APPLICATION NOTE No. 462

High-Density *Escherichia coli* Fed-batch Fermentation Using the SciVario[®] twin Bioreactor Control System

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Abstract

In upstream bioprocessing, the feed strategy is an important parameter to achieve high cell densities. In this study we optimized high cell density fed-batch fermentation of *Escherichia coli* (*E. coli*) using the SciVario twin bioreactor control system equipped with a 1 L glass bioreactor. By improving the medium composition and feeding strategy, we increased the optical density (OD₆₀₀) more than two-fold compared

to a previous study [1], where we used the same *E. coli* strain. These experiments highlighted SciVario twin's capabilities to control complex processes achieving high cell densities. We propose this application note as a starting point for further optimization of high biomass based *E. coli* bioproduction.

Introduction

E. coli fermentation is a predominant platform for recombinant protein production for the biopharmaceutical market. To achieve high cell densities and notable yields in microbial fermentations performed in stirred-tank bioreactors, the bacterial strain, the medium composition, the bioprocess control system, as well as the bioprocess control strategies must all be optimized.

The process window, where growth and/or product formation are maintained at their optimum state is usually quite narrow. Therefore, the ability to tightly control the bioprocess parameters is critical. This means that a bioprocess controller should cover a variety of bioprocess protocols and deliver robust control at a wide range of bioreactor volumes and conditions.

In this study, we used the SciVario twin bioreactor control

system for high-density *E. coli* fermentation. We describe the setup of the bioprocess system and its principal components. We also explain how we improved process control, medium composition, and feed strategy.

The improvements led to high cell densities at OD readings above 300, more than twice the value reported in a previous application note on fed-batch culture [1].

This application note was developed to allow users to achieve success with high-density culture. The presented experiments provide preliminary results addressing feasibility without extensive optimization and thus do not represent the maximum fermentation potential. However, they serve as a starting point for further process optimization.

Material and Methods

Microbial strain

We used E. coli K12 W3110 (DSM 5911), purchased through the Leibniz Institute - DSMZ-German Collection of Microorganisms and Cell Cultures GmbH.

Fermentation media

The chemicals and components can be acquired from different suppliers such as Merck KGaA, Germany, as well as Carl Roth GmbH + Co. KG, Germany. To find the appropriate chemical from your supplier we provide you the CASnumbers for a fast search.

Complex medium for preculture

To prepare the inoculum, E. coli was cultivated in complex Luria-Bertani (LB) medium which contains peptone, yeast extract, and sodium chloride.

LB medium

Tryptone	10 g/L	CAS 91079-40-2	
Yeast extract	5 g/L	CAS 8013-01-2	
Sodium chloride	10 g/L CAS 7647-14-5		
Dissolve ingredients in dH O and sterilize by autoclaving			

Dissolve ingredients in dH_0 and sterilize by autoclaving.

Chemically defined medium for main culture

Chemically defined media are favored in industrial bioprocessing because the scientist is able to control which carbon source the bacteria metabolize. Furthermore, batchto-batch variation of complex media components is greatly reduced. There are many recipes for chemically defined media that have been extensively evaluated over a wide range of conditions. Below we describe the preparation of the medium that we routinely use in our applications lab.

Media stock solutions

First, prepare the following stock solutions:

Thiamine stock solution

Thiamine-HCI	5 g/L	CAS 67-03-8
Dissolve ingredients in dH ₂ O. Sterili	ze by filtration	1.

Magnesium-sulfate stock solution

$MgSO_4 \cdot 7 H_2O$	100 g/L	CAS 10034-99-8
Dissolve in dH ₂ O. Sterilize by autoclaving.		

2 M magnesium-sulfate stock solution

$MgSO_4 \cdot 7 H_2O$	493 g/L	CAS 10034-99-8
Dissolve in dH ₂ O. Sterilize by autocl	aving.	

10x PAN medium stock solution

$CaCl_2 \cdot 2 H_2O$	0.15 g/L	CAS 10035-04-08	
KH ₂ PO ₄	30 g/L	CAS 7778-77-0	
K₂HPO₄	120 g/L	CAS 7758-11-4	
(NH ₄) ₂ SO ₄	50 g/L	CAS 7783-20-2	
$FeSO_4 \cdot 7 H_2O$	0.75 g/L	CAS 7782-63-0	
Tri-sodium-citrate $\cdot 2 H_2 0$	10 g/L	CAS 6132-04-3	
Dissolve in dH O. Sterilize by autoclaving			

Dissolve in dH₂O. Sterilize by autoclaving.

PAN trace elements solution

Al ₂ (SO ₄) ₃ · 18 H ₂ O	2 g/L	CAS 17927-65-0
$CoSO_4 \cdot 7 H_2O$	0.8 g/L	CAS 10026-24-1
$CuSO_4 \cdot 5 H_2O$	2.5 g/L	CAS 7758-99-8
H ₃ BO ₄	0.5 g/L	CAS 10043-35-3
$MnSO_4 \cdot 1 H_2O$	24 g/L	CAS 10034-96-5
$Na_2MoO_4 \cdot 2 H_2O$	3 g/L	CAS 10102-40-6
$NiSO_4 \cdot 6 H_2O$	31.5 g/L	CAS 10101-97-0
$ZnSO_4 \cdot 7 H_2O$	15 g/L	CAS 7446-20-0
H ₂ SO ₄ , 25 %	2.4 mL/L	CAS 7664-93-9

Dissolve ingredients in dH₂O. Sterilize by filtration.

50 % (w/v) glucose stock solution

Glucose · 1 H ₂ 0	550 g/L	CAS 77938-63-7
Discolve in dH O. Sterilize by auteol	nuina	

Dissolve in dH₂O. Sterilize by autoclaving.

Culture medium

Prepare 1 L of culture medium from the stock solutions.

1x PAN-medium with additions, 1L

10x PAN-medium stock solution	100 mL
10 % Struktol J-673	20 mL
dH ₂ O	735 mL ^a I 755 mL ^b
Add components to the vessel and s cooling, add the following heat-labil using a syringe filter.	terilize by autoclaving. After e components through a feed tube
Magnesium-sulfate stock solution	3 mL
50 % glucose solution	40 mL ^a l 20 mL ^b
Thiamine stock solution	1 mL
PAN trace elements solution	1 mL

^a: Fed-batch process with 20 g/L initial glucose concentration (exponential feed)

^b: Fed-batch process with 10 g/L initial glucose concentration (linear ramp-up feed)

The volumes of the medium components add up to 950 mL. The final working volume is reached with the addition of the inoculum (10 % of the working volume).

Feed medium

The feed medium consists of a 60 % glucose solution, PAN trace elements, magnesium sulfate, and thiamine solution.

Feed solution

$Glucose \cdot 1 H_2 0$	660 g/L	CAS 77938-63-7
Dissolve in dH ₂ O. Sterilize by autoc	laving. After	cooling, add the fol-
lowing components aseptically.		
PAN trace elements solution	2 mL/L	-
Thiamine stock solution	10 mL/L	-
2 M magnesium sulfate stock	00 ml /l	
solution	90 IIIL/L	-
Storilize by filtration		

Sterilize by filtration.

Bioprocess system and process control

The following describes *E. coli* fermentation using the SciVario twin bioreactor control system equipped with DASGIP[®] glass bioreactors (Figure 1). The main bioreactor components are described in Table 1. The bioreactor configuration is illustrated in the appendix (Figure 10).

Table 1: System components required for the setup of the process

Function	Component		
Bioreactor control system	SciVario twin		
Bioreactor	Glass bioreactor (order number: 76SR10000DLS) Working volume: 0.4 L – 1.5 L		
Temperature control, cooling and heating	Temperature control well		
pH monitoring	pH sensor (polarographic, analog)		
DO monitoring	DO sensor (Clark sensor, analog)		
Temperature monitoring	Platinum RTD temperature sensor (Pt100)		
Anti-foam control	Level sensor		
Agitation	Overhead drive MD40 (100 – 1,600 rpm)		
Impeller	Two Rushton-type impellers		
Baffles	Baffle cage with four baffels		
Gassing	Macro-L-sparger		
Sampling	Sampling tube with valve		
Pump head tubing	Bioprene tubing; inner diameter 0.5 mm		
Feed lines	PTFE feed lines; inner diameter 0.8 mm		
Options for liquid	Short dip tube for anti-foam addition;		
addition	long dip tube for pH agents (acid, base)		
Exhaust cooling	Liquid condenser		



Fig. 1: SciVario twin bioreactor control system

Technical Features

The SciVario twin bioreactor control system can control two glass or single-use bioreactors, either individually or in parallel, across a wide range of vessel sizes from small- to bench-scale. It was developed for both microbial fermentation and cell culture applications.

For more technical information on the SciVario
 twin, please visit
 www.eppendorf.group/sci-vario

E. coli fed-batch fermentation

Batch fermentation is a process conducted in a closed system. At time t=0, the sterilized nutrient solution (supplemented with anti-foam agent) in the fermenter is inoculated with microorganisms and incubation can proceed at a suitable temperature and gaseous environment for a suitable period. During the entire fermentation, no medium or feed solution is added.

A fed-batch fermentation starts with a small amount of medium inside the fermenter. It is characterized by the addition of a defined amount of concentrated medium during the process. The addition starts with a trigger point of the process (e.g. the *E. coli* glucose depletion peak as defined in this study) or after a certain time point. These substances continue to be added during the fermentation process.

Recommended controller settings

To maintain optimal growth conditions during fermentation, the SciVario twin's built-in bioprocess controller provides integrated online control of temperature, dissolved oxygen concentration, gas flow, agitation, pH, as well as feeding. However, optional integration with DASware[®] control enables more sophisticated possibilities for process automation. Examples are the DO spike-based automatic feeding start or an agitation-based triggered feed start. The SciVario twin has standard parameters programmed in its system for the standard control loops such as temperature, pH and DO. Control parameters and setpoints used in this study are summarized in Table 2.

 Table 2: Control parameters and setpoints for the fed-batch

 bioprocess

Fed-batch volume		Process start	0.5 L	
Fed-batch volume		Process end	1 L	
		Temperature setpoint	37 °C	
lemperature	control	Proportional value	80 %	
		Integral value	0.2 %/h	
		pH setpoint	7.4 (dead band 0.05)	
		Proportional value	85 %	
		Integral value	4080 %/h	
		Deadband	0.05	
		Controller out min	-100 %	
nH control		Controller out max	100 %	
pricontion		Acid	25 % phosphoric acid	
		Set maximum flow of acid pump	40 mL/h	
		Base	25 % ammonia	
		Set maximum flow of base pump	40 mL/h	
		DO setpoint	50 %	
		Proportional value	0.4 %	
Dissolved ox	ygen	Integral value	19.2 %/h	
control		Deadband	0 %	
		Controller out min	0 %	
		Controller out max	100 %	
Inactive until:		Input level signal	35 μs	
		Anti-foam pump	0 mL/h	
Foam	Active from:	Input level signal	35.01 μs	
CONTROL		Anti-foam pump	40 mL/h	
		Sensing time	1 s	
		Pause time	2 s	
		Anti-foam dosage	Headspace	

DO control

The DO was controlled at 50 % using a DO cascade (Figure 2, Table 3).

Compared to a previous study [1] the dissolved oxygen setpoint was increased from 30 % to 50 % to guarantee sufficient O_2 supply till the end of the cultivation. Together with the DO setpoint, the DO cascade (Figure 2, Table 3) was slightly changed in comparison to Application Note 433 [1]. Here, the main differences were the reduction of the maximum stirring speed to 1,500 rpm (compared to 1,600 rpm) and adoption of a maximum of 46 % O_2 in the inlet gas stream (compared to 100 %), while maintaining the maximum gas flow using 120 sL/h. Smaller changes were also made in the DO cascade distribution of the actuators. Therefore, the rates of the increase in the different actuators outputs could be smoothed out.



Table 3: DO cascade and feeding for the fed-batch bioprocess

		Controller output		Actua	tor output
	Acitation	X1	0 %	Y1	500 rpm
	Agitation	X2	50 %	Y2	1,500 rpm
DO cas-	Gassing	X1	50 %	Y1	36 sL/h
cade	rate	X2	75 %	Y2	120 sL/h
-	NOC	X1	75 %	Y1	21 %
	XU2 _{in}	X2	100 %	Y2	46 %
Feed	Feed control	Script-controlled after reaching glucose depletion or script-controlled after reaching 7 hour fermentation time			
	Feed pump [mL/h]	Script-controlled using a combination of exponential feed phase and steps or script-controlled using a linear ramp-up feed			

pH control

The SciVario twin offers the possibility to establish a twosided pH control. We used 25 % ammonia and 25 %phosphoric acid solutions to adjust the pH for the 1 L cultures.

Compared to a previous study [1] the pH setpoint was increased from pH 7.0 to pH 7.4 according to Pinhal et al. [4] where the inhibition of *E. coli* growth was examined as a function of different acetate concentrations and pH. Here, pH 7.4 was clearly shown to decrease the inhibitory effect of acetate compared to lower pH 6.4.

Anti-foam control

The SciVario twin offers the possibility to establish an anti-foam control. We used 10 % anti-foam solution using Struktol® J-673.

Struktol J-673	50 g
dH ₂ O	450 mL
Sterilize by autoclaving.	

Feed medium and feed strategy

The feed media were optimized in comparison to the feed medium introduced in our Application Note 433 [1] by increasing the thiamine amount and by adding an additional component, magnesium sulfate, as in a previous study [2]. Addition of magnesium sulfate to the feed medium was previously described as factor to achieve high cell densities [3].

The feed strategy was also optimized, taking into account adjustments to the feed medium and change of process setpoints. In this study we used two different approaches for comparison, in order to achieve an optical density of around 300.

The first approach was to use an exponential feed phase after the batch phase. This was initiated after a drop in the agitation speed that results from an increase in the DO, when the carbon source of the first phase is depleted. After the exponential feed we initiated a short break of an hour duration to give the *E.coli* time to consume the excess glucose, before we initiated a stepwise increase of the glucose feed (Figure 3). This approach was programmed using a script with DASware control (see Appendix).

In the second approach, a linear ramp-up feed was used (Figure 4). This can either be operated through the script via DASware control (see Appendix), or a simple feed profile table. Using this approach, since we wanted the feed profile as simple as possible, we did not want to wait for depletion of the carbohydrate source but instead started the linear feed ramp after 7 h following inoculation until 24 h of fermentation time. The feed rate was adjusted to avoid overfeeding, based on the results of previous studies.







Fig. 4: Resulting linear feed ramp-up based on the script described in the Appendix

Preculture and inoculation

To generate enough biomass for the inoculation of the bioreactor, we prepared the preculture using shake flasks. To prepare the initial preculture, 25 mL of sterile LB medium had to be filled into a glass or single-use shake flask with a total volume of 500 mL. We recommend using flasks with baffles. Then, we inoculate the shake flask with *E. coli* K12 from one cryovial [5] and incubated the preculture overnight, at 37 °C and 200 rpm using an Innova® S44i shaker.

For preparation of the inoculum, we filled 100 mL of

sterile LB medium into a shake flask with a total volume of 1 L in order to expand the initial preculture. Here, we also recommend using flasks with baffles. We then inoculated the 1 L flask with 5 mL of the preculture, and incubated for approximately 7 hours, at 37 °C and 200 rpm (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 to inoculate 2 L of main culture.

We transferred the final inoculum culture to a sterile beaker to expedite drawing the culture into a syringe. Precultures were pooled in case we prepared several shake flasks of precultures.

We inoculated the main culture to an OD_{600} of ca. 0.5 using an inoculum culture with an OD_{600} of 6 – 8, corresponding to to 5 % to 10 % 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.

Optical density measurement

We measured the optical density of the culture using a photometer. 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.

Results

To validate the suitability of the controller settings, we recorded process values and controller output of pH, temperature, and DO in the microbial runs. Additionally, we analyzed the OD₆₀₀ of the culture offline.

Bacterial growth and glucose addition

For the fed-batch process a glucose feed with supplementations was used as indicated in the materials section. In contrast to our previous study [1] the feed protocol was changed to a combination of exponential feed followed by a feed pause followed by a stepwise increase of the feed (Figure 3).

The fed-batch run using the SciVario twin together with the DASware control 6 and the exponential feed resulted in a maximum OD_{600} of 320 (Fig. 5). For the alternative approach, using the linear ramp-up feed, we achieved a maximum OD_{600} of 285. For the process using the exponential feed, 213 g (356 mL) glucose were added until reaching the OD peak and 222 g (371 mL) until reaching the process end. For the process using the linear ramp-up feed, the cells were fed ca. 204 g (340 mL) glucose until the OD peak was reached and 229 g (383 mL) until the end of the process, respectively.

In the following, we review data from the exponential feed-based process.





DO control and feed trigger

We carried out the DO control strategy as planned for the fed-batch processes; the results are shown in Figure 6 and 7. As result, the growth of the *E. coli* microorganism can be indirectly seen as anticipated by the behavior of the DO-cascaded actuators. Until reaching a DO controller output of 50 % DO the stirring speed increases for enough dispersion of gas bubbles. After the maximum of the stirring speed was

reached, the gas flow of air increased. After the gas flow reached its maximum, the concentration of oxygen increased as expected. After the end of the batch phase all actuators decreased, and the DO signal increased.

The data presented in Figure 7 also display results of a script-based, agitation-triggered fed-batch experiment with the SciVario twin and DASware control. The script is presented in the appendix. To start the feed, we used an agitation-triggered approach instead of a DO-triggered approach [1]. This decision was based on the fact that in some cases the DO controller output is unstable. In this case here, after the agitation signal increases to an upper threshold of 850 rpm (high trigger threshold) and reaches a lower threshold of 650 rpm (low trigger threshold) the feed script was started. This behaviour of the agitation signal



Fig. 6: DO control of the *E. coli* fed-batch bioprocess. Dissolved oxygen process value (DO.PV) and dissolved oxygen controller output value (DO.out) are shown.



Fig. 7: Dissolved oxygen controller actuator output – stirring speed (N.PV), oxygen concentration (XO2.PV), gas flow rate (F.PV (Gas)) and Medium Feed rate (F.PV (Feed)).

indicated the end of the batch phase when all glucose was consumed. The output of the actuators (stirring speed, oygen concentration, and gas flow rate) increased again, indicating a further growth stimulated by the new bolus of glucose. As we monitored the down-regulation of the actuators when the feed break was initiated, the step required great care, in order not to fully deplete the glucose source. The break step and reinitiation of the glucose feed represent a possible starting point for optimization. For example, after the exponential feed one can directly feed at a constant rate or with a linearly increasing feed under script control.

Temperature control

The temperature control can be manipulated within an extremely narrow range around the setpoint (Fig. 8). The deviation using the temperature control block for the 1 L vessel led to a deviation of 0.04 K around the setpoint of 37 °C with a range between the minimum and maximum value of about 0.5K.

pH control

Typically, the pH of an *E. coli* fermentation producing acetic acid can be controlled via a one-sided pH control using base. However, we used a two-sided pH control in the fed-batch fermentation process, as we described it before [1].

In contrast to our previous study, we used a pH deadband of 0.05. This resulted in a spiking behavior of the pH signal (Fig. 9 A) that differed from the displayed value in the Application Note 433 [1]. In Figure 9 B higher activity of the pumps is noted with developing process times. This indicates a phase of high growth performance of the *E. coli*.



Fig. 8: Temperature control of the E.coli fed-batch fermentation. Temperature process value (T. PV), setpoint (T.SP), and controller output (T.Out) are shown.

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Conclusion

In this application note, we described an improved method for a high cell density fed-batch fermentation protocol using *E. coli* as a reference organism. The results of the process described in this and in our previous study [1] are summarized in Table 4.

Table 4: Process comparison

Parameter	Process described in this study	Process described previously [1]
Cultivation time until OD ₆₀₀ peak	26 h	40 h
Maximum OD ₆₀₀	320	105
Feeding strategy	exponential	constant
Glucose added until reaching maximum OD ₆₀₀	213 g	180 g

We achieved very high biomass levels with OD_{600} value peaked around 320, more than doubled the biomass as seen

in the previous fed-batch culture. It should be noted, however, that the optical density does not allow a direct conclusion on the cell viability. Furthermore, with the fedbatch process presented here, we achieved the results within a lower process time leading to a process of higher effectivity and efficiency.

The main differences between the two processes were the addition of magnesium sulfate and the increased thiamine amount in the feed medium. Furthermore, the process pesented here used an exponential feed (results are shown in Table 4) and a linear ramp-up feed, respectively, whereas the previous process used a constant feed. Slight adaptions included increasing the DO setpoint to 50 % and the pH setpoint to 7.4. Furthermore, the DO-cascade was evened out.

DASware control software was integrated, enabling a more sophisticated process automation control – with agitationbased feed start, after glucose depletion, of an exponential feed combined with constantly increased feeding steps.

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Appendix



Fig. 10: Bioreactor configuration of the 1 L microbial bioreactor 76SR10000DLS.

The arrangement of the equipment options in the head plate is flexible. Please refer to the DASGIP Bioreactors user manual for more information.

	Port	Port accessory	Associated device	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	DO monitoring
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	Acid addition Base addition Anti-foam 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 exhaut gas cooling
5	Pg 13.5	Triple port position 1 Triple port position 2 Triple port position 3	Long dip tube Long dip tube Short dip tube	Sample port 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 and baffles	Motor MD30 or MD40 and baffle cage	Agitation and baffle
9	M6	Thermowell	Platinum RTD temperature sensor (Pt100)	Temperature monitoring

Exponential feed script

The following visual basic script provides the opportunity to start the feeding based on the decrease in agitation when the DO signal increases due to depleted carbon source – which occurs during the end of the batch phase. Other triggers can be used to initiate the feed phase.

As indicated in the discussion of the results, this script is suggested process optimization protocol. It can, of course, be varied according to the needs of the investigator. For instance, the break phase can be eliminated and the protocol can move directly to the constant feed step.

Copy and paste the following script into the DASware control as vessel script. As noted in the script, the pump that is referenced in the DASware control section as Pump D was used as a feed pump.

'Script parameters Dim startdelay h '[h] delay after start inoculation time as double = 1/60Dim HighNPVTrg as double = 850 'high NPV trigger level Dim lowNPVTrg as double = 650 'low NPV trigger level Dim ExpoFeedDuration_h as double = 8 '[h] expo. feed duration Dim ConstFeedDuration as double = 3 '[h] const feed duration ' Exponential Feed parameters ' multiply by 1000 for L to mL, unit of flow rate (SP is in ml/hr) ' Feed Equation ' Feed = 1000 * (mu * CFW * V * exp(mu*(t h+1)) / (Con * YXS) 'Definition of internal variables Dim Con as Double = 600 'Feed bottle glucose concentration [g/L] Dim mu as Double = 0.18 'growth rate [1/h] Dim V as Double = 0.5'Total volume [liters] Dim YXS as Double = 0.8'Yield coefficient [g/g] as Double = 45.0 'CFW [g/L] Dim WCW 'Feed rate [mL/h] Dim FeedSP as Double = 0.0Dim t_h as double 'time delay [h] Dim NPV as double 'Stirring speed [rpm] if P is Not Nothing Then With P NPV = .NPVSelect Case .phase Case 0 'Init .LogMessage("Entering phase " & .phase & ": Wait for inoculation timer > " & format(startdelay_h,"#.##") & " [h]") .phase = .phase + 1Case 1 'start delay if .inoculationtime_h > startdelay_h then .phase = .phase + 1 .LogMessage("Entering phase " & .phase & ": Wait for high agitation trigger N.PV > " & HighNPVTrg & " [rpm]") end if

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```
Case 2 'high NPV TRG
    if NPV > highNPVTrg then
      .phase = .phase + 1
     .LogMessage("Entering phase " & .phase & ": Wait for low agitation trigger N.PV > " & lowNPVTrg & " [rpm]")
    End If
   Case 3 'low NPV trigger
    if NPV < lowNPVTrg then
      .phase = .phase + 1
      .LogMessage("Entering phase " & .phase & ": Start exponential feed; feed duration = " & format(ExpoFeedDuration
h,"#.##") & " [h]")
    End If
   Case 4 'exponential feed phase
    t_h = .Runtime_h - .phaseStart_H +1
    ' multiply by 1000 for L to mL, unit of flow rate (SP is in ml/hr
    FeedSP = 1000 * (mu * CFW * V * exp(mu*t_h)) / (Con * YXS)
    if t h > ExpoFeedDuration_h then
     .phase = .phase + 1
      .LogMessage("Entering phase " & .phase & ": Start const. feed steps; feed duration = " & format(ConstFeedDurati
on,"#.##") & " [h]")
    end if
   case 5 ' feed break for 1 hour
    t_h = .Runtime_h - .phaseStart_H
    FeedSP = 0
    if t h > 1 then
     .phase = .phase + 1
     .LogMessage("Entering phase " & .phase & ": Constant feed 1")
    end if
   case 6 ' const feed phase 1
    t_h = .Runtime_h - .phaseStart_H
    FeedSP = 20
    if t_h > ConstFeedDuration then
     .phase = .phase + 1
     .LogMessage("Entering phase " & .phase & ": Constant feed 2")
     end if
   case 7 ' const feed phase 2
    t_h = .Runtime_h - .phaseStart_H
    FeedSP = 25
    if t_h > ConstFeedDuration then
      .phase = .phase + 1
     .LogMessage("Entering phase " & .phase & ": Constant feed 3")
     end if
```

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```
t_h = .Runtime_h - .phaseStart_H
FeedSP = 30
if t_h > ConstFeedDuration then
.phase = .phase + 1
.LogMessage("Entering phase " & .phase & ": Stop")
end if
case 9 ' stop feed
FeedSP = 0
End Select
'Select correct feed pump
.FDSP = FeedSP
```

end With end if

Simple linear feed script

'Script

```
If p.InoculationTime_H > 7 and p.InoculationTime_H < 24
    p.PumpDActive = true
    p.FDSP = 1.1293 * p.InoculationTime_H + 5.9389 'linear ramp-up
End if
If p.InoculationTime_H > 24
p.FDSP = 0
End if
```

Literature

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Ordering information

Description	Order no.
SciVario® twin Fermenter/Bioreactor Control System, base unit for 2 vessels	7600 100 001
DASGIP® vessel, 400 mL – 1.5 L, L-sparger, overhead drive, 2 Rushton-type impellers	76SR10000DLS
DASware [®] control, including PC, OS, and licenses	78600188

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