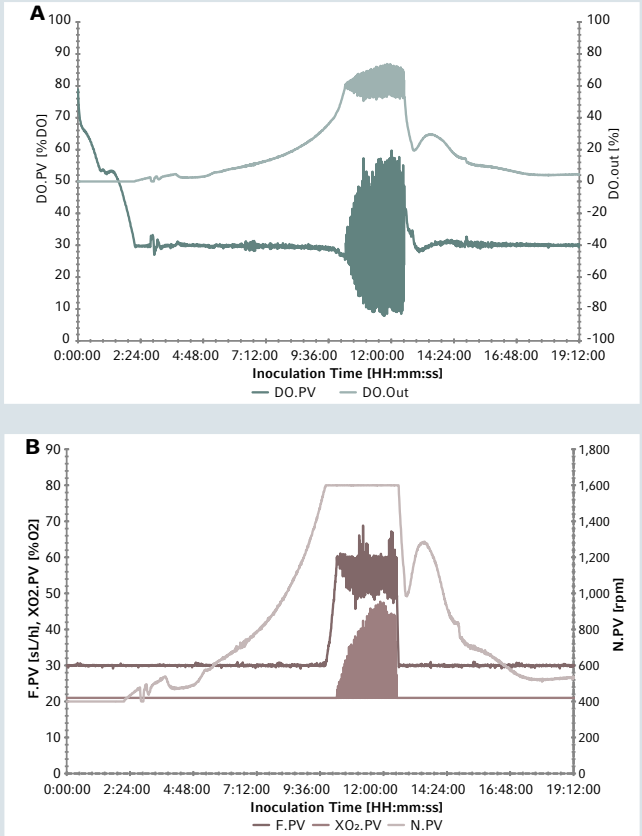
Appendix Figure 1: Typical head plate for the DASGIP Bioblock Stirrer Vessel with a working volume of 400 mL – 1.5 L (76SR1000ODLS). The arrangement of the equipment options in the head plate is flexible. Please refer to the DASGIP Bioreactors user manual for more information. Please note that for the other vessel types the port accessories may differ.

Appendix Table 2: Standard vessel configuration of the 3 L microbial bioreactor DR03F (shown configuration differs from standard configuration)

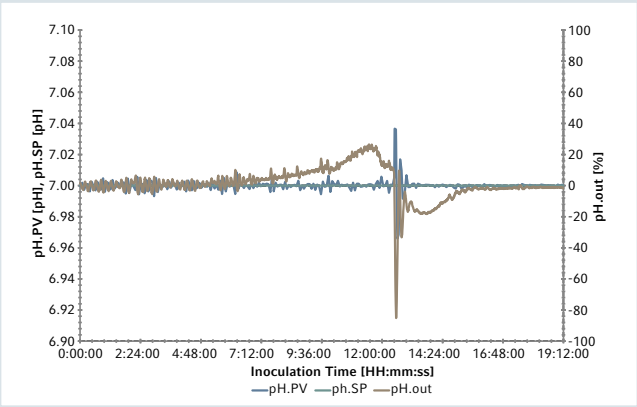
Port	Port Accessory	Associated Equipment	Purpose
1 M10	Compression fitting, I.D 6 mm	L-Sparger with 50 mm silicone tubing and 0.2 µm inlet gas filter	Submerged gassing
2 M10	M10 to Pg 13.5 adapter	DO Sensor	pO_2 Monitoring
3 M10	Triple port position 1 Triple port position 2 Triple port position 3	Long dip tube Long dip tube Long dip tube	Inoculation/ Sampling Acid addition Base addition
4 M10	Compression fitting I.D.12 mm	Condenser with 50 mm silicon tubing and 0.2 µm filter Capsule	Water based exhaust gas cooling
5 M10	Compression fitting I.D. 4 mm	Level Sensor	Foam monitoring
6 M10	M10 to Pg 13.5 adapter	pH Sensor	pH Monitoring
7 M10	Triple port position 1 Triple port position 2 Triple port position 3	Short dip tube Short dip tube Short dip tube	Antifoam addition Feed addition Free
8 M10	Compression fitting ID12	Cooling finger	Temperature Control
9 M30	Lip seal stirrer Assembly	Motor MD30 or MD40	Agitation
10	M6 male thread	None. Additional harvest and addition tubes can be added to the vessel	
11	Thermowell	Platinum RTD temperature sensor (Pt100)	Temperature monitoring
12	-	2nd ground connector for Antifoam detection	Antifoam



Appendix Figure 2: Typical head plate for the DASGIP Benchtop Stirrer Vessel with a working volume of 750 mL – 2.7 L (DR03F).
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 the port accessories may differ.



App. Fig 3: DO-Control of the *E.coli* Batch Fermentation run in the DASGIP 1L vessel.
A: Dissolved oxygen process value (DO.PV) vs. Dissolved oxygen controller output value (DO.out)
B: Dissolved oxygen controller actuator output – stirring speed (N.PV), oxygen concentration (XO_2.PV), gas flow rate (F.PV).



App. Fig. 4: pH- Control of the 1 L *E.coli* Batch Fermentation in the DASGIP 1L vessel.
pH setpoint (pH.SP) and pH process value (pH.PV) vs. pH controller output value pH.out.

Appendix Table 3. CAS numbers for fast search

Chemical	CAS number
Tryptone	CAS 91079-40-2
Yeast Extract	CAS 8013-01-2
Sodium chloride	CAS 7647-14-5
D-Glucose Monohydrate	CAS 77938-63-7
Thiamine hydrochloride	CAS 67-03-8
Calcium chloride dihydrate	CAS 10035-04-08
Potassium dihydrogen phosphate	CAS 7778-77-0
Dipotassium hydrogen phosphate	CAS 7758-11-4
Ammonium sulfate	CAS 7783-20-2
Iron(II) sulfate heptahydrate	CAS 7782-63-0
Trisodium citrate dihydrate	CAS 6132-04-3
Magnesium sulfate heptahydrate	CAS 10034-99-8
Aluminum sulfate octadecahydrate	CAS 17927-65-0
Cobalt(II) sulfate heptahydrate	CAS 10026-24-1
Copper(II) sulfate pentahydrate	CAS 7758-99-8
Boric acid	CAS 10043-35-3
Manganese sulfate monohydrate	CAS 10034-96-5
Sodium molybdate dihydrate	CAS 10102-40-6
Nickel(II) sulfate hexahydrate	CAS 10101-97-0
Zinc sulfate heptahydrate	CAS 7446-20-0
Sulfuric acid, 25 %	CAS 7664-93-9

Ordering information

Description	Order no.
SciVario® twin Fermenter/Bioreactor Control System, base unit for 2 vessels	7600100001
DASGIP® Bioblock Stirrer Vessels, 1 L Vessel, 0.2 mL - 1.0 mL working volume, 2x Rushton-type impeller	76SR0700ODLS
DASGIP® Benchtop Bioreactors for Microbiology, 3.5 L Vessel, 0.8 L - 3.8 L working volume, 3x Rushton-type impeller	76DR04F

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