How to set up a cultivation with pH and DO control in the SB10-X bioreactor

Kuhner AppNotes

by Kuhner Shaker

This application note provides a step by step guide through the entire set-up procedure of a new cultivation in the Kuhner SB10-X bioreactor. It summarises all important work steps and gives you helpful application tips.

For detailed information about the SB10-X and its Kuhner Insight application software please read these manuals:

Machine:
"User Manual OrbShake Bioreactor SB10-X" (Doc. No. 52409)

Software:
"User Manual Kuhner Insight" (Doc. No. 56725) and
"User Manual Kuhner Insight Add-ons for OrbShakes" (Doc. No. 69219)

1. Carefully unpack the single-use bag. Be sure you don't damage the single-use bag when opening the outer packaging.

> Do not discard the calibration parameters for the pH and DO sensors which are on the sticker attached to the outer packaging of the single-use bag.

2. If required, modify the bag according to yours needs (welding of additional exhaust filters or feeding lines, connecting medium bags or neoprene tubing, etc.). Regarding peripheral attachments (couplings) of the bag see the *User Manual OrbShake Bioreactor SB10-X (6.4 Connecting and filling the bag)*.

> All the bag tubing is thermoplastic, which means weldable! MPC connections need to be done at a sterile work bench.

3. Start the Kuhner Insight software. For detailed information about the software see the User Manual Kuhner Insight.

4. Install and inflate the bag according to the *User Manual OrbShake Bioreactor SB10-X (6.2 bag set-up)*. Make sure that all connected gas bottles are open, filled enough for the whole cultivation and that the inlet gas pressures are between 1.5-2.5 bar!

> SB10-X: With a max. flow rate of 2 L/min. it takes approx. 12 min. to fully inflate the bag. Use the time and enjoy a cup of coffee or tea (not in the lab of course).

5. Calibrate the required pumps as described in *User Manual Kuhner Insight Add-ons for OrbShakes, 4.9.2 Pump Calibration*. At least pump B for the addition of base must be calibrated!

6. Fill the bag with the minimum volume of medium (3L) as preparation for the sterile run. When filling the bag with medium make sure the exhaust gas line is opened to ensure pressure equalisation.

> Prior to filling push the harvest line clamp as close as possible to the bag entrance to avoid trapping medium and cells in the tube.

7. Set up the light protection jacket as described in the *User Manual OrbShake Bioreactor SB10-X, 6.5 Setting up the light protection jacket*, if you have a light sensitive medium components or product.

> If the cultivation time is >2 weeks, I recommend using the light protection jacket. Shading the optical pH sensor extends its life span.

8. Install the optical fibres (for pH/DO measurement) as described in the *User Manual OrbShake Bioreactor SB10-X, 6.7 Setting up DO and pH measurement*.

9. Make sure all tubing and optical fibres are arranged correctly and safely (see *User Manual OrbShake Bioreactor SB10-X, 5.4 Cable and tube management*).

AN1A

Kuhner shaker
www.kuhner.com

How to set up a cultivation with pH and DO control in the SB10-X bioreactor

Kuhner AppNotes

by Kuhner Shaker

[1] Decreasing the LED current extends the life span of the pH sensor. Starting with a LED current of 50 % is recommended.

[2] A quick test to check for bacterial contamination is to switch off the gassing and immediately close all bag tubing. Continue shaking. If the DO drops significantly (but some noise below 100 % is normal), that could be a sign of contamination.

[3] To control the pH, a liquid base, CO_2 AND air (or N_2 if air is not present) are required. To control the DO, O_2 (air or/and O_2 mix) AND N_2 are required. If you want to control the full range of pH and DO all 4 gases (and a base) are required. Note: to control the DO without N_2 is NOT possible.

10. Start a new file in the Kuhner Insight software. Choose a file length which you are certain is long enough for the whole cultivation incl. the sterile run. Some buffer time is recommended (for example: if you expect a cultivation time of 10 days choose the 2 week file length).

11. Define the properties in the Kuhner Insight software: Set a pH/DO sampling rate that allows for the cultivation time incl. the sterile run and some buffer time (the pH sensor has a life span of approx. 10,000 measurements with 100 % LED current). A sampling rate of 120 s is recommended. Check the amplitudes of the pH and DO sensor (must be > 500). For detailed information see *User Manual Kuhner Insight Add-ons for OrbShakes, 4.8 Sensor drift of optical sensors.* [1]

12. Carry out the sterile run for at least 12 hours (see *User Manual OrbShake Bioreactor SB10-X, 6.6 Sterile run*). The sterile run can be combined with other tasks to save time:

a) **Preheat the medium** (see *User Manual OrbShake Bioreactor SB10-X, 6.8 Heating up the medium*) to the desired cultivation temperature. Set the medium temperature in Kuhner Insight.

b) During the sterile run the **optical chemosensor** (for measuring pH and DO) can **equilibrate** (needs 4-12 hours).

c) **Set the calibration parameters of the sensors** after the sensor equilibration (see *User Manual Kuhner Insight Add-ons for OrbShakes, 4.5.2 and 4.7.2 Calibration of DO/pH sensor*)

d) After the calibration parameters are set, **take offline samples and re-calibrate the sensors** (see *User Manual Kuhner Insight Add-ons for OrbShakes, 4.5.3 and 4.7.3 Re-Calibration of DO/pH sensor*). Note: during the sterile run the medium is shaken and aerated with air and x % CO_2 if a $NaHCO_3$ based medium is used. The percentage of CO_2 depends on the $NaHCO_3$ concentration. Set the gas mixture manually! If there is no contamination the medium is fully saturated with air after some hours. Therefore, the DO sensor is recalibrated to 100 % DO (100 % O_2 saturation in medium which is possible in air atmosphere). In addition, the measured DO can be verified using a calibrated reference sensor (e.g. Clarke electrode or blood gas analyser). Also recalibrate the pH sensor: take a sample (avoid outgassing of CO_2 out of the sample!) and measure the pH with a calibrated reference sensor (pH electrode). Set a pH offset in Kuhner Insight if necessary. [2]

13. Increase the medium volume to the desired final working volume minus the inoculation volume. Preheat the medium.

14. Start the pH/DO controller with the desired set points for the initial pH/DO (set the desired gas flow rate in advance). Note: Initially (after a new start of Kuhner Insight) the pH/DO controller must adapt. The O_2 and CO_2 in the gas mixture might increase for a short time until the controller adapts to the situation. For detailed information about the pH/DO controller please see the *User Manual Kuhner Insight Add-ons for OrbShakes, 4.4 DO and pH control setup process steps.* [3]

15. Program: If you set a program (see *User Manual Kuhner Insight, 5.5 Tab: Program*) all functions (incl. pH/DO controller) must be switched on prior to starting the program.

16. The SB10-X is ready for the inoculation!

AN1A

Kuhner shaker
www.kuhner.com