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A Guide to Calibration on the BioFlo® 120 and BioFlo® 320: Dissolved Oxygen Sensors

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Abstract

Dissolved oxygen (DO) is a critical process parameter in microbial fermentation and cell culture bioprocesses. The Eppendorf BioFlo 120 and BioFlo 320 bioprocess control stations support standard analog polarographic DO sensors and Intelligent Sensor Management (ISM®)-enabled sensors with optical or polarographic sensing technology. This protocol describes the proper calibration procedure for each DO sensor type and the function of each of the options on the calibration screen.

Introduction

DO levels are often a limiting factor in achieving high cell densities in fermentation, and critical for mammalian cell cultures as well. As cells grow and the demand for DO rises, it is imperative that the DO sensor communicates the DO concentration to the control software in an accurate and timely manner. Since the release of the BioFlo 120 and 320, the types of sensors that are supported by the Eppendorf Bioprocess Control Software (BCS) has increased. Not only are the controllers compatible with standard analog polarographic DO sensors (sometimes referred to as Clark cell), but they also support Intelligent Sensor Management (ISM)-enabled sensors with both optical and polarographic sensing technology.

This document is intended to describe the proper calibration procedure for each DO sensor type and the function of each of the options on the calibration screen. Navigate to the chapter that relates to your particular sensor for detailed information on calibrating that sensor. The contents within were designed for use with BCS software, and not for Reactor Process Control (or RPC) software, which is available on the BioFlo/CelliGen® 510 and BioFlo 610. For instructions on DO sensor calibration using RPC software, please refer to the operating manual.

Polarographic Sensor Technology

Polarographic DO sensors can be digital (ISM) or analog. Regardless of the communication protocol, the mechanism of O_2 sensing is identical for both types (Figure 1). The polarographic DO sensor is filled with an electrolyte solution which is separated from the culture medium by an O_2-permeable membrane at the tip. Polarization of the sensor uses a voltage supplied by the control station to establish an anode and a cathode within. As O_2 diffuses through the membrane, redox reactions at the cathode and anode result in the generation of current at a rate that is proportional to the amount of dissolved O_2 in solution.

At the cathode, the following reaction takes place:

$$O_2 + 2\ H_2O + 4\ e^- \rightarrow 4\ OH^-$$

At the anode, the following reaction takes place:

$$4\ Ag + 4\ Cl^- \rightarrow 4\ AgCl + 4\ e^-$$

The measured current is proportional to the partial pressure of O_2 (pO_2) in solution, however, the % DO that is displayed in the control software is a relative value that is calculated based on the user-defined calibration. As such, % DO as displayed on the control station is not identical to pO_2 or concentration of O_2 in solution, which is commonly measured by offline analyzers. Instead, it is a percentage of the calibrated saturation, that is user-defined.

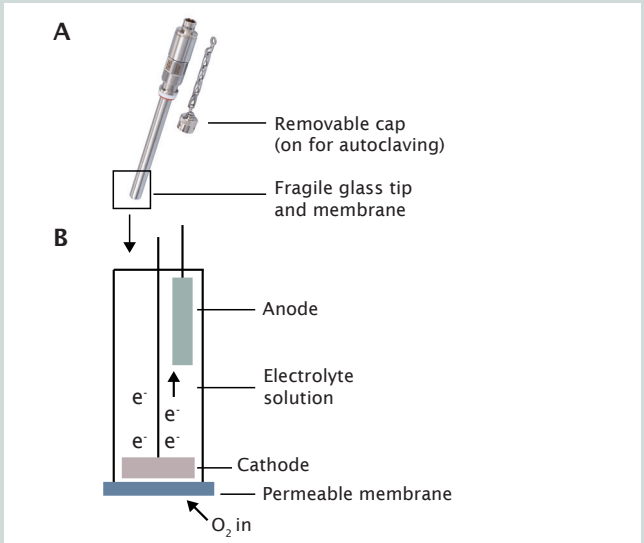

Fig. 1: Illustration of polarographic DO sensor function.

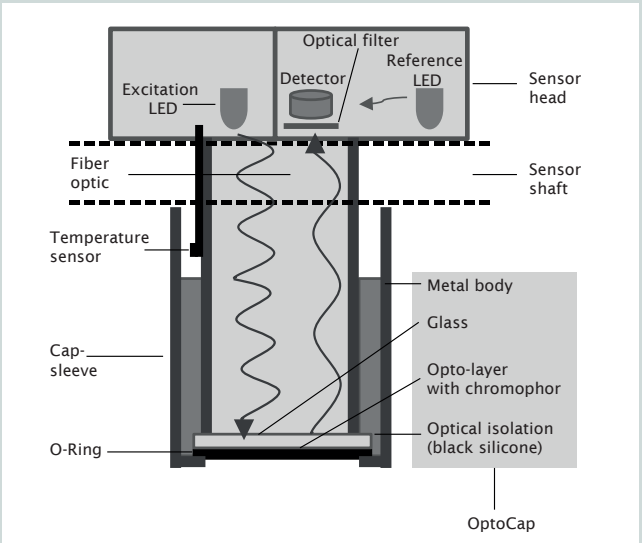

Fig. 2: Illustration of optical DO sensor function. Adapted from [1].

Optical sensor technology

Optical DO sensors are, by nature, digital sensors. Unlike the polarographic sensor, the optical DO sensor does not rely on chemical redox reactions to measure O_2 in solution. Instead, the measuring principle is based on determining dynamic quenching of fluorescence light (Figure 2): Chromophores on the sensor tip are excited with blue light. A chromophore absorbs this energy and is transferred to a higher energy level. A part of the energy is transferred as heat. As part of this process, the chromophore emits a red fluorescence light and returns to its ground state. If an O_2 molecule collides with the chromophore in its excited state, the energy can be transferred to O_2 (dynamic quenching). In this case, no fluorescence light is emitted because O_2 itself can transfer this energy as heat without light emission. The emission of fluorescence light is therefore dependent on the pO_2 at the chromophore layer. The amount of fluorescence detected a short time after excitation is used in the control software, along with the user-defined calibration Zero and Span values (Phi 0 and Phi 100, respectively), to calculate % DO. As such, % DO as displayed on the control station is not identical to pO_2 or concentration of O_2 in solution, which is commonly measured by offline analyzers. Instead, it is a percentage of the calibrated saturation, that is user-defined.

Table 1 offers a summary of the key differences between each sensor type.

Table 1: Comparison of key features for each sensor type

	Analog polarographic	Digital polarographic	Optical
Analog/digital	Analog	Digital	Digital
Sensing type	Clark cell (electrochemical)	Clark cell (electrochemical)	Optical (dynamic quenching)
Polarization period required	Yes	Yes	No
Zero/offset calibration required	Yes	No	Yes
Compatible with electronic zero method	Yes	No	No

Methods

Calibration of analog polarographic DO sensors

The protocol offered below is for the calibration of a Mettler Toledo® analog polarographic DO sensor of the InPro® 6800 series. When using analog polarographic DO sensors with the BioFlo 120 or BioFlo 320, a 2-point calibration involves an offset value and a saturation value. In the BCS software the offset value is termed Zero and the saturation value Span value. A Restandardization button is available to recalculate the offset based on changing process parameters. Figure 3 offers a graphical representation of the function of these buttons.

Upon setting Zero (Figure 4), the user defines the offset value used to remove background noise from the DO signal. Setting Span determines the maximum or saturation value for DO in the current matrix at the current temperature and pressure. The slope of the calibration curve is calculated from these values. Restandardize allows the user to perform a 1-point calibration in case of changing process parameters or matrix. When Restandardize is used, the offset is recalculated, but the slope of the calibration curve remains the same.

Calibration protocol

Preparations

> Examine the analog DO sensor tip sfor any damage to the sensitive O_2-permeable membrane at the tip. For instructions on how to replace the membrane, consult the Mettler Toledo operating manual [1] for your sensor.

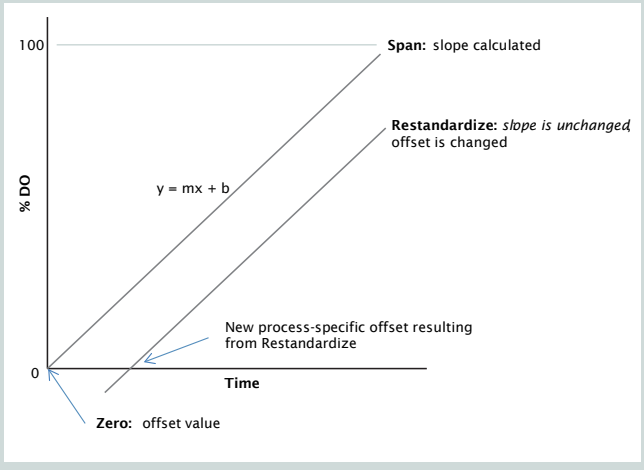

Fig. 3: Graphical representation of analog polarographic DO sensor calibration.

> The electrolyte in the sensor tip should be replaced any time the sensor has been stored for a long period of time, or any time erratic or slow response times are noted. To be certain of proper sensor functioning, the electrolyte can be replaced before each calibration, but never after calibration or during an experiment. For instructions on how to replace the electrolyte, consult the Mettler Toledo operating manual [1] for your sensor.

> A polarographic DO sensor needs to be polarized (see Introduction for more details) before calibration. The control station is designed to provide the correct voltage for polarization. Simply connect the sensor to the control station with the provided cable for at least 6 h. If the sensor is then disconnected from the cabinet for any length of time greater than 5 min, polarization should be redone. The polarization period can occur inside of a sterilized vessel post-autoclave, or on the bench in open air for BioBLU® Single-Use Vessel applications.

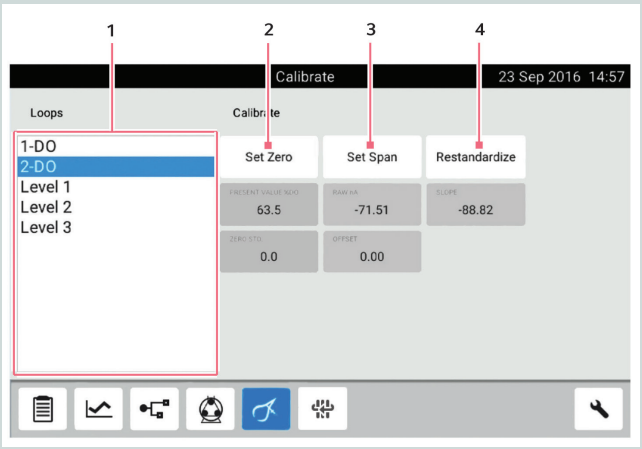

Fig. 4: Analog polarographic DO calibration screen (BioFlo 120 shown. Note that the screen is nearly identical to what is seen on the BioFlo 320.)

1. Loops field: List of available loops for user calibration. Select the desired loop to populate the appropriate calibration information on the right side of the screen. In this case, sensor 2 is an analog DO sensor which has been properly polarized.
2. Set Zero: Touch the edit box and enter the first calibration value (i.e. 0 %).
3. Set Span: Touch the edit box and enter the second calibration value (i.e. 100 %).
4. Restandardize: Touch to reset the Span value after 2-point calibration has been completed. See text for notes on restandardizing polarographic DO sensors.

> Equilibrate the vessel to process temperature before beginning the calibration.

Calibration

> To establish the correct loops on the controller, consult the BioFlo 120 or BioFlo 320 operating manual, as appropriate. Figure 4 displays the calibration screen for a connected analog polarographic DO sensor.

> To set the Zero point, or the offset value, using the electronic method, perform the following:

1. Disconnect the DO sensor from the cable or control station.
2. Navigate to the Calibrate screen and select 2-DO from the Loops field.
3. Allow the DO PV measurement to stabilize.
4. Touch the Set Zero edit box and then use the onscreen touchpad to enter 0. Touch the check mark button to confirm.
5. Reconnect the DO sensor to the cable or control station.

> To set the Zero point using the N_2 sparge method (recommended), perform the following:

1. Connect the DO cable to the DO electrode (previously installed in vessel) and the control station.
2. Go to the Calibrate screen and touch DO.
3. Turn only the N_2 loop on and select the desired flow rate for vessel saturation.
4. In approximately 10 – 30 minutes, the present value reading for DO will stabilize.
5. Touch the Set Zero edit box and use the touchpad to enter 0. Touch the check mark button to confirm.
6. Turn the N_2 loop off.

> To set the Span value, or slope, perform the following:

1. In the Agitation Gauge screen, set the speed to the desired rpm. Set the mode to Auto. Note that Eppendorf recommends setting Span at the maximum process parameters.
2. Vigorously sparge air into the vessel via the sparge inlet port on the head plate until the process value for DO is stable for approximately 10 minutes (this may take up to 30 minutes).
3. In the Calibrate screen, touch 2-DO.
4. Touch the Set Span edit box and use the touchpad to enter 100. Touch the check mark button to confirm.

> For polarographic DO sensors, the restandardize function is only recommended for use when changes in process parameters result in changes to O_2 saturation values. If restandardization (= 1-point offset calibration) is required, perform the following:

1. To restandardize the DO sensor, navigate to the Calibrate screen.
2. Select 2-DO from the Loops field.
3. Touch the Restandardize button, and then enter the desired value in the touchpad (e.g. 100 % for air saturation), and touch the check mark button to save changes.

Calibration of digital (ISM) polarographic DO sensors

Intelligent Sensor Management (ISM) technology from Mettler Toledo offers a range of benefits over the standard analog polarographic DO sensor, while the method of O_2 sensing remains identical. Most relevant to calibration is that the most recent calibration data is displayed on the BioFlo 120 and BioFlo 320 Calibrate screen (Fig. 5).
In most cases Mettler Toledo recommends a 1-point calibration of their digital polarographic DO sensors, that involves a saturation value. This value is termed Span in the BCS software.The customer is not required to zero the sensor. The offset, or Zero, is set at the factory and Mettler Toledo recommends that Zero calibration is only performed if the customer's application requires high resolution at very low (<5 %) DO levels. Further, performing a 2-point calibration including the Zero point may cause erratic readings. Figure 6 shows the function of calibration buttons and how they differ from analog sensors.

Setting Span determines the maximum or saturation value for DO in the current matrix at the current temperature and pressure. The slope of the calibration curve is calculated from these values and is stored on the sensor. It should also be noted that the ISM software performs temperature, humidity, and pressure compensation on the data automatically. These algorithms may result in the Span process value appearing slightly higher or lower than the 100 % that is entered by the customer. Rest assured that this is a normal part of the calibration process. Restandardize allows the user to perform a 1-point calibration in case of changing process parameters or matrix. When Restandardize is used, the offset is recalculated, but the slope of the calibration curve remains the same. In the case of the digital polarographic DO sensor, the restandardized offset value is not saved to the sensor as it is process-specific. The Zero that is factory-set remains on the sensor. Again, the data are compensated for temperature, humidity, and pressure.

If a Zero value must be entered for your process, ensure that the corresponding nA (value from the sensor displayed on the calibration screen) is within the acceptable range of 0 +/- 0.3 nA. Improperly setting a Zero point for ISM polarographic DO sensors will create an out of range offset value which can cause erratic sensor behavior, resulting in inaccurate DO values. If the Zero point is improperly recorded outside of the recommended range, please use the Restandardize function to reset your Zero, which will clear the offset value.

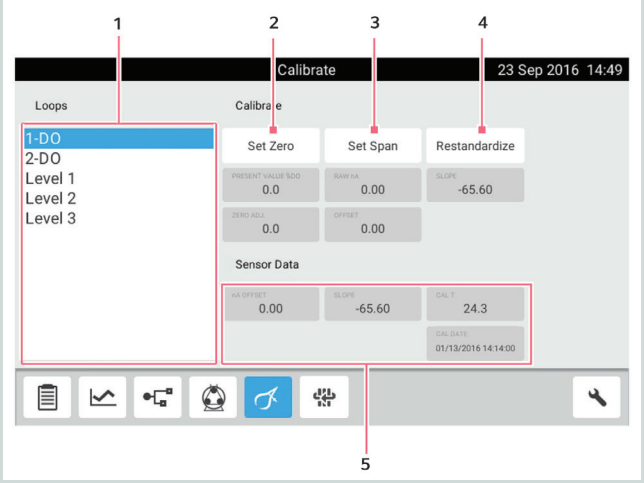

Fig. 5: Digital polarographic DO calibration screen (BioFlo 120 shown. Note that the screen is nearly identical to what is seen on the BioFlo 320.)
1. Loops field: List of available loops for user calibration. Select the desired loop to populate the appropriate calibration information on the right side of the screen. In this case, sensor 1 is a digital DO sensor which has been properly polarized.
2. Set Zero: Touch the edit box and enter the first calibration value (i.e. 0 %).
3. Set Span: Touch the edit box and enter the second calibration value (i.e. 100 %).
4. Restandardize: Touch to reset the Span value after 1-point calibration has been completed. See text for notes on restandardizing polarographic DO sensors.
5. Sensor Data field: Displays the information stored on the sensor.

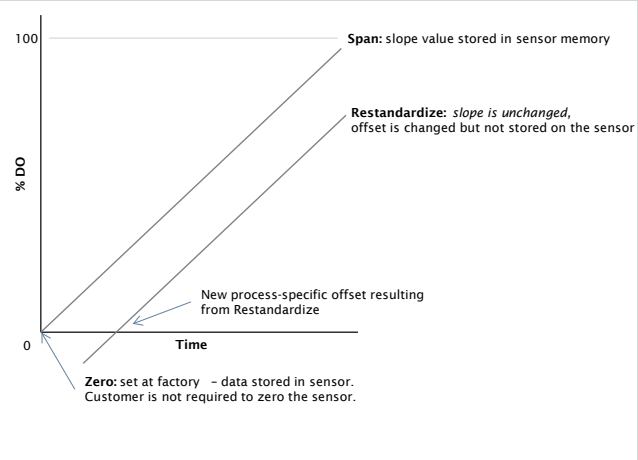

Fig. 6: Graphical representation of digital (ISM) polarographic DO sensor calibration.

Calibration protocol

Preparation

> The DO sensor tip should be examined for any damage to the sensitive O_2-permeable membrane at the tip. For instructions on how to replace the membrane, consult the Mettler Toledo operating manual for your sensor.

> The electrolyte in the sensor tip should be replaced any time the sensor has been stored for a long period of time, or any time erratic or slow response times are noted. To be certain of proper sensor functioning, the electrolyte can be replaced before each calibration, but never after calibration or during an experiment. For instructions on how to replace the electrolyte, consult the Mettler Toledo operating manual for your sensor.

> A polarographic DO sensor needs to be polarized (see Introduction for more details) before calibration. The control station is designed to provide the correct voltage for polarization. Simply connect the sensor to the control station with the provided cable for at least 6 h. If the sensor is then disconnected from the cabinet for any length of time greater than 5 min, polarization should be redone. The polarization period can occur inside of a sterilized vessel post-autoclave, or on the bench in open air for BioBLU Single-Use Vessel applications.

> Equilibrate the vessel to process temperature before beginning the calibration.

Calibration

> To establish the correct loops on the controller, consult the BioFlo 120 or BioFlo 320 operating manual, as appropriate. Fig. 5 displays the calibration screen for a connected digital ISM polarographic DO sensor.

> REMINDER: It is not necessary or recommended by the sensor manufacturer to Zero the sensor. This is only required if your application requires high resolution at very low % DO values (< 5 % DO). Therefore, after polarization, proceed directly to the set Span (slope) procedure:

1. In the Agitation Gauge screen, set the speed to the desired rpm. Set the mode to Auto. Note that Eppendorf recommends setting Span at the maximum process parameters.
2. Vigorously sparge air into the vessel via the sparge inlet port on the head plate until the display is stable for approximately 10 minutes (this may take up to 30 minutes).
3. In the Calibrate screen, touch 1-DO (or whichever sensor number is your digital sensor).
4. Touch the Set Span edit box and use the touchpad to enter 100. Touch the check mark button to confirm.

> For polarographic DO sensors, the Restandardize function is only recommended for use when changes in process parameters result in changes to O_2 saturation values. If restandardization, or 1-point offset calibration, is required, perform the following:

1. To restandardize the DO sensor, navigate to the Calibrate screen.
2. Select 1-DO from the Loops field.
3. Touch the Restandardize button, and then enter the desired value in the touchpad (e.g. 100 % for air saturation), and touch the check mark to save changes.

NOTE: Pressure and temperature compensation may cause the displayed Span value to appear higher or lower than what was entered. This is normal.

Calibration of optical DO sensors

Since the optical sensor works by emitting a pulse of light, an electronic Zero procedure is not appropriate. In addition, unlike the digital polarographic sensor where it is not recommended to perform a Zero or offset calibration, the optical sensor requires the Zero (Phi 0) calibration as part of the protocol. In the case of this sensor type, the software on the BioFlo 120 or BioFlo 320 will guide you through the process, even indicating whether or not the signal is stable enough to proceed. There is an additional button available on the optical DO calibration screen: the Process Cal button. This allows the user to perform an additional calibration curve adjustment in which the Span (or Phi 100) value is adjusted (Fig.7).
In this case the Zero point is unaffected. See Figure 7 for a graphical representation of this phenomenon. Ensure the optical DO sensor is connected to the sensor 2 port on the utility panel. In case the secondary sensor module is present on the BioFlo 320, it can be connected to either port 2 or port 4. Optical DO sensors are not compatible with the sensor 1 port. Consult the operating manual for the appropriate control station (BioFlo 120 or 320) for detail on connecting the sensor before proceeding to calibration.

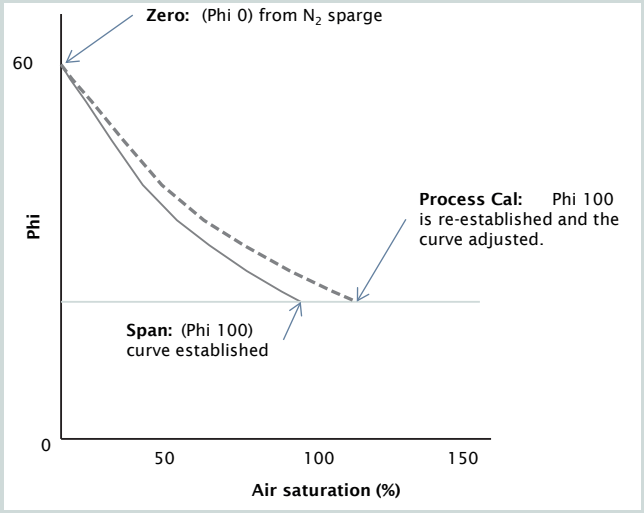

Fig. 7: Graphical representation of optical DO sensor calibration

The optical sensor requires both Zero (Phi 0) point and Span (Phi 100) calibration to establish the calibration curve. Both the Zero and Span values are stored on the sensor. When the user sets Span, the process value is compensated for using default temperature, pressure, and humidity values and can therefore differ slightly from the value entered by the customer. In addition, if the user sets a Span value that is either out of expected range, or not within expected delta from the Zero point, the calibration will be rejected and an error message is received. The Process Cal button allows the user to adjust the calibration curve to account for a new saturation value. This button is often used by customers who perform the 2-point calibration in open air and wish to adjust the calibration post-autoclave with culture medium at process parameters. The Process Cal value is used to recalculate the calibration curve and is also compensated for current conditions such as pressure, temperature, and humidity. See Figure 8 for illustration of the optical DO sensor Calibrate screen.

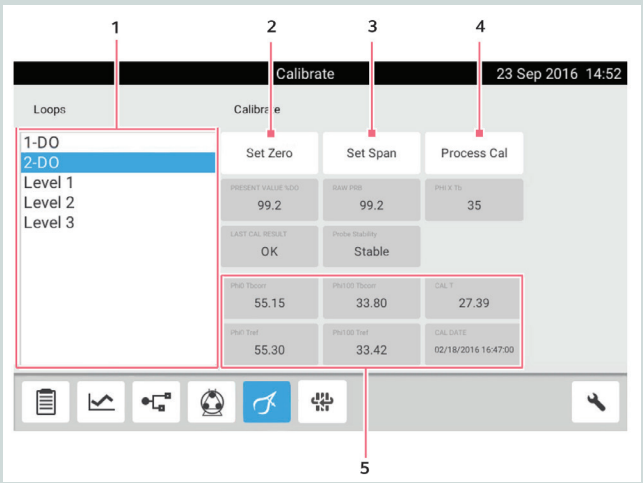

Fig. 8: ISM optical DO sensor calibration screen (BioFlo 120 shown. Note that it is almost identical on the BioFlo 320.)

1. Loops field: List of available loops for user calibration. Select the desired loop to populate the appropriate calibration information on the right side of the screen. In this case, sensor 2 is a digital optical DO sensor which has been properly polarized.
2. Set Zero: Touch the edit box and enter the first calibration value (i.e. 0 %).
3. Set span: Touch the edit box and enter the second calibration value (i.e. 100 %).
4. Process Cal: Touch to reset the Span value after 2-point calibration has been completed.
5. Sensor Data field: Displays the information stored on the sensor.

Calibration protocol

Calibration

> To set the Zero calibration using the N_2 sparge method perform the following:

1. Go to the Calibrate screen and touch DO.

2. Turn the N_2 loop on and select the desired flow rate.
3. In approximately 10 – 30 minutes, the current value reading for DO will stabilize.
4. The Probe Stability field will indicate that the reading is "Stable" when it is recommended to proceed to the set Zero step.
5. Touch the Set Zero edit box, and use the touchpad to enter 0 and confirm.
6. Turn the N_2 loop off and proceed to Setting Span.

> To set the Span or saturation value, perform the following:

1. In the Agitation Gauge screen, set the speed to the desired rpm.
2. Set the mode to Auto.
3. Vigorously sparge air into the vessel via the sparge inlet port on the head plate until the display is stable for approximately 10 minutes (this may take up to 30 minutes total).
4. The Probe Stability field will indicate that the reading is "Stable" when it is recommended to proceed to the set Span step.
5. Touch the Set Span edit box and use the touchpad to enter 100 and confirm.

> If a Process Cal is required, a 2-point (Zero/Span) calibration is required prior to use of the Process Cal function. To perform a Process Cal:

1. Navigate to the Calibrate screen, and then select the desired Optical DO sensor from the Loops field.
2. Touch the Process Cal button and then enter the desired value in the onscreen touchpad (e.g. 100 % for air saturation), and confirm.

Literature

[1] InPro 6800 Series O_2 Sensors Instruction Manual (#52 200 953), Mettler-Toledo AG.

Ordering information

Description	Order no.
BioFlo® 120, Advanced	
Plug type B (USA, Canada, Mexico, Japan)	B120ACS000
Plug type CEE 7/7 (EU (except UK, Ireland, Switzerland), Russia, Korea	B120ACS001
Plug type I (Australia, New Zealand, China, Argentina)	B120ACS002
Plug type J (Switzerland)	B120ACS003
Plug type G (UK, Ireland)	B120ACS004
Plug type N (Brazil)	B120ACS005
Plug type D (India)	B120ACS006
BioFlo® 320 Base Control Station, all configured units include the same base control station	
Base Control Station	1379963011
DO Sensor, Mettler Toledo® Ingold (InPro® 6830)	
120 mm, angled T-82 connector	P0720-6280
160 mm, angled T-82 connector	P0720-6580
220 mm, angled T-82 connector	P0720-6282
225 mm, straight T-82 connector	P0720-6526
320 mm, angled T-82 connector	P0720-6283
355 mm, straight T-82 connector	P0720-6525
420 mm, angled T-82 connector	P0720-6284
526 mm, straight T-82 connector	P0720-6529
DO Sensor, Hamilton® OxyFerm™ FDA	
625 mm, straight T-82 connector	P0720-6520
Polarographic DO Sensors, ISM®	
12/120 mm	P0720-6652
12/220 mm	P0720-6653
12/320 mm	P0720-6654
12/420 mm	P0720-6655
Optical DO Sensor, ISM®	
12/120 mm	P0720-6651
12/220 mm	P0720-6660
12/320 mm	P0720-6661
12/420 mm	P0720-6662

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