Technical Note

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E. coli fed-batch cultivation using the BioLector® Pro

Introduction

The BioLector® Pro device combines the scalable BioLector® technology of monitoring biomass, pH, dissolved oxygen concentration (DO) and diverse fluorescence with a microfluidic chip. The system is based on standard ANSI (SBS) microtiter plate (MTP) format and operates with non-invasive optical sensors. It is ideally suited for aerobic cultures with high cell density due to the continuous shaking during the optical measurements throughout the entire cultivation time. The micro valves and micro channels on the microfluidic chip enable pH control and fed-batch cultivation in 32 parallel experiments. Four different feeding strategies are available: constant, linear, exponential feed or a signal triggered pulsed feed are applicable for each individual cultivation well. The massive acquisition of relevant bioprocess data allows precise and reliable bioprocess development, strain characterization, media optimization and clone screening in short time frames.

This technical note gives an overall understanding of the use of the BioLector® Pro on the example of a standard *Escherichia coli* fed-batch cultivation experiment with one-sided pH-control. Standard experiment conditions are indicated and the standard protocol settings of the BioLection software are shown. Furthermore, the data analysis of the experiment is presented.

Cultivation in the microfluidic FlowerPlate® format

For fed-batch and pH controlled cultivation experiments the microfluidic microtiter plate in the FlowerPlate® format (see figure 1) is the MTP of choice. It is equipped with flower shaped wells which perform like baffles in a shake flask to ensure better oxygen transfer into the culture broth. A schematic illustration of the microfluidic channels is given in figure 2.

Figure 1: Microfluidic FlowerPlate®

Rows A and B represent the reservoir wells that can be filled either with feeding solutions or the pH adjusting agents. The wells from row C to row F are the cultivation wells which are filled with the culture broth. In total, 32 cultivation wells and 16 reservoir wells are placed on one MTP. The microfluidic channel system connects the reservoir well A or B with the four cultivation wells located below.

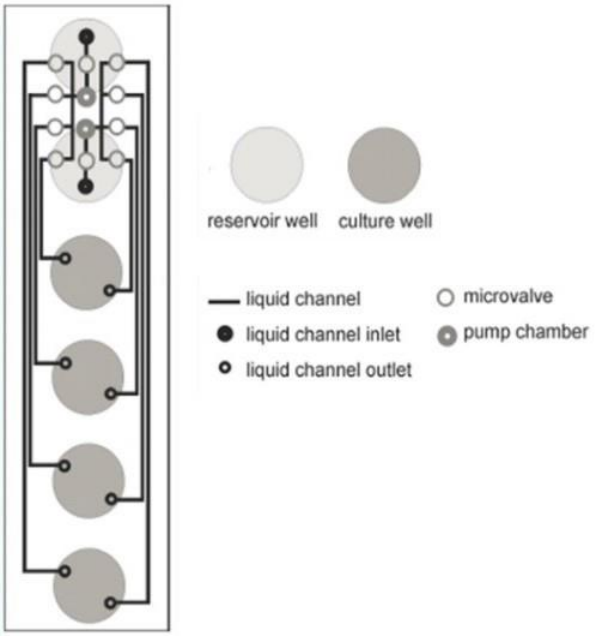

Figure 2: Schematic illustration of the microfluidic channels of the microfluidic FlowerPlate®

The minimal pump volume is 120 nL. Hence, a maximum feed rate of 80 µL/h is feasible. Three different types of the microfluidic FlowerPlate® are available and depending on the application it is a choice between three different types: The *MTP-MF32-BOH1* suitable for the use of colourless

media equipped with the standard optodes HP8 (pH) and Pst3 (DO).The *MTP-MF32-BOH2* type equipped with LG1 optodes for pH measurements in a range between pH 4.8 and 7.2 and RF optodes for the DO measurement in a higher wavelength range in order to reduce the background noise of coloured media. The *MTP-MF32-BOH3* type for applications in lower pH ranges (pH 4-6).

For the *E. coli* cultivation experiment described here the MTP type *MTP-MF32-BOH1* is chosen since it is ideally suited for the standard *E. coli* fed-batch cultivation conditions in colourless media. It comes with two kinds of optodes, located in each well on the bottom of the MTP. The HP8 optodes are designed for the optical online monitoring of the pH in the range of pH 5.0 and 7.0 and the PSt3 optodes for the optical measurement of the DO. The *MTP-MF32-BOH2* can be chosen alternatively. However, in this technical note we focus on the use of the BOH1 type. An overview of the technical properties can be found in the technical data sheet *TS-020_Microfluidic-FlowerPlate®.pdf*.

Cultivation conditions and Software settings

The standard cultivation conditions for an one-sided pH controlled *E. coli* fed-batch cultivation are described in table 1. In the following text, experiment instruction and media preparation are also explained. A preculture of *E. coli* BL21 wild type in terrific broth (TB) medium needs to be prepared. For this, inoculate 25 mL fresh TB media with an *E. coli* BL21 wild type cryo culture and set the initial optical density (OD_{start}) to 0.1. Cultivate it at 37 °C in a 250 mL shake flask at 250 rpm for 6 h.

Prepare two master mixtures of the Wilms MOPS minimal medium with both 20 g/L glucose and 10 g/L glycerol for the main culture in the BioLector® Pro. The OD_{start} should be set to 0.1.

A glycerol and a glucose feed solution are prepared. For the glucose feed a mixture of 10 mL glucose solution (500 g/L glucose) and 2 mL of N-Source (250 g/L $(NH_4)_2SO_4$, 25 g/L NH_4Cl) is used. For the glycerol feed a mixture of 10 mL glycerol solution (500 g/L glycerol) and 2 mL of N-Source (250 g/L $(NH_4)_2SO_4$, 25 g/L NH_4Cl) needs to be prepared. 3 M NaOH is used for the pH adjusting agent (MTP row B).

Table 1: Cultivation conditions in the BioLector® Pro

Parameter	Condition
Cultivation system	*E. coli* BL21 wild type (OD_{start} = 0.1)
Media	Wilms MOPS buffered (50 mM)
Temperature	37 °C
MTP type	MTP-MF32-BOH1
Culture volume	800 – 1200 µL per well
Shaking frequency	1200 rpm with a 3 mm shaking diameter
Humidity control	75 $\%_{rH}$
pH-adjusting agent	3 M NaOH (control to pH = 7.0)
Feeding solutions	Glucose feed: 50 % Glucose + 16 % N-Source Glycerol feed: 50% glycerol + 16 % N-Source

Protocol settings in the BioLection Software

The BioLection is the software associated with the BioLector® Pro. The correct handling with the software version 3.17 is described below. Before getting started with the BioLector® Pro experiment the related layout and protocol settings must be created using this software. The software settings for the *E. coli* fed-batch experiment are explained using screenshots as examples.

First, ensure a working LAN connection between the BioLector® Pro and the computer by clicking on the BioLector® symbol in the upper right edge of the taskbar as marked in figure 3. Then, perform the synchronization step via clicking on the synchronization symbol. During this step, protocols will be transferred from the BioLection software to the BioLector® Pro and vice versa.

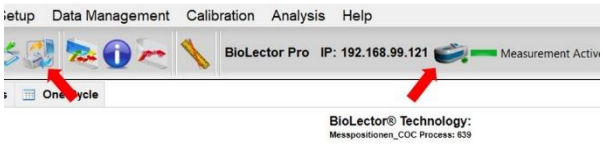

Figure 3: Location of the synchronization button in the BioLection main window

Next, update the calibration settings. Every single MTP comes with a lot. number for the optodes located on the well bottom of the MTP. Keep the calibration settings up to date to obtain the correctly calibrated online values for the pH and DO measurements. A click on the *Calibration* tab in the taskbar opens the *Calibration settings* window. Via *Get Calibration Files from Web* the most recent calibration files for the pH and DO optodes are loaded from the internet (see figure 4).

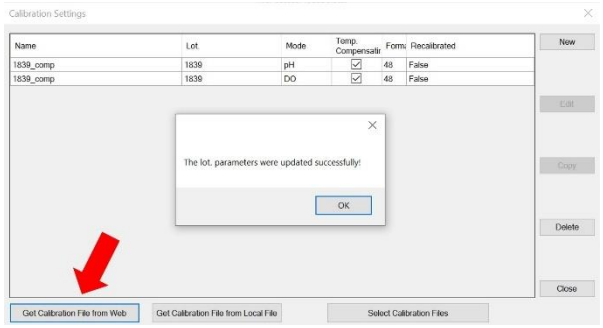

Figure 4: Calibration settings menu

Then, the required lot. numbers can be selected from the list by clicking on the “Create” tab next to the certain lot. number as shown in figure 5. Afterwards, close the *Calibration* settings menu.

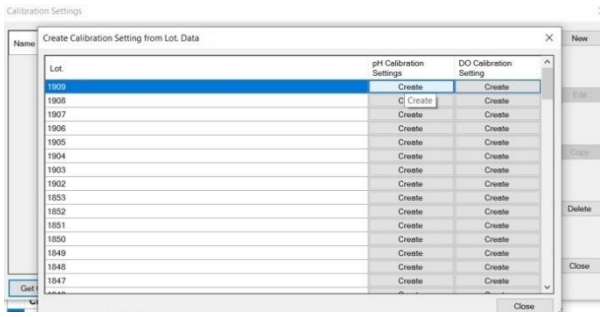

Figure 5: Create calibration settings from lot. data

The layout configuration (see figure 6) is the next step. Open the *Layouts* window via *Experiment Setup* and click on *New to* open the *Layout settings* menu. Select *microplate format 32* and a layout name. Determine the samples by marking all culture wells from C1 to F8 in the well map. The microfluidic MTP is a disposable product and it is not suitable for multiple usage. Therefore, it is recommended to use the whole plate in one experiment. Save this layout by clicking *OK*. A sample description can be created by clicking the *Sample Description* button below if desired.

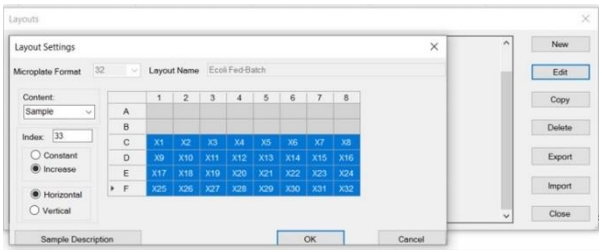

Figure 6: Layout settings

Now proceed to the protocol setting menu (shown in figure 7) by clicking on the *Experiment Setup* tab and *Protocols* and *New*.

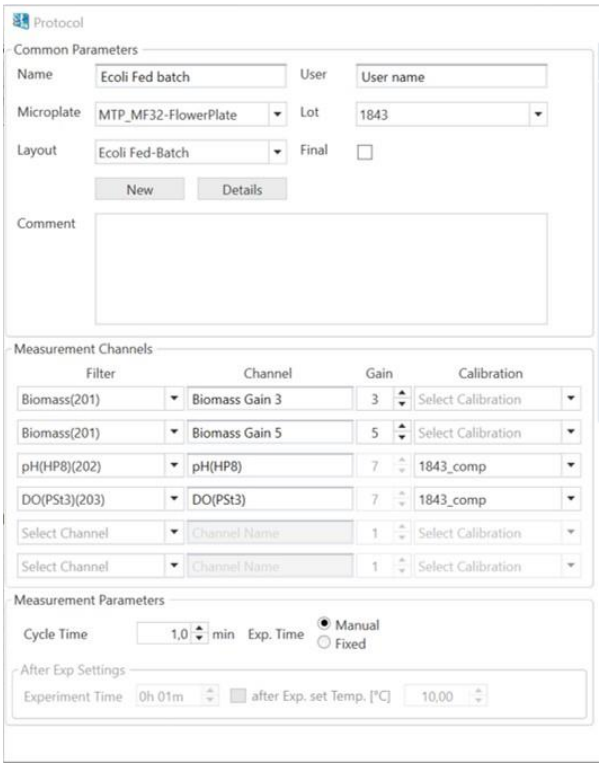

Figure 7: Protocol setup of common measurement parameters and measurement channels

A protocol contains mandatory information like MTP type, number and type of measurement channels and filter modules, cycle time, cultivation temperature, shaking frequency and additional,

optional information like the user name and comments.

For this fed-batch experiment setup choose *MTP_MF32-FlowerPlate* in the *Microplate* dropdown menu and the respective *Lot* number of the MTP. Load the *Layout* you created over the respective dropdown menu.

When configuring the measurement channels one and the same filter module can be selected more than once when different gains are defined for each channel. In general, the gain is the signal amplification of the photodiode. Low gain values indicate low sensitivity which is preferred for strong light signals (like high fluorescens signals or high optical densities in the stationary phase). High gain values increase the photodiode sensitivity and are recommended for low light signals. The gain range for biomass and fluorescence filters is between 1 and 13. To find out the right configuration for the used strain it is recommended to choose gain values between 3 and 5 for the biomass channel. The channel names must be renamed, for example to “biomass gain 3” and “biomass gain 5” (see figure 7). The gain settings for pH and DO filter modules are fixed and cannot be changed.

Next, choose a cycle time. The minimal required cycle time depends on the number of filter channels being used, on the number of cultivation wells being measured and on the shaking frequency. If less time is selected than is necessary, the device will automatically measure as fast as possible and thus ensures an optimal pH control. If less data points are needed a higher cycle time can be selected. In this case select one minute for the cycle time.

In the environment parameter settings (figure 8), the humidity control is enabled, the temperature is set to 37 °C and the shaking frequency is set to 1200 rpm.

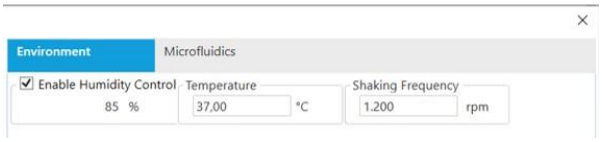

Figure 8: Protocol setup for environment parameters

Continue with the microfluidic settings menu (see figure 9) by clicking on *Microfluidics* and on *Settings* in the *Protocol* menu.

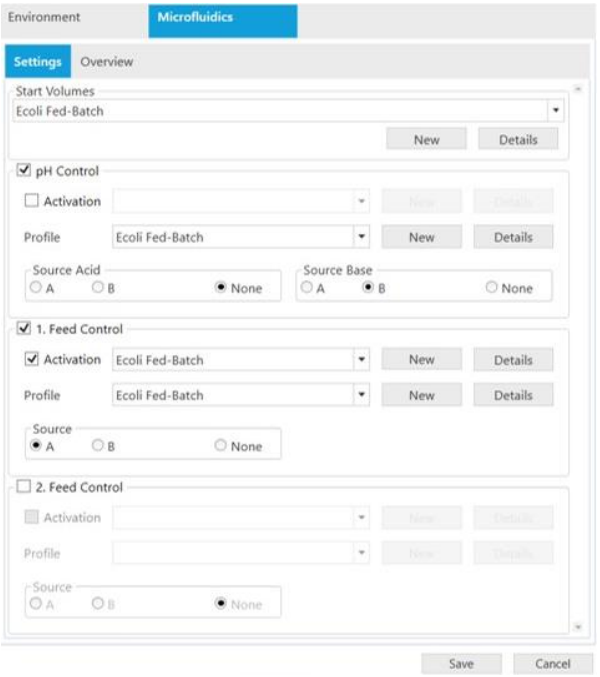

Figure 9: Microfluidic settings menu

In *Start volumes* the filling volume profiles can be defined. To open the *Fill Volume Profiles* menu (see figure 10), click on *New* in the *Start Volume* paragraph.

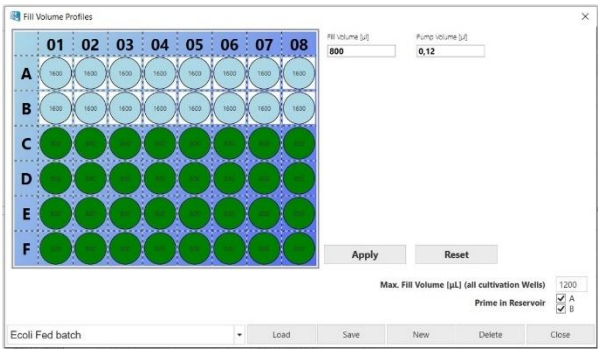

Figure 10: Fill Volume Profiles

Select 800 µL as the initial starting volume and 1200 µL for the maximum filling volume in the cultivation wells. For the reservoir wells choose 1600 µL as the start volume. The pump volume

(0.12 µL) should not be changed unless otherwise directed. Always set the filling volumes in the shaking frequency related specification range listed in table 2. You can find the information in the MTP-datasheet *TS-020_Microfluidic-FlowerPlate®.pdf*. Then select *Priming in Reservoir* A and B by ticking the checkboxes. During the priming process the microfluidic channels will be filled with the fluids from the reservoir right at the start of the experiment.

Table 2: Operating filling volume ranges in the microfluidic FlowerPlate®

Shaking frequency	Max. filling volume (rows A + B)	Max. filling volume (rows C – F)	Min. filling volume
800 rpm	1800 µL	1900 µL	800 µL
900 rpm	1800 µL	1700 µL	800 µL
1000 rpm	1800 µL	1500 µL	800 µL
1100 rpm	1800 µL	1500 µL	800 µL
1200 rpm	1800 µL	1400 µL	800 µL
1300 rpm	1800 µL	1200 µL	800 µL
1400 rpm	1800 µL	1000 µL	800 µL
1500 rpm	1800 µL	900 µL	800 µL

The pH control setup is located in the second section of the microfluidic settings (see figure 9). Tick the associated checkbox to activate the pH control. Choose reservoir B as source for the base by clicking bullet point *B.*

To open the *pH Profiles* menu (see figure 11) click *New* next to the profile dropdown menu. Then, select all wells in the well map and set the pH profile parameters to $t_0 = 1$ h and $pH_0 = 7$ and additionally activate the checkbox right next to it. The pH control should not be activated until at least 1 h after experiment start. This waiting time is important since the pH optodes in the MTP need to adapt to the media environment. To confirm the settings, click on *Apply* and save them by overwriting the current profile name. An activation trigger for each pH regulation is not necessary for this example experiment. But in general, for each pH regulation, an activation trigger can be chosen. For unlocking the *Activation Trigger* settings, tick the corresponding checkbox next to *Activation* in the pH Control menu box. From the drop-down menu next to *Activation*, you can select previously saved activation trigger profiles. When selected, you can view the respective trigger profiles via *Details*. To create new trigger profiles, select *New*. The *Activation Trigger* settings menu opens. A further detailed description of the activation trigger setup is written in the feed regulation settings below.

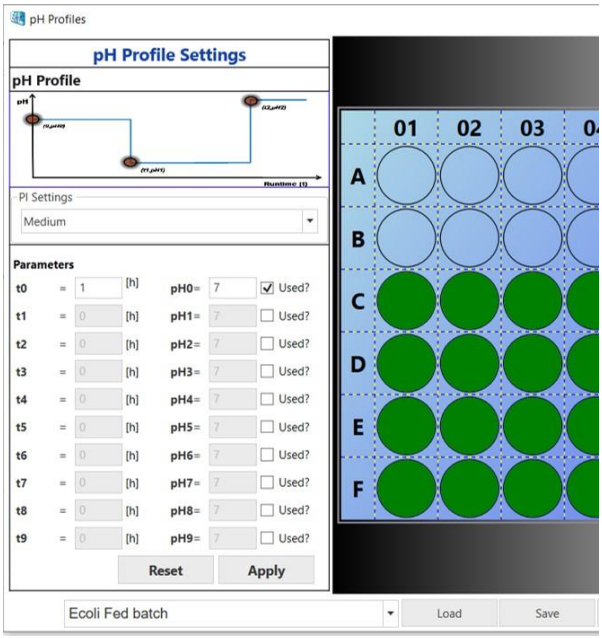

Figure 11: Configuration of the pH profile settings

To enable the feed control tick the corresponding checkbox in the *Feed Control* section of the *Microfluidic Settings* menu (see figure 9). The four different feeding strategies constant, linear, exponential and pulse feed are applied accordingly to the well map plan shown in figure 12. In the left half of the MTP (columns 1-4) the glycerol feeding takes place. The glucose feeding is applied in the right half of the MTP (columns 5-8).

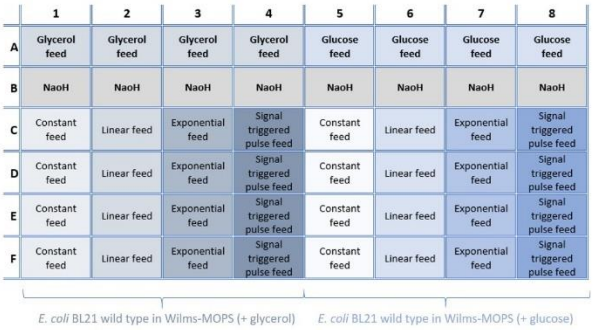

Figure 12: Well map description for the layout of feed strategies

The feed profile settings for our standard *E. coli* fed-batch experiment are described below. The described settings for the different feeding strategies are exemplary for the use of our *E. coli* BL21 wild type strain. So principally, you need to find out the right feeding settings for your strain.

Next, click on *New* next to the feed profile dropdown menu (figure 9) to open the *Feed Profile Settings* (see figure 13).

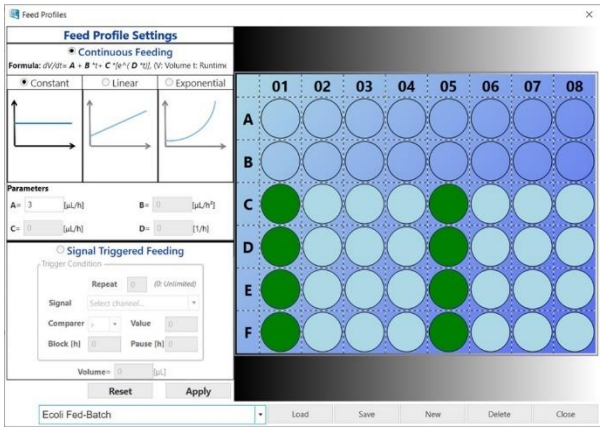

Figure 13: Feed profiles menu

In the right section of this window a well map is shown where the wells can be selected individually. Here it is possible to choose between the constant, linear and exponential feed profiles. Moreover, a signal triggered pulse feed profile can be selected.

The individual formulas to the respective feed profiles with the parameters A $[\mu L/h]$, B $[\mu L/h^2]$, C $[\mu L/h]$ and D $[1/h]$) are listed in table 3.

Table 3: Mathematic formulas of the feed strategies

Feed Strategy	Formula
Constant feed	$\frac{dV}{dt} = A$
Linear feed	$\frac{dV}{dt} = A + B \cdot t$
Exponential feed	$\frac{dV}{dt} = A + B \cdot t + C \cdot e^{D \cdot t}$

The constant feed strategy will take place in MTP columns 1 and 5. To set up this feed strategy, refer to the *Feed Profile Settings* window and mark column 1 and column 5 as shown in figure 13. Then select the constant feed related bullet point and choose 3 µL/h for parameter A as presented in figure 14. Afterwards, click on *Apply* to confirm.

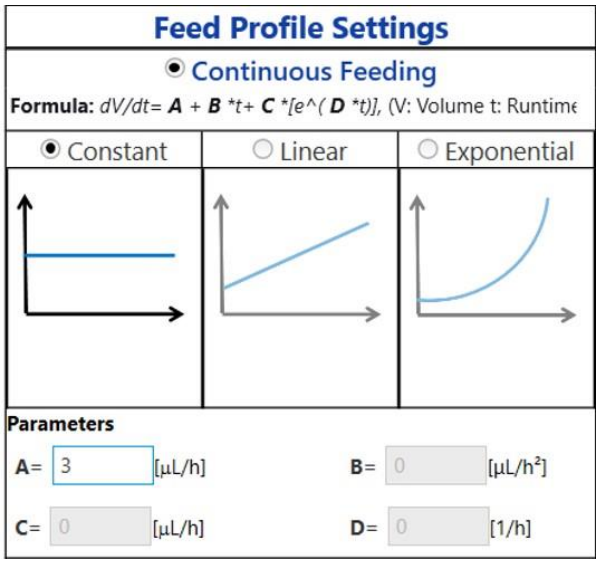

Figure 14: Constant feed profile settings

Then select column 2 and column 6 for the linear feed. Choose 3 µL/h for parameter A and 0.1 µL/h² for parameter B (see figure 15). Again, confirm with *Apply*.

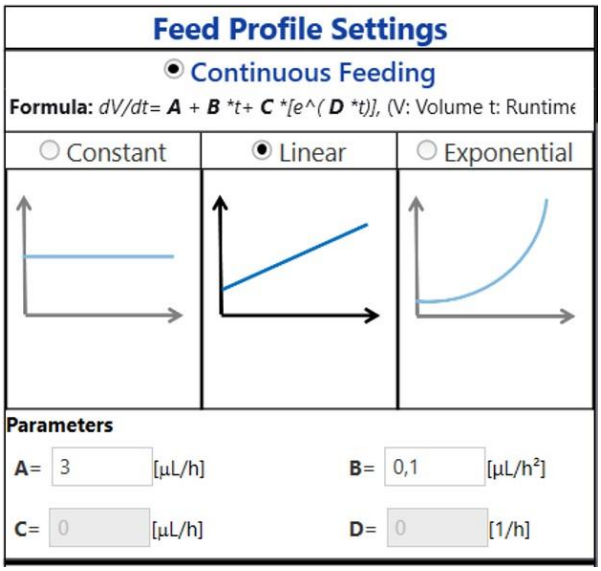

Figure 15: Linear feed profile settings

The exponential feed takes place in column 3 and column 7. The value for parameter C is set to 0,5 µL/h and for D = 0,1 h^{-1} (see figure 16).

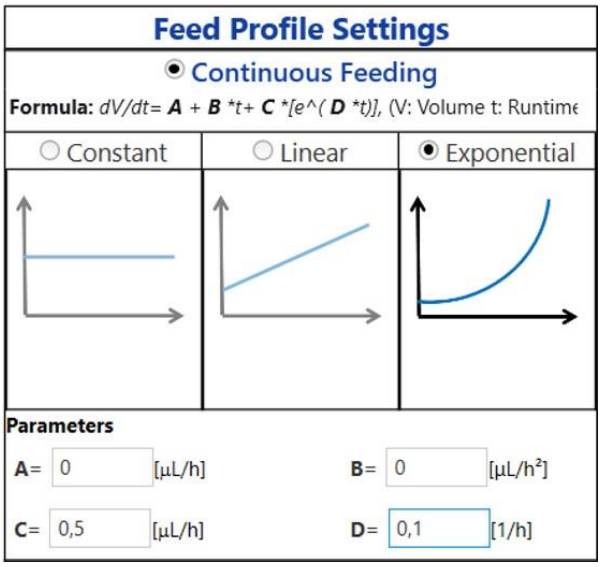

Figure 16: Exponential feed profile settings

Finally, the signal triggered feeding strategy is chosen for columns 4 and 8. In the *Signal* dropdown menu the calibrated DO (*Cali.DO*) channel is selected as the trigger signal. Select > 70 as the comparer to enable pulse feed when the DO value exceeds 70 %. The pulse volume is set to 4 µL as shown in figure 17.

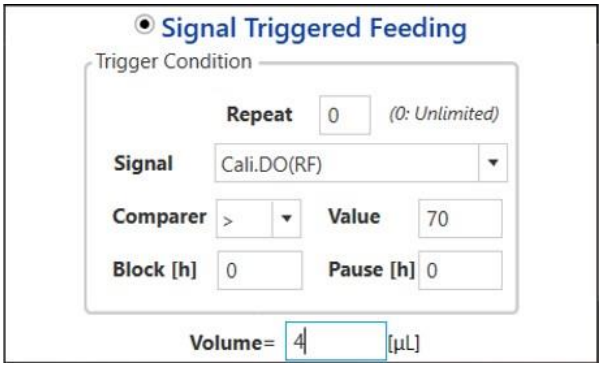

Figure 17: Signal triggered pulse feed

Since you usually do not want your feed to start right at the beginning of your experiment but when the batch phase is over, you should set an activation trigger. To set up the activation trigger profile click on *New* next to the *Activation* dropdown menu of the *Feed Control* section (see figure 9). The DO is chosen as activation trigger signal for the feed control. At the beginning of the cultivation the cell number is low and thus the DO in the culture broth is about 100 %. Due to the increase of the cell count more and more oxygen is consumed over the time. Consequently, the DO drops in the course of the cultivation. The DO increases back up towards 100 % again when the stationary phase is initiated.

For the constant, linear and exponential feed strategies two different start conditions are set up. The first start condition needs to be met before the second start condition will be applied. Also, activation trigger will only work once. After being activated the respective trigger condition will not be checked anymore. Here, in the first start condition the trigger signal *Cali.DO (PSt3)* is set to < 30 %. In the second start condition the trigger signal *Cali.DO (PSt3)* is set to > 70 % (figure 18). Hence, the feed starts as soon as the DO has dropped below 30 % and then increases above 70 % again.

Figure 18: Activation trigger settings for continuous feeding

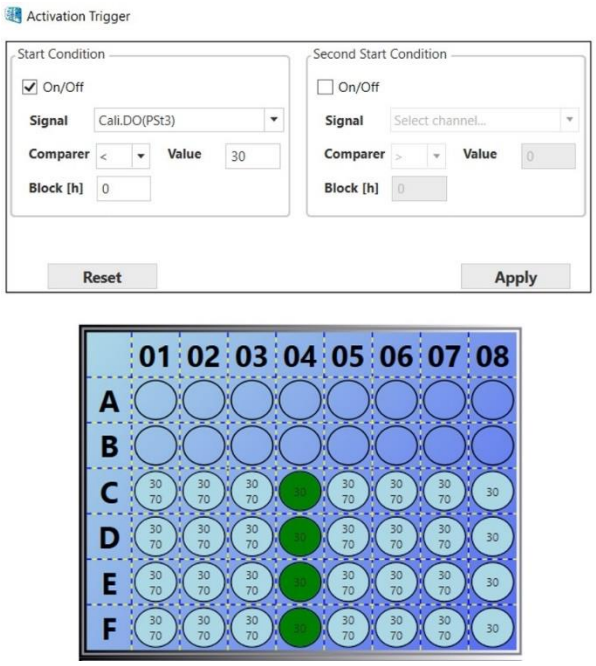

Figure 19: Activation trigger settings for signal triggered feeding

For the signal triggered feed pulse only the first start condition is activated and set to < 30 % DO as shown in figure 19. In the signal triggered feed options described before in figure 17, the feed start condition is already set to deliver a bolus addition at a DO > 70 %. Hence, a second start condition is not necessary. Here, it means that first the DO needs to drop below 30 % once for activation. The feed pulse will then always be initiated whenever the DO is read above 70 % during the cultivation process. After the start conditions are defined, press *Apply*. Finally, save the entire generated activation trigger profile under the respective profile name.

To activate the *Activation Trigger Profile,* tick the corresponding checkbox next to *Activation* in the *Feed Control* menu box. Now the microfluidic settings for the experiment protocol are complete. Go back to the microfluidic settings menu (see figure 9). Select reservoir A as feeding source by clicking the *tick box A* and save the entire protocol. Transfer the protocol to the BioLector® device by repeating the synchronization step as described in figure 3.

MTP inoculation and placement

Now, the MTP can be inoculated. The MTP must always be filled and sealed inside a biosafety cabinet to avoid contaminations. Inoculate the two prepared Wilms-MOPS media mixtures with the *E. coli* preculture to a start OD of 0.1 and transfer them to MTP rows C-F according to the well map presented in figure 20. Fill the reservoir wells in row A with 1600 µL feeding solution and in row B with 1600 µL NaOH (3M). The starting volume in the cultivation wells is 800 µL.

	1	2	3	4	5	6	7	8
A	Glycerol feed	Glycerol feed	Glycerol feed	Glycerol feed	Glucose feed	Glucose feed	Glucose feed	Glucose feed
B	3 M NaoH	3 M NaoH	3 M NaoH	3 M NaoH	3 M NaoH	3 M NaoH	3 M NaoH	3 M NaoH
C	WM-Glycerol	WM-Glycerol	WM-Glycerol	WM-Glycerol	WM-Glucose	WM-Glucose	WM-Glucose	WM-Glucose
D	WM-Glycerol	WM-Glycerol	WM-Glycerol	WM-Glycerol	WM-Glucose	WM-Glucose	WM-Glucose	WM-Glucose
E	WM-Glycerol	WM-Glycerol	WM-Glycerol	WM-Glycerol	WM-Glucose	WM-Glucose	WM-Glucose	WM-Glucose
F	WM-Glycerol	WM-Glycerol	WM-Glycerol	WM-Glycerol	WM-Glucose	WM-Glucose	WM-Glucose	WM-Glucose

Figure 20: Well map description for the media layout on the MTP

After the inoculation, attach the gas permeable F-GPRSMF32-1 cover to the MTP to maintain evaporation reduction, sterility and microfluidic functionality. In figure 21 the correct attachment of the foil to the MTP is shown.

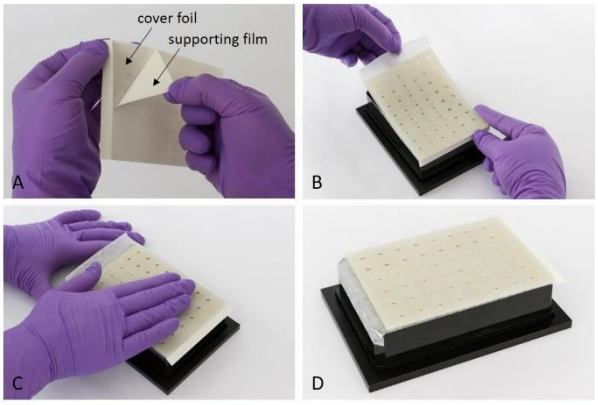

Figure 21: Attaching the F-GPRSMF32-1 foil to the MTP

First, remove the supporting film carefully without touching the sterile foil itself (see figure 21A). Then stick the foil with the silicone layer on top and align its right upper corner to the right upper corner of the microtiter plate (see figure 21B). Carefully smooth the foil over the whole MTP while making sure that every single well can be seen through the cover foil (see figure 21C). Make sure that the pressure holes are applied centrically above the reservoir wells. Remove the remaining supporting foil at the left end of the cover foil and fix the overlapping ends to the MTP (see figure 21D).

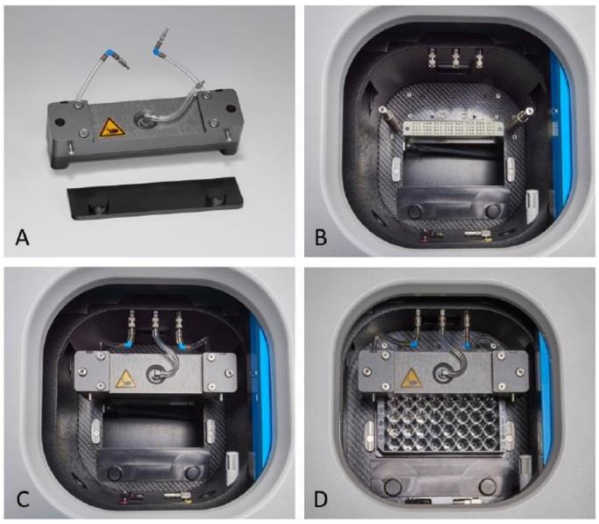

Figure 22: Placing of the MTP into the BioLector® Pro

Next, position the MTP into the incubation chamber of the BioLector® Pro. In figure 22A the MTP fixation and the positioning plate are shown. First, place the positioning plate onto the magnets at the front of the shaking table and attach the two springs to the vertical brackets (see figure 22B). Insert the MTP fixation by holding it horizontally and pushing it downwards onto the brackets (figure 22C). During this process, the two metal pins of the MTP fixation need to be pushed in. The correct positioning of the MTP fixation is ensured if afterwards green O-rings are visible on these metal pins and the MTP fixation is immovable. Finally, the MTP-fixation must be connected with the three colorless tubes to the three snap locks located in the back of the incubation chamber wall (figure 22D). You can read a more detailed description of the MTP fixation in the BioLector® Pro user manual in section 3.4.3. This user manual is available via support@m2p-labs.com.

Now, the prepared MTP can be placed into the BioLector® Pro incubation chamber. It will be fixed firmly by clicking on the *MTP-FIX* button on the BioLector® Pro HMI screen (see figure 23).

Clicking the *Select Protocol* button opens the protocol list. Here the protocol for the *E. coli* fed-batch cultivation can be chosen and the experiment can be started.

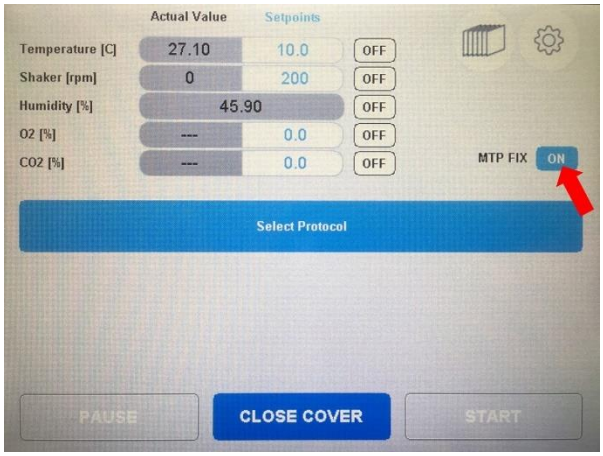

Figure 23: MTP FIX button in the BioLector® Pro HMI

Cultivation results and data analysis

In the *History Plot* tab of the BioLection Software the live data of the running experiment can be monitored. During the running experiment the produced data is stored automatically on the BioLector® Pro device in csv format. This raw data file can be transferred to the computer by clicking on the *Data Management* tab (see figure 24A). Also, already downloaded data can be loaded into the BioLection after the experiment is over via the *Load Data* button (see figure 24B).

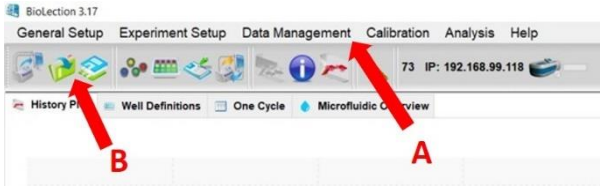

Figure 24: BioLection main window – Location of the Load Data button and the Data Management tab

The raw data csv file is only intended for using it with the BioLection. Of course, the loaded data can also be transformed for further data analysis into more user-friendly csv or Excel file formats. The transformation to the user-friendly csv file can be executed by clicking on the *Data Management* tab and then on the *Save as* button. To transform it to the Excel file format simply click on *Export to Excel*.

In the section *Chart Options* wells can be individually chosen in the well map (figure 25A). The filter channels are listed in the table *Channel* right next to the well map (figure 25B) To show the non-calibrated values and the pumped MF volumes activate the checkbox next to *Show Raw Data Channel* above the filter Channel list.

Additionally, *Parameters* like temperature, humidity and shaker frequency as well as the O_2 and CO_2 concentration in the head space, user comments and system events can be selected (see figure 25C).

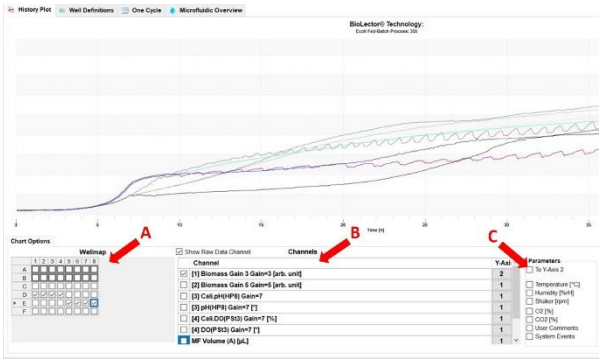

Figure 25: BioLection history plot - Chart Options

As example, the results of the fed-batch cultivation of *E. coli* BL21 wild type in Wilms-MOPS (with 10 g/L glycerol) performed in the BioLector® Pro are demonstrated in figure 26. Here the biomass curves and the related glycerol feed volume courses of different feeding strategies are shown in duplicates.

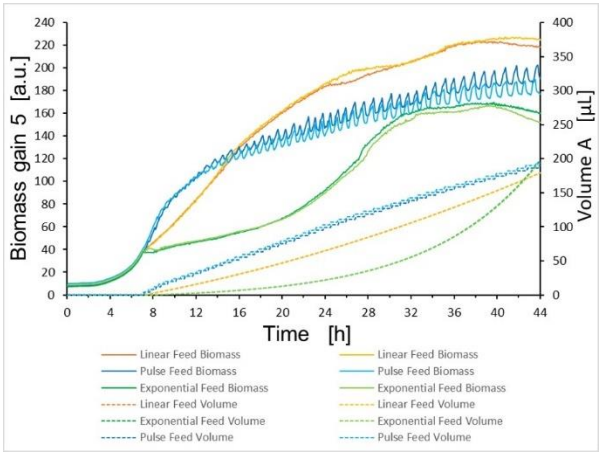

Figure 26: Fed-batch cultivations of *E. coli* BL21 wild type in Wilms-MOPS (with 10 g/L glycerol). The cultivations took place in a microfluidic FlowerPlate® (MTP-MF32-BOH1) with V_0 = 800 µL and n = 1200 rpm at 37 °C using the BioLector® Pro.

All replicates show the same growth behaviour up to the end of the batch phase at 7 h. With the

beginning of the stationary phase the DO-dependent glycerol feeds start. As mentioned before, the feed is activated when the DO values first drop below 30 % and then increase again above 70 %. After the batch phase the biomass growth behaves differently depending on the feeding strategy.

As an example, in figure 27 the DO and the pumped feed volume (Volume A) of a linear feed are plotted for the first 12 h of the cultivation. The linear feed $\left(\mathrm{dV/dt} = 3\frac{\mu L}{h} + 0{,}1\frac{\mu L}{h^2} \cdot t\right)$ starts as soon as the second trigger condition is met.

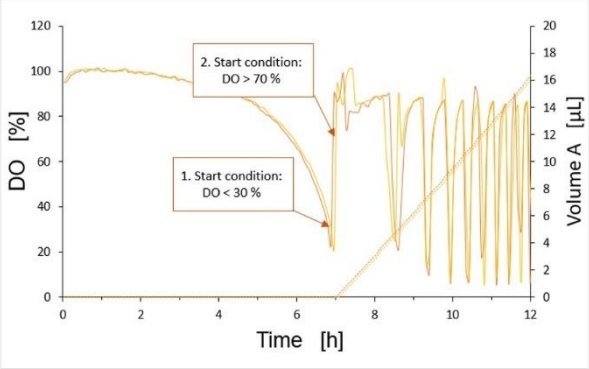

Figure 27: Fed-batch cultivation of *E. coli* BL21 wild type in Wilms-MOPS (contains 10 g/L glycerol) with linear glycerol feed . The cultivations took place in a microfluidic FlowerPlate® (MTP-MF32-BOH1) with V_0 = 800 µL and n = 1200 rpm at 37 °C using the BioLector® Pro.

The DO triggered feed pulse of 4 µL shown in Figure 28 is now analysed more in detail. In the upper chart the biomass signal is shown over 44 h. In the middle graph the volume of the pH adjusting agent (Volume B) and the corresponding pH course are plotted. The pH control was started after 1 h to adapt the pH value to pH 7. In the lower graph the history plot of the DO and the glycerol feed volume (Volume A) are presented.

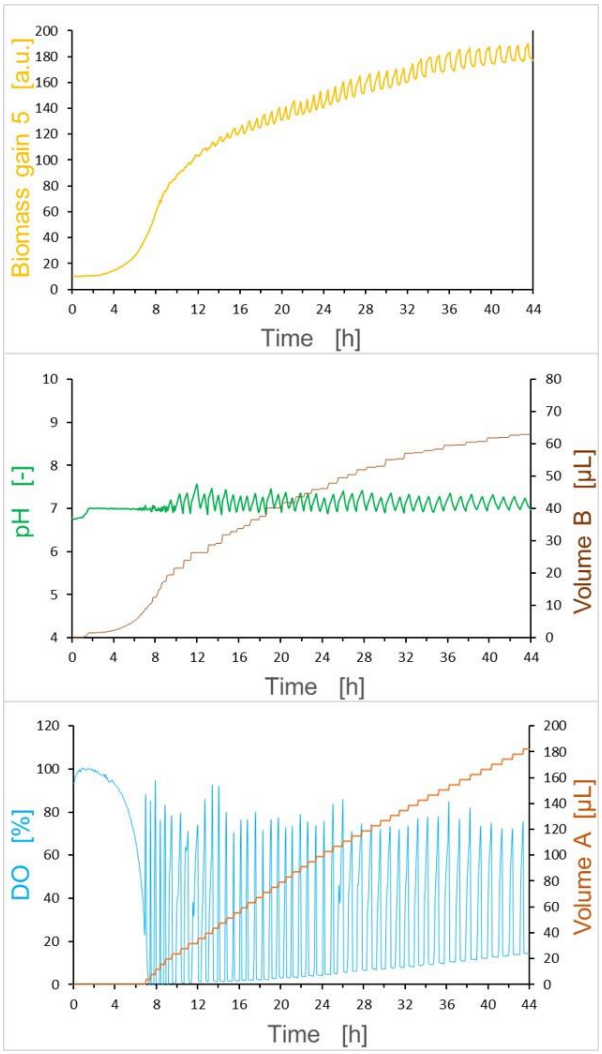

Figure 28: Fed-batch cultivation of *E. coli* BL21 wild type in Wilms-MOPS (contains 10 g/L glycerol) with a DO triggered glycerol feed. The cultivations took place in a microfluidic FlowerPlate® (MTP-MF32-BOH1) with V_0 = 800 µL and n = 1200 rpm at 37 °C using the BioLector® Pro.

In the batch phase at the beginning of the experiment it is shown that the DO drops, correlating with the exponential growth of the *E. coli* culture. The stationary phase is initiated at about 7 h, recognizable by a rapid increase of the DO due to a slower growth rate resulting in a lower or no oxygen consumption of the culture. Related to the DO value, the feed control is started and consequently the growth continues. As expected, when the DO drops below 30 % once, the feed pulse is continuously initiated whenever the DO increases above 70 %. Therefore, the feed curve is step-like increasing dependent on the DO fluctuations whose impact can also be seen in the biomass signal.

On account of the ongoing addition of glycerol and thus the further bacterial growth, the culture produces acetate. This leads to a growth dependent drop in the pH value. Hence the volume of the pH adjusting agent NaOH (Volume B) increases according to the growth and glycerol consumption related acetate production.
With the DO triggered pulse feeding strategy, strong fluctuations of the pH value can occur due to the high fluctuations of the DO. In order to reduce these pH fluctuations, it is recommended to choose a constant feed. Figure 29 shows the constant glycerol feed with 3 µL/h. Here we see that there are fewer fluctuations in the DO compared to DO triggered pulse feed, leading to smaller fluctuations in the pH value as well.

In conclusion, the BioLector® Pro is ideally suited for microbial fed-batch cultivation processes. Up to 32 pH controlled fed-batch cultivations can be performed in one experiment at the same time. Together with the user-friendly BioLection 3 software, data analysis is easy. Thus, the online parameter of different feeding strategies can be immediately compared during the experiment, *e.g.* the biomass signal, pH value, DO as well as the pumped volumes.

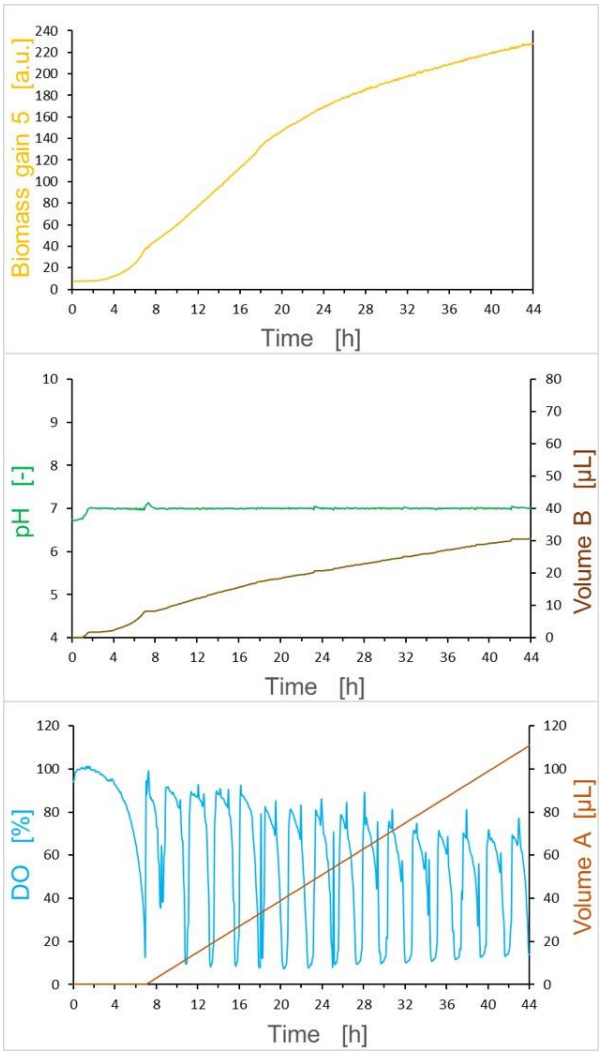

Figure 29: Fed-batch cultivation of *E. coli* BL21 wild type in Wilms-MOPS (contains 10 g/L glycerol) with constant glycerol feed. The cultivations took place in a microfluidic FlowerPlate® (MTP-MF32-BOH1) with V_0 = 800 µL and n = 1200 rpm at 37 °C using the BioLector® Pro.

Additional information

Please visit the website for further information: www.m2p-labs.com, www.beckman.com

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