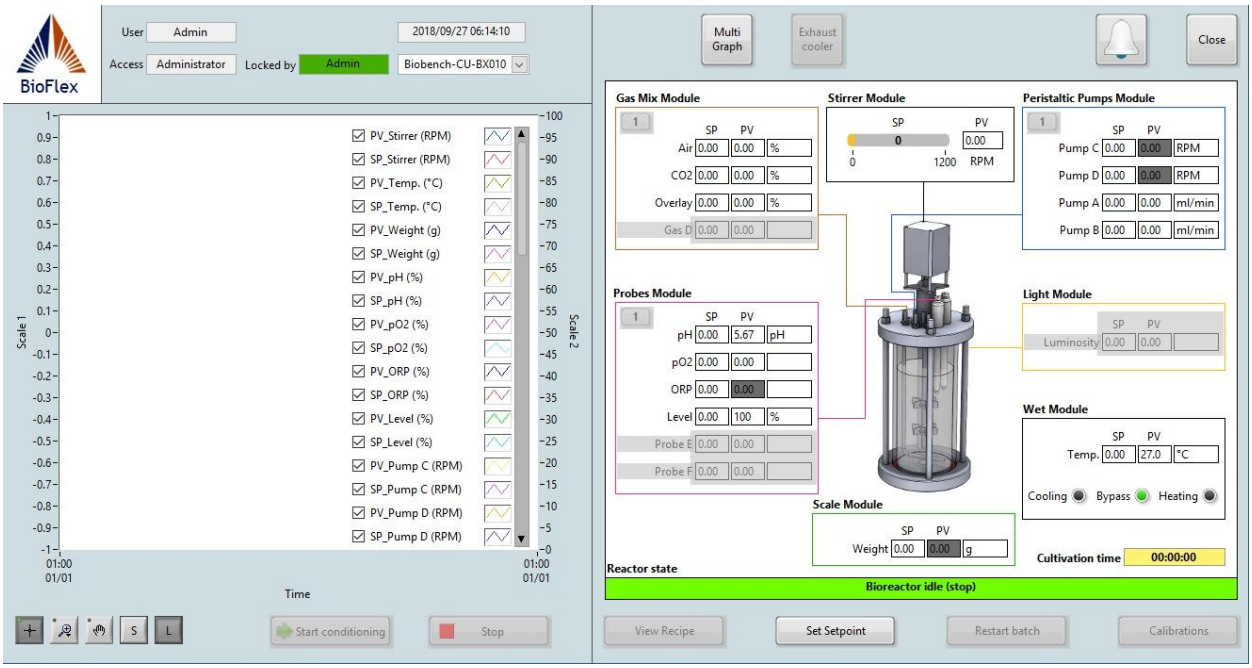
BIOFLEX software



Operating manual Ver. 3.3.1

CE

Index:

1. General information

All the operations on cultivation with Biobench can be carried on through Bioflex interface.

Bioflex is a software application that laboratory researchers and plant operators use to control, monitor, record, and analyze processes, such as fermentation and cell culture. Bioflex facilitates the configuration of devices and experiments, integrates all sources of data into a unified collection, allows flexible control methods, and expedites post-run reports and analysis.

Bioflex is a networked application. Any number of bioreactor control stations (computers) can be tied together through a shared central database for the purposes of experimentation and managing device configuration, as well for real-time sharing of process data.

Bioflex has been designed to allow groups of researchers to share information and experiment configurations. Similar devices and data associations can be configured with minimal additional effort. Experiments, once configured for specific recipe control, can be quickly duplicated.

Before starting a cultivation process, operator have to set up a recipe. It is intended to have the group of set point parameters for the intended cultivation. If fully configured, a recipe can automatically manage the cultivation, without the need of a manual operation on parameters. Setpoints can be varied during a cultivation, to follow the steps of cell growth; user can also set up a variation of parameters during the time of cultivation. Configure a “Cascade” for a parameter, such as dissolved oxygen or pH, will set automatic actions that the system performs to correct the parameter to set value. However it is possible, for the operator, to act on all aspects of cultivation at any moment, operating through an immediately comprehensible interface. Variations of parameters during cultivation are shown as real-time graphs, if selected.

Operator can review cultivations performed, recipes prepared, and all the events occurred during a cultivation. All the data and graphs can be exported.

1.1. Software specifications:

Description	Value
HMI	Colour touch screen
Operating system	Windows 7 pro, Windows 8, Windows 10
Software	Bioflex 3.3.1

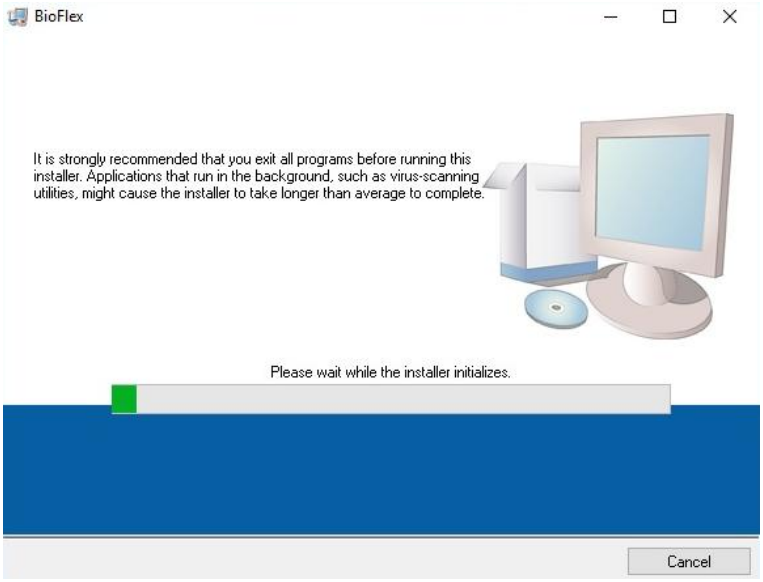
2. Installation HMI

The software (Bioflex) will be installed in the HMI device, which comes with the container. If the software was not installed, you must follow the procedures given below:

2.1. Procedure to install:

Once the instruments are arrived at client's place, the client have to mail to Kbiotech at service@kbiotech.eu, asking information about the missing software and after we will give you the link for the software download, after receiving the following information's: Serial number of the instruments received, client's details. Once the client received, the download link, will be from dropbox. Double click on Bioflex.exe icon, will open a window, showing the status of Bioflex as it initializes, as shown in picture below.

Then the program allows the user to choose directories for the program; it is recommended, do not modify the default directories. When the main overview display opens, Bioflex is ready to use.



2.2. Configuration

To configure the bioflex software, connect all the probes properly and also connect it to the power supply of 220/230V AC.

After connecting to the supply, switch ON the master unit, control switch on the rear panel and release the emergency button if pressed on the front panel. After, press the "Blue" push button on the front panel in order to start the machine.

Once the system is ON (Master unit). Connect the Ethernet cable from the rear panel of the master unit to the PC where the software Bioflex is installed.

2.2.1. IP address configuration

It is necessary to configure the IP address of the PC . In order to communicate the PC with the Bioflex. It is recommended to follow the steps below to configure the IP address.

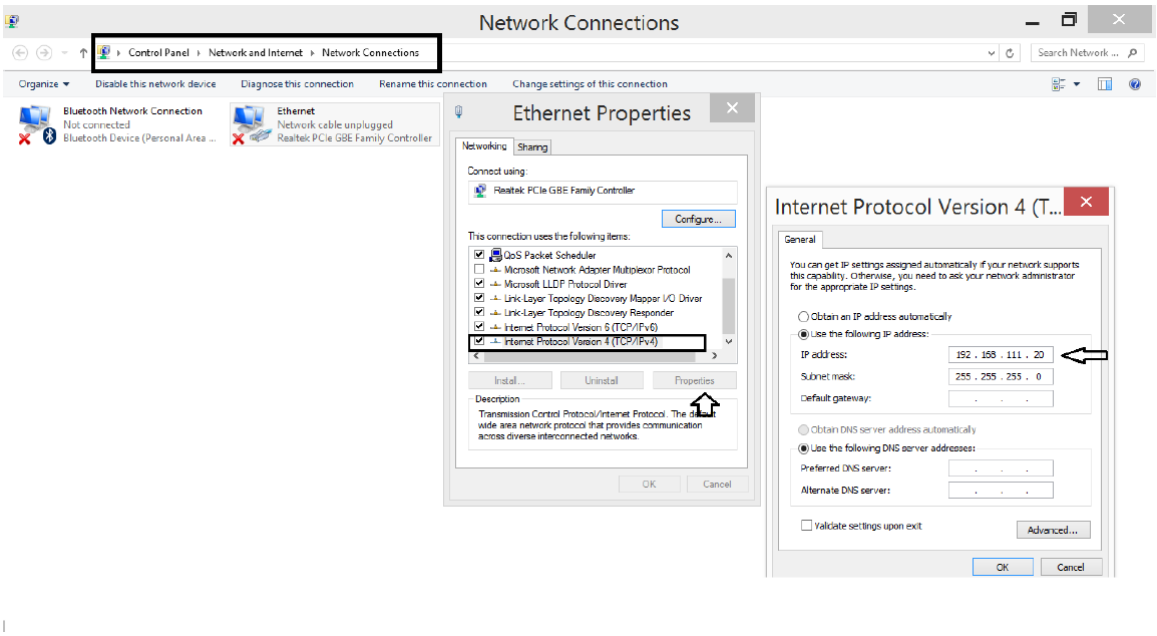
From the Control panel, select Network and Internet, then Network connections.

Right click on Ethernet, select properties.

On Network window, select “Internet protocol version 4 (TCP/IPv4)”; a new window named “Internet protocol version 4 (TCP/IPv4)” will open.

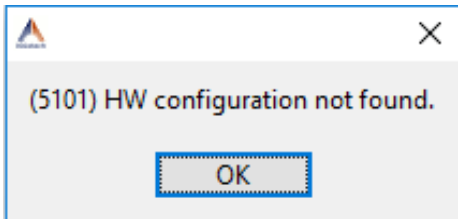
Select “Use the following IP address” and enter “192.168.111.20”.

Confirm by clicking OK.



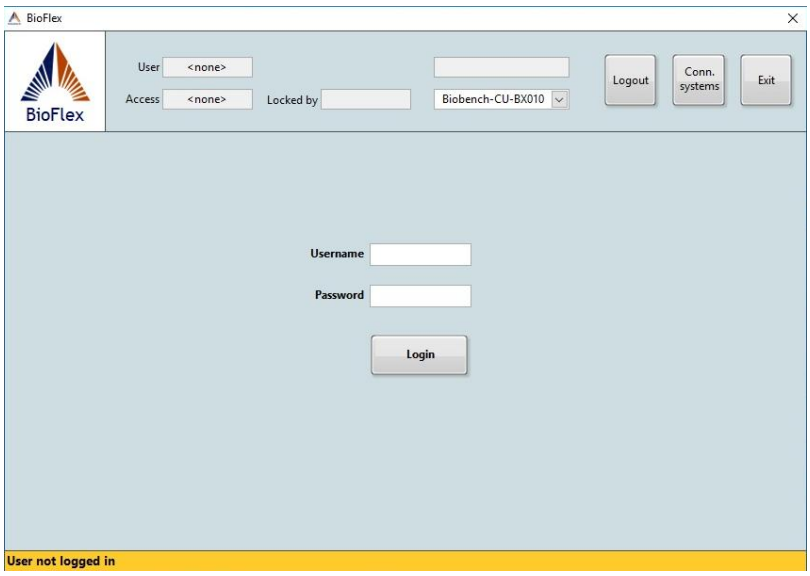
2.2.2. Vessel configuration

If the error message shown as the image below, an update of the Vessel Configuration is required. Upload a proper configuration file through “Update Vessel Configuration” function (see chapter 4.4.8).



3. Getting started

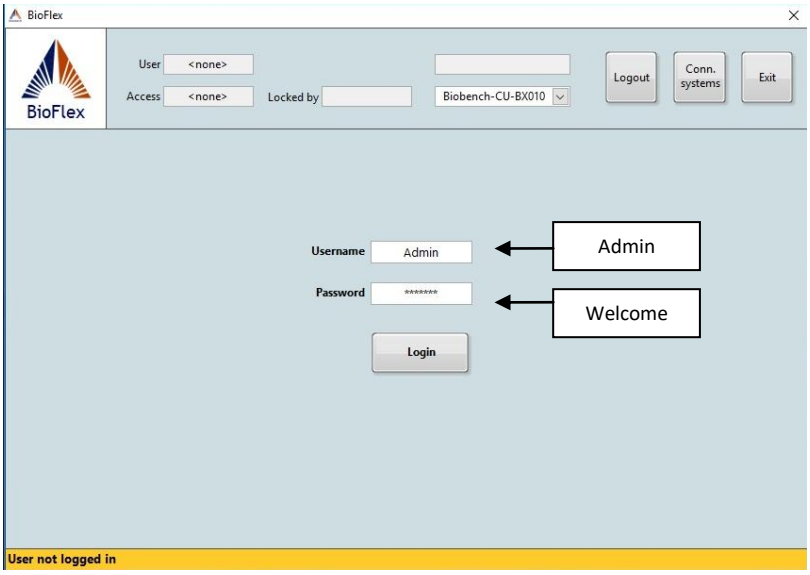
When the User start the software, it comes up with the following window.



In the top are displayed:

- Username
- User Access Level
- Creator of the current User
- Bioreactor selector
- Connected systems
- Logout
- Exit Button (alternative to the (X) windows button at the top right)

The following procedure will explain the way to enter into the software.

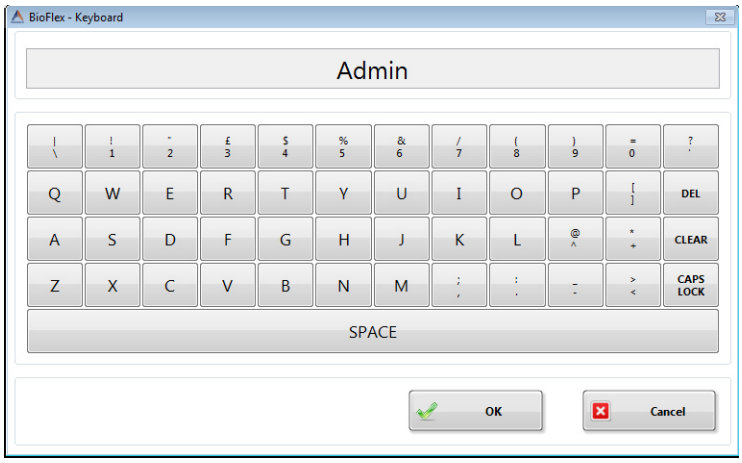


Clicking on the tabs username and password, will make a keyboard appears, by entering Username and Password, as shown in the figure above, you can enter into the software.

On the first boot, only configuration made, is an administrator.

Default username :"Admin"

Default password: "Welcome".

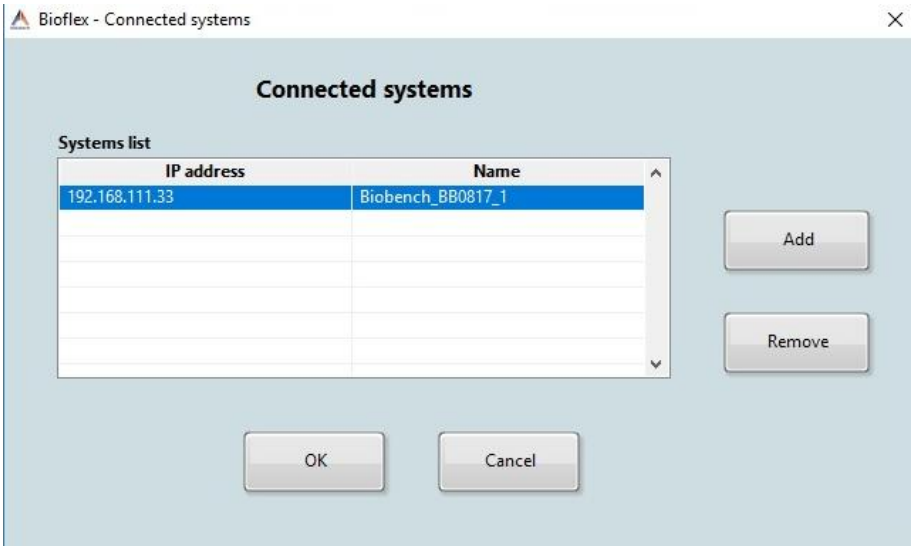


If the software does not connect, check Ethernet cable connection to Control Unit Module; when turning Control Unit On, wait until Status Led on the rear of the module blinks. When it starts blinking, the system is ready to operate, and the connection state indicator on Bioflex start page will turn green.

Once you have selected Exit, you will be asked to confirm that you want to stop Bioflex. Click Yes to exit, No to cancel and continue running Bioflex. If an experiment is running, its recipes (if any) must stop when you exit Bioflex. In this case, you will be asked to confirm that you want to stop the recipes, if yes, stop the entire experiment, or cancel to continue running Bioflex.

In case the group of employees, who wants to work in the bioflex the administrator of the software can set up user profiles and user groups for operating in Bioflex. Different users and groups may be given different level of accessibilities to the functions. Users identify themselves to the Bioflex system by logging in on a station. A station may be operated with no user logged in, but the functionality is usually limited to viewing data without the ability to make modifications. If another user is currently logged in, first select Logout from the File menu.

Click on the connected systems to select/ modify the type of vessel. In that window you can also add the systems IP address by which, we will get to know the configuration of the vessel chosen. It can be seen in the following image:



4. Tabs section

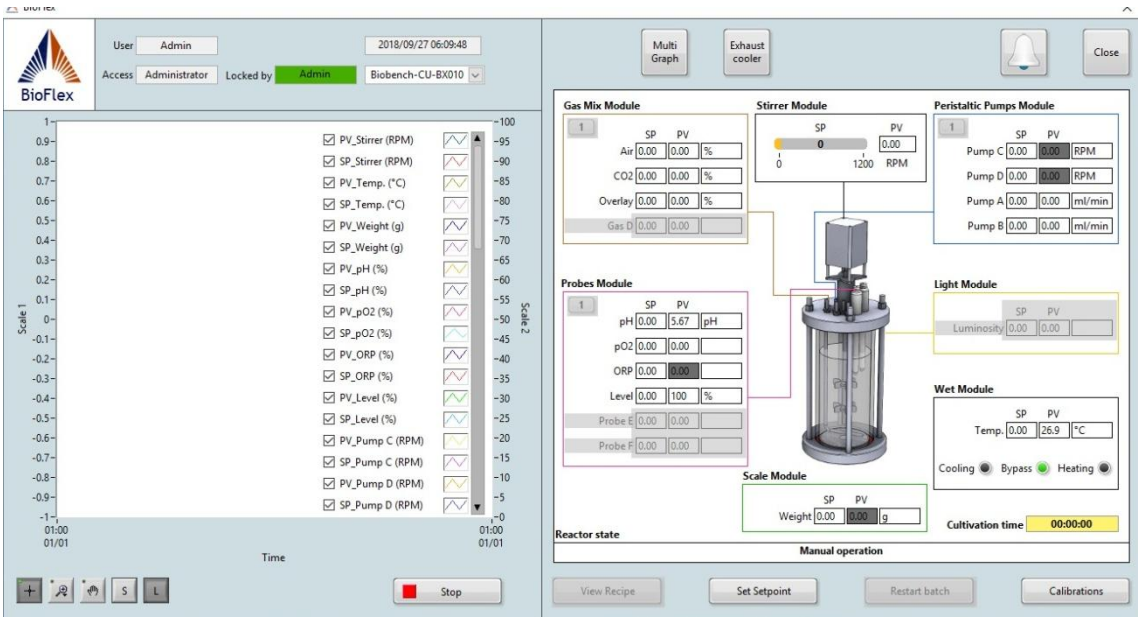
Once logged in, the program will open the above window.

- Manual test – Used to control Manually all the devices which is connected to bioreactors,
- Recipes and calibrations – Used to define the specific parameters for cultivation,
- Cultivation – Used to view the current cultivation in process. Remain active only during the cultivation process.
- Batches – Used to define the duration and name of the process,
- Start Batch – Used to start the batch, which turns into a cultivation
- Review acquired data – Used to view saved Recipes and Batches
- CIP – Cleaning in Process remain inactive until the customer asks for it,
- SIP – Sterilization in process remain inactive until the customer asks for it,
- System configuration – used to define Users profiles: Username, passwords, and level of access, wet part manual control etc..,

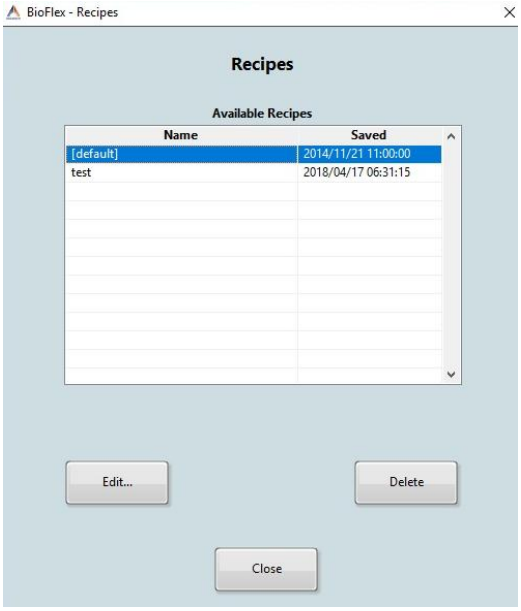
Details of every single tabs were explained below:

4.1. Manual Test

Once the customer login to the bioflex, will note the image as seen in the section 4 above. If the customer decides to verify the functionalities of the machine. He can enter into Manual test mode, in which the synoptic window will appear where he/she can verify the functionalities by entering some values, in order to make it work. If, he/she decides to stop the operation, click the stop button, then, the bioreactor state becomes stable or idle.



4.2. Recipes tab



The recipes tab contains the list of recipes stored on the bioreactor. At the first start the only recipe available will be the default recipe, which has the set point are all at 0 and this recipe cannot be deleted. The default recipe serves as a guide for the user to fill in new recipes that will be saved in the database of recipes.

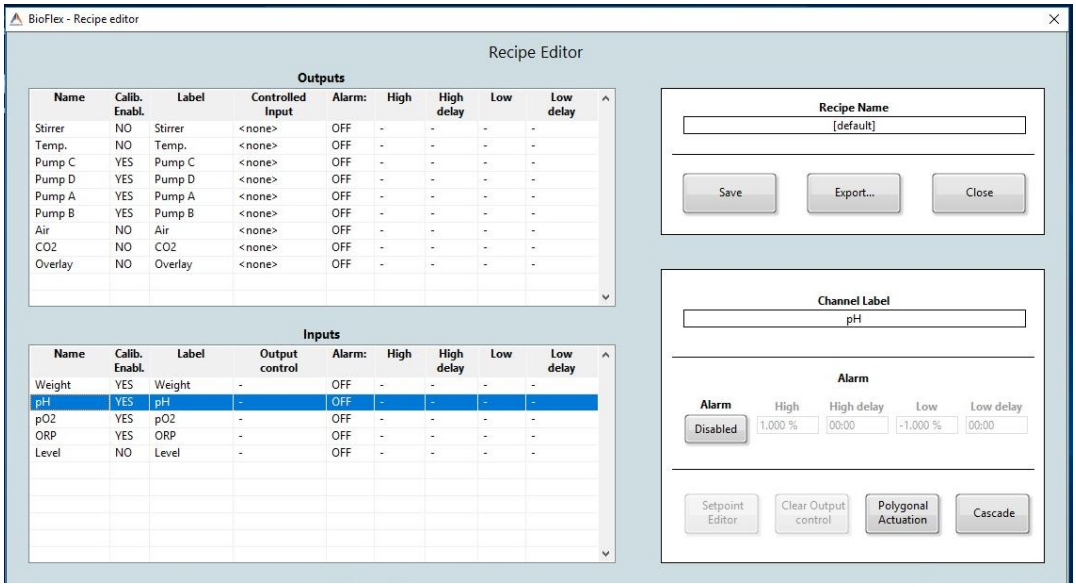
The window lists the devices present in the bioreactor, divided into inputs and outputs.

For outputs, it is possible to insert the set point by clicking the “Set point" editor. For inputs, it is possible to set a set point, only after inserting the values in the cascade or inserting the values in the polygonal actuation, which allows you to select the output to indirectly control the input; it is possible to insert the parameters by clicking on the “cascade” or “polygonal actuation” button.

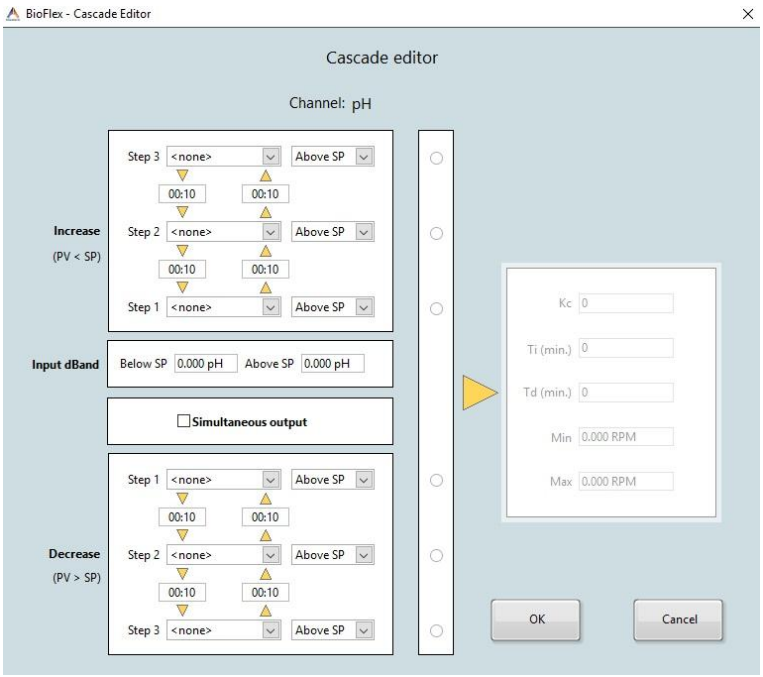
In "Recipe name" field you can specify the name of the recipe (new or existing).

It is possible to make a new recipe by selecting “Edit”, while the "Delete" button lets you delete the selected recipe. In order to modify the recipe, you have to select either the new recipe or the existing recipe. By selecting the necessary recipes, you can modify the recipe, by clicking “Edit”, which will open the following window (recipe editor).

In the recipe editor window, you can select either any of the inputs nor any of the outputs. After selecting any of the necessary options in the recipe editor, the cascade or the set editor becomes active. By clicking cascade, the window named cascade editor will get opened, where you can specify the values and the operation to be followed based on the set point. By clicking set point editor, will open a window “Set point editor” where you can directly set the values for the outputs which controls the inputs.



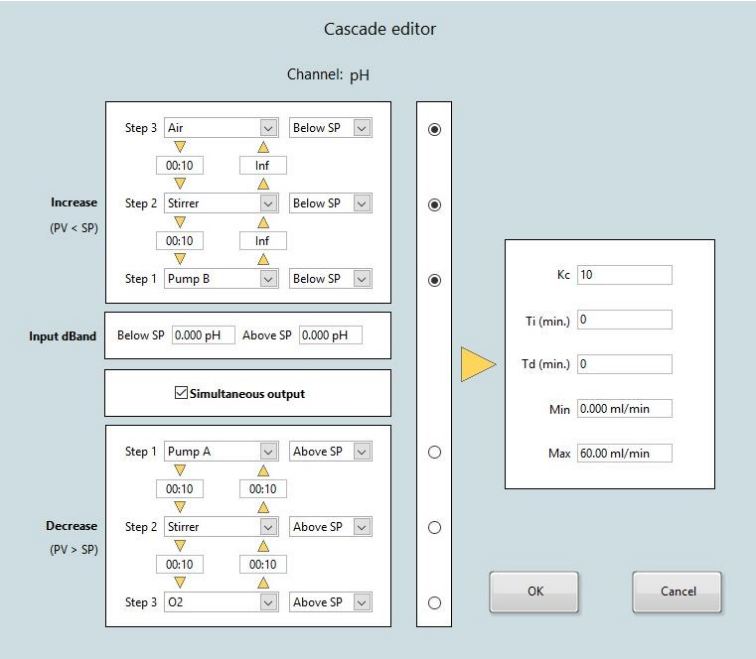
If you select any of the inputs as shown in the figure above, the cascade and polygonal actuation becomes active. You can select any one of the actions as per your requirement. The following are the details of these windows:



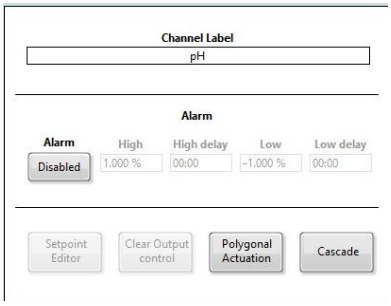
In this window, it is possible to assign the parameters on every step on both the Increase and the decrease segments by using the dropdown menu. After assigning all the parameters, we can select any of the actions based on the requirements from the drop down menu in PID controller. For example, Let's assume that, we are going to maintain the pH value of 7. To do that, we have to select the pH as the input, then click on the cascade will open the above window. In the above window you have to select the parameters for every step. We in this example, named the pump A as pump acid and pump B as pump base for the simplicity of the process. We calibrated and found that my projects pH value is below the set point. If the pH value is below the set point then select the pump B/pump base in the increase(PV<SP) and in the second step put the stirrer to mix well the mixture and in the third step put air as an input. You can also maintain the transition time between the every step of the operation by inserting the time in hours or minutes or seconds.

If the pH value is above the set point, then select the pump A/ Pump acid in the decrease (PV>SP) and vice versa as shown in the below figure:

You can also specify the PID values from the drop down menu for Increment/Decrement the process.



4.2.1. Channel label and alarm



It is possible to enable an alarm on each device using the "Alarm" field.

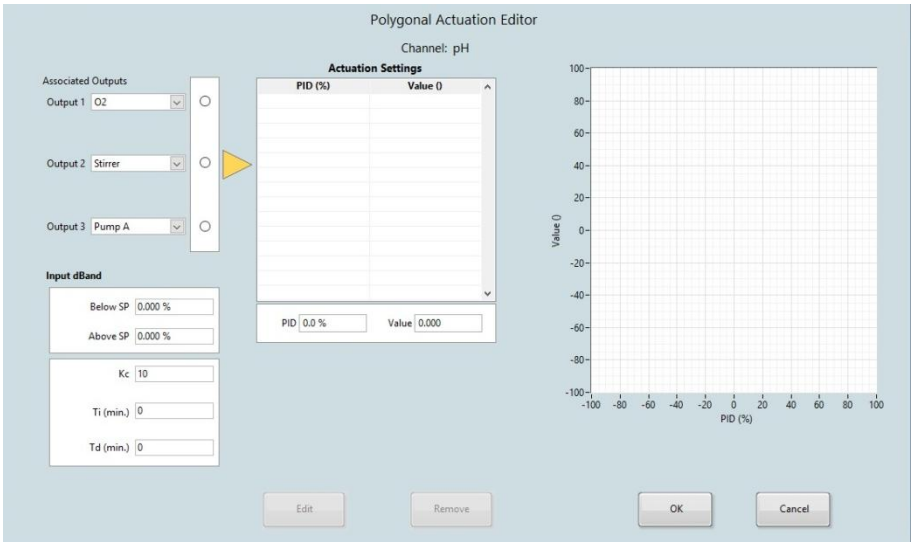
This window allows you to enable and disable an alarm by entering minimum and maximum values, with appropriate delays. If the PV of the selected device remains below the "low" value for longer than the time delay "Low", the PV field on the synoptic will be colored blue and will log an event to the event log. Similarly, if the PV of the selected device remains above the "High" value for a time greater than the "High delay", the PV field on the synoptic will be colored pink and will log an event to the event log.

To set the alarm, you have to insert your timings, while inserting, the button “Disabled” has to be remained. Once the alarm is set, you have to press the button “Disabled” to enable the alarm.

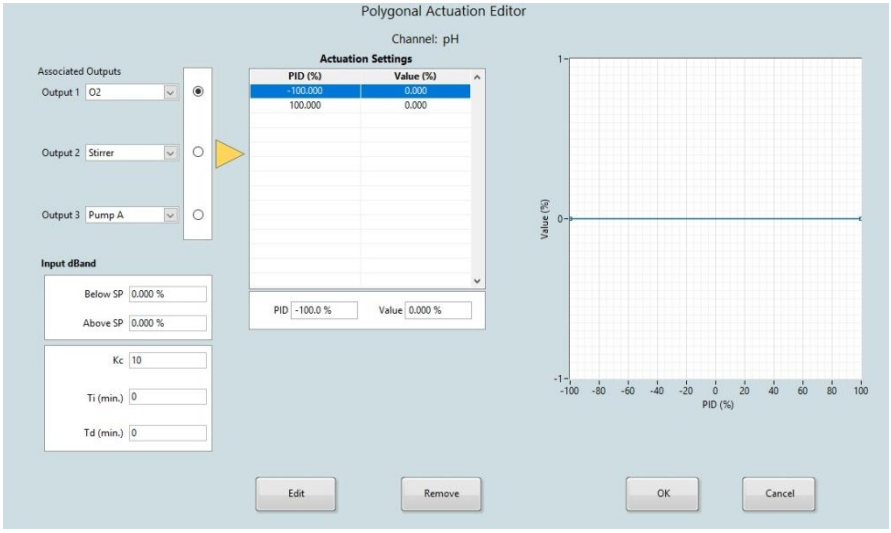
For the selected recipe, it is possible to change the name of the channel through the Channel Label field by clicking on the empty space will open a keyboard window where the user can enter the name for the channel. The name chosen will appear in the synoptic during cultivation. It is useful to recall the utilities with label allowing a fast and clear recognition; i.e. if pump A is used to add acid, it might be helpful to change the value of the label as ” Pump Acid".

4.2.2. Polygonal actuation:

Polygonal actuation tab allows the user to set the specific PID values for his project, if necessary. In this tab, the user can specify the parameters which he wanted to add during the cultivation. The following figure shows the polygonal actuation tab:

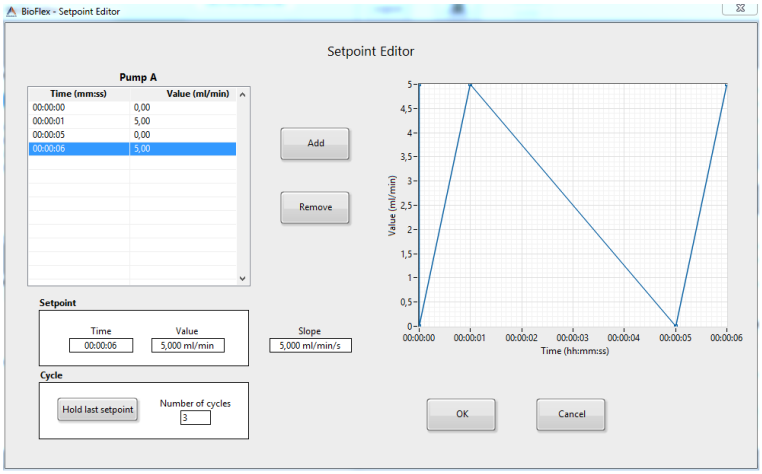


The below figure shows the polygonal editor window, where the PID% values has been added to maintain the cell culture. The figure is just an example.



4.2.3. Set point editor

The "Set Point Editor" button opens the following window.



In this window, it is possible to define the selected parameter, then, the setpoint trend as a function of time, adding pairs of points / time value.

Click on “Add” icon to add a setpoint value to the trend. Click on “Remove” to delete a setpoint.

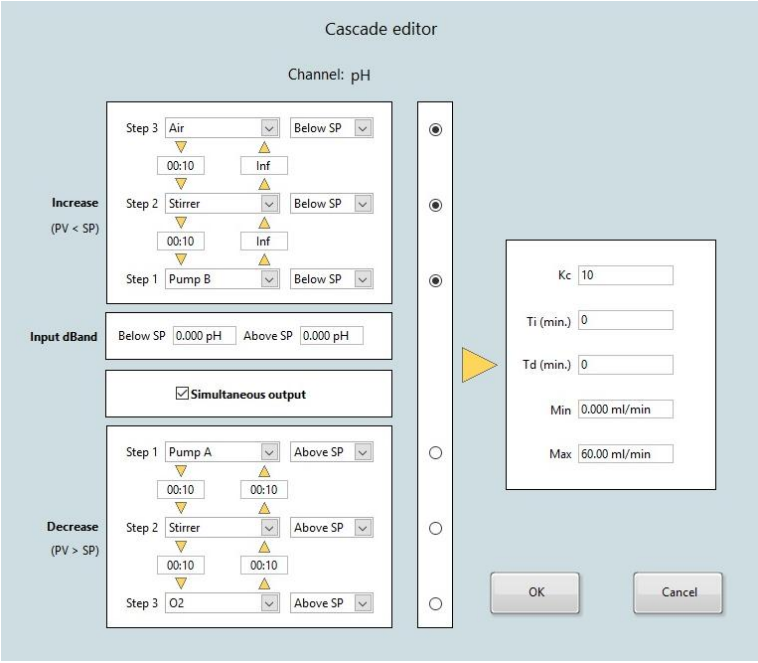
In “Setpoint” editor window, you can enter Time and Value for each setpoint. The Time showed refers to the minutes and seconds from the start of cultivation. For example: A set- up of the pump 5 ml/min at 00:00:01, will led the system to increase the pump speed linearly from 0 to 5 ml/min, in order to reach the 5 ml/min value at the time set (00:00:01). To maintain a value for a period of time, enter the value at start time and the same value at end time (i.e. to maintain 5 ml/min for 1 minute set 5 ml/min at 00:00:00 and 5 ml/min at 00:01:00, if starting from zero).

In “Cycle” window, operator can specify whether to perform the sequence indefinitely or whether to repeat the sequence designed for a defined number of times (even one). In this situation, if the time of cultivation exceeds the moment in which, it is implemented the last cycle, the setpoint is frozen at the last set value

Once the setpoint trend has been configured, click on “OK” to save changes made, or “Cancel” to discard changes.

4.2.4. Cascade

The cascade option is designed to set up a series of actions to be carried on automatically by the system to control input parameters during cultivation; the user can choose for example a blind action to control a parameter, and, in case, if not sufficient, the system will proceed with the second action set, more drastic, and so on.



Clicking the "Cascade" will open the following window (in the specific case of a possible cascade to control the "pH" input). In this window, it is possible to assign the output to the selected input control.

In “Increase” and “Decrease” tabs. An operator can specify up to three outputs used one after the other (step 1, step 2 and finally step 3) to increase or decrease the value of the PV.

In the tab "Increase" specify the output sequence to be used to increase the PV, when PV is too low, while in the "Decrease" field specify the output sequence to be used to decrease the PV, when PV is too high.

The “Increase” tab needs to be set on "Below SP", while the “Decrease” tab needs to be set on "Above SP" in order to maintain the values at neutral.

Between step 1 and step 2 and between step 2 and step 3, it is possible to specify the transition times. The engine of the cascade goes to the next step if the current step remains at the maximum setting for a time greater than specified and returns to the previous step in case rests to a minimum of adjustment to more than the specified time. In the moment in which the engine of cascade passes to step 2, the setpoint of the step 1 remains suspended at the maximum value (in the case where “above SP” is set), or minimum (in the case in where “below-SP” is set).

In the "Input dBand" tab, it is possible to specify a dead band value around the setpoint, where the system does not perform any action; if the PV is in the dead band, the cascade will not interfere with the recipe setpoint values of controlled output . System will start to action only when PV is greater than Above SP dBand, and when PV is less than Below SP dBand.

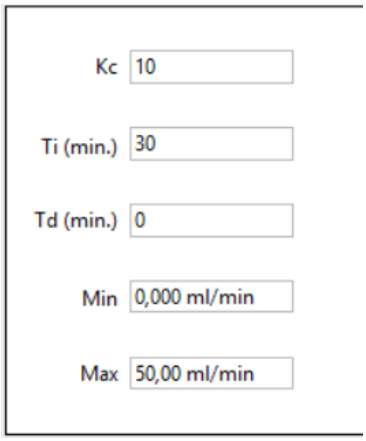
Once you set the values and selected the appropriate pumps in the cascade editor window and clicked OK. You will see the set point editor button becomes active in the recipe editor window. By clicking it you can edit the set point editor values based on your preferences.

Note:

If one of the outputs in the recipe editor (Ex: stirrer, pump or gas etc.,), is used as the input steps (1-2-3) of the cascade editor, It is not possible to use the same in the other cascade input.(Ex: If I use the stirrer in the pH cascade, it is not possible to use the stirrer in oxygen cascade).

4.2.5. PID window

A Proportional–Integral–Derivative controller (PID controller) is a control loop feedback mechanism. A PID controller continuously calculates an error value as the difference between a desired set point and a measured process variable and applies a correction based on proportional, integral, and derivative terms (sometimes denoted as P, I, and D respectively) which give their name to the controller type.



The three actions of PID are calculated separately and simply added by selecting the tabs (K_c, Ti, Td) algebraically. The engine of the cascade uses a PID to adjust the current output value. User can specify the parameters by selecting one of the options from the drop down menu on the top of PID window. K_c – Constant, corresponds to the Proportional Gain term, Ti corresponds to the Integral term, Td corresponds to the Derivative term.

In most of the situation it is sufficient to use the parameter K_c, starting from default K_c value and increasing it gradually if its effect is not sufficient.

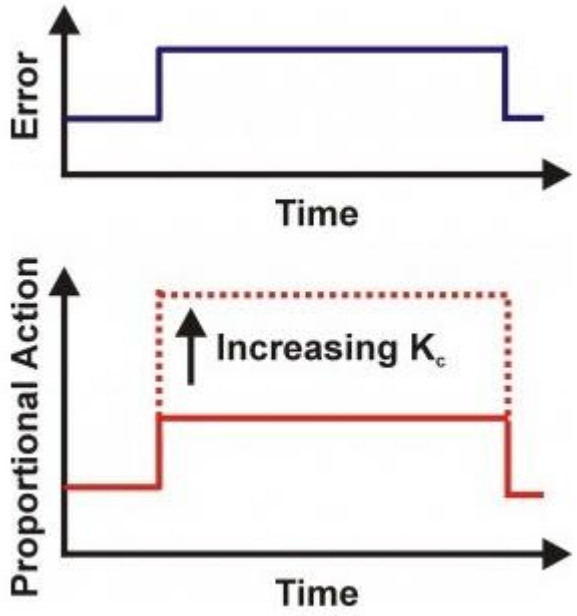
The “PID” window also let the user to set the upper and lower limits of the device, which are related to the two-step in question: in increase will be important to specify the maximum value, while in the decrease will be important to specify the minimum value.

The three actions of the controllers were explained briefly below:

Proportional controller:

The proportional control mode is in most cases the main driving forces in a controller. It changes the controller action in proportion to the error. If the error gets bigger, the control action gets bigger. The adjustable setting for proportional control is called the controller gain (K_c). A higher controller gain will increase the amount of proportional control action for a given error. If the controller gain is set too high the control loop will begin oscillating and become unstable. If the controller gain is set too low, it will not respond adequately to disturbances or set point changes.

$$P = K_c \times E$$



While most controllers use controller gain (k_c) as the proportional setting, some controllers use proportional band which can be seen in the below table.

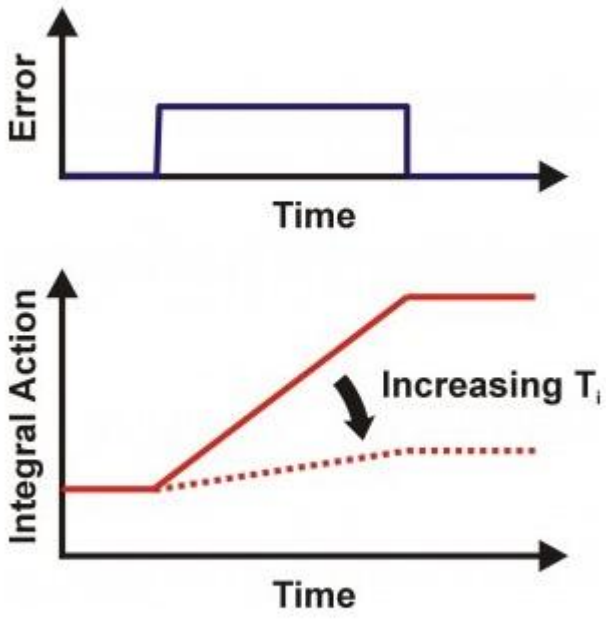
Controller Gain (K_C)	Proportional Band (PB) %
0.1	1000
0.2	500
0.5	200
1	100
2	50
5	20
10	10

Integral controller:

As long as there is an error present (Process variable not at set point), the integral control mode will continuously increment or decrement the controller's output to reduce the error. This mode will drive the controller output far enough to reduce the output to zero, if given enough time.

If the error is large, the integral mode will increment/decrement the controller output fast, if the error is small, the changes will be slower. For a given error, the speed of the integral action is set by the controller's integral time setting(T_i). A large value of T_i results in a slow integral action and a small value of T_i results in a fast integral action. If the integral time is set too long, the controller will be sluggish, if it is set too short, the control loop will oscillate and become unstable.

$$I = I_{previous} + K_C \times E \times \frac{T_S}{T_I}$$



$$CO = K_C \left(\frac{1}{T_I} \int E \, dt \right)$$

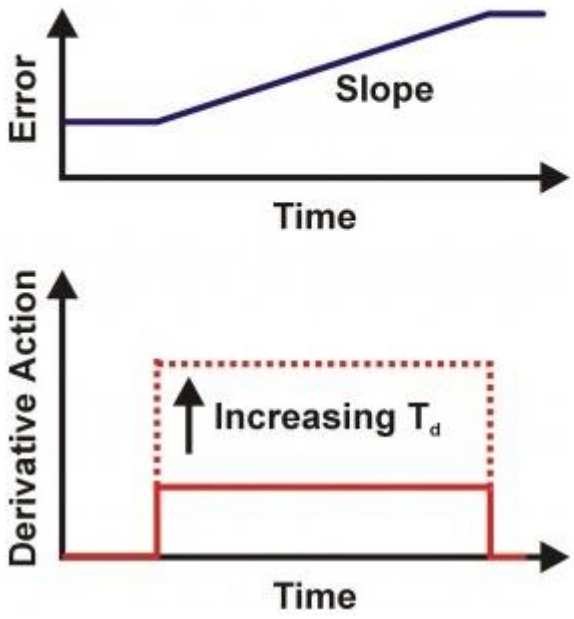
Most controllers use integral time in minutes as the unit of measure for integral control, but some others use integral time in seconds, integral gain in repeats per minute or repeats per second which can be seen in the table below:

Integral Time		Integral Gain	
Minutes	Seconds	Rep / Min	Rep / Sec
0.05	3	20	0.333
0.1	6	10	0.167
0.2	12	5	0.0833
0.5	30	2	0.0333
1	60	1	0.0167
2	120	0.5	0.00833
5	300	0.2	0.00333
10	600	0.1	0.00167
20	1200	0.05	0.00083

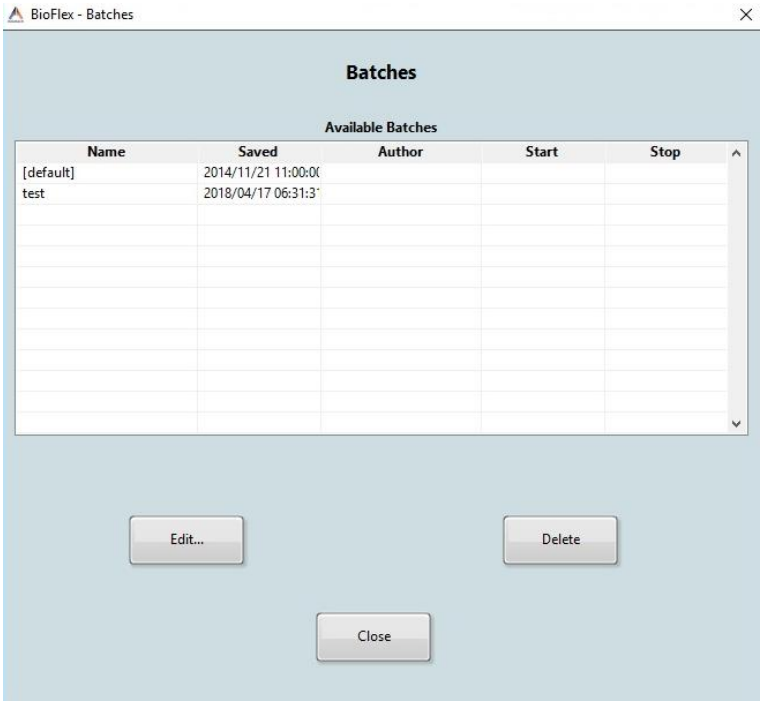
Derivative control mode:

The third control mode is the derivative mode in PID controller. The derivative control is rarely used in controlling processes, but it is used often in motion control. For process control, it is not absolutely required, because it is very sensitive to measurement. The derivative mode produces an output based on the rate of change of the error. This mode produces more control action if the error changes at a faster rate. If there is no change in the error, the derivative action is zero. It has the adjustable setting called Derivative time (T_D). The larger the derivative time setting, the more derivative action is produced. A derivative time setting of zero will effectively turns off this mode. If the derivative time is set too long, oscillations will occur and the control loop will run unstable. T_s is the controller's execution interval.

$$D = K_C \times \frac{T_D}{T_S} \times \left(E_{previous} - E_{now}\right)$$



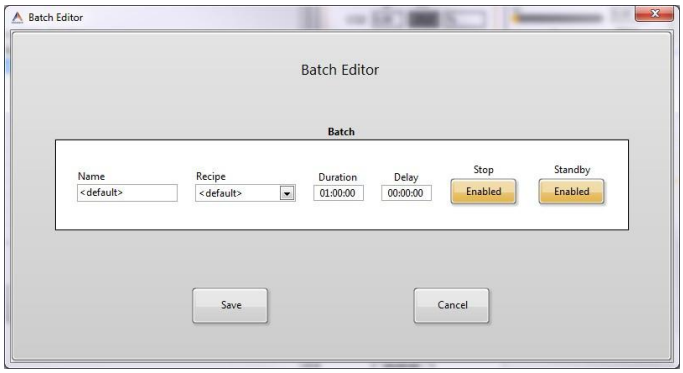
4.3. Batches tab



The batches tab contains the list of operations made and saved on the Bioflex software.

Selecting a batch opens the above window, allowing the user to operate the following actions.

- The "Delete" button delete the selected batch (with the exception of the default batch).
- The "Edit" button opens a window for setting the characteristics of the batch, in terms of time duration, delay, as shown in the following figure.

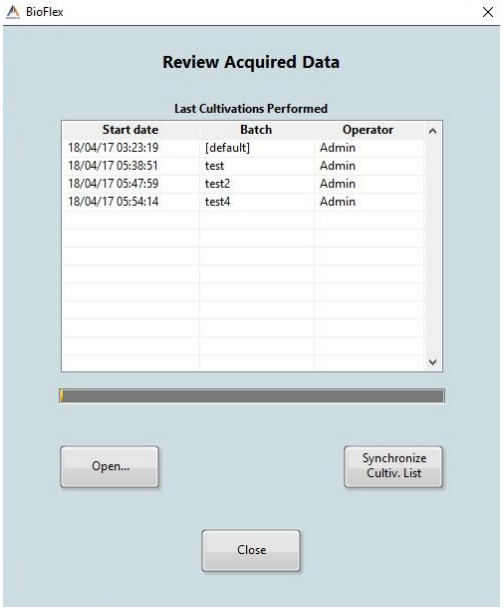


It is necessary to specify a batch name, select the recipe to which, the batch is related, specify a total duration of cultivation and an initial delay.

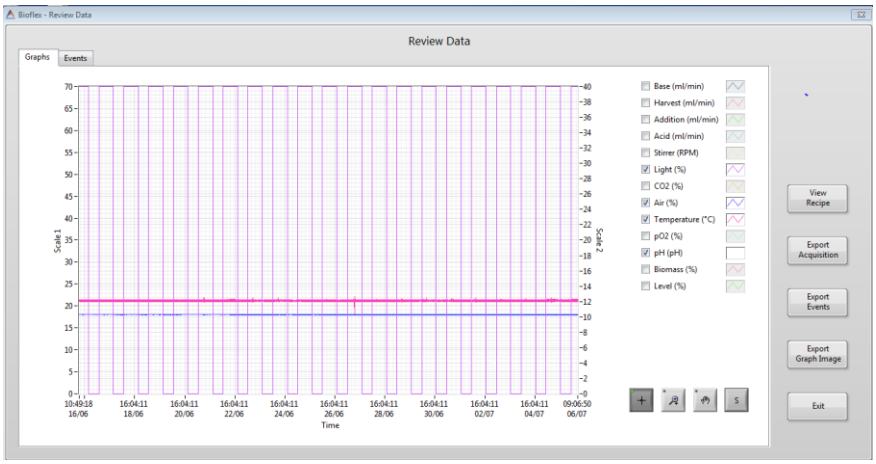
After pressing "start cultivation" set points remain locked to their initial value until the time of delay expires.

The image on the left shows the “Batch Editor” setting window. In this window, it is possible, for the administrator, to authorize the "operator" to stop or put on standby the cultivation at any time, by not modifying the settings. In order to put the process in standby or to stop, the buttons has to be remained “Enabled”. When “Disabled”, it is not possible for the User to stop the cultivation or to put in standby.

4.4. Review Acquired Data:

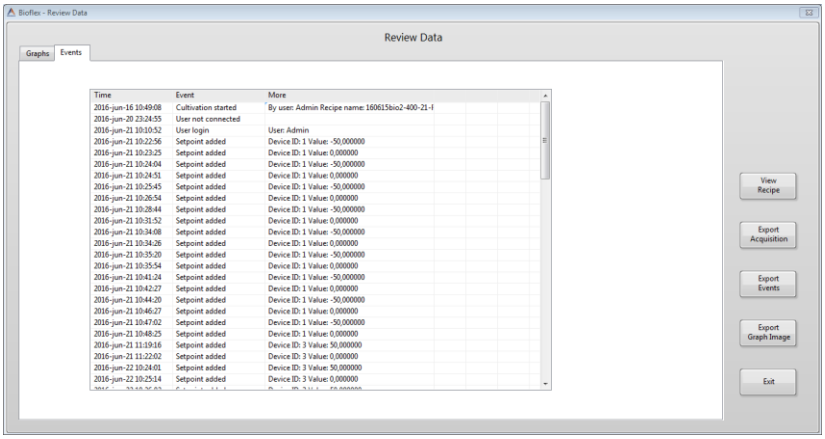


"Review Data" tab let the user have access to the list of cultivations performed by bioreactor. The "Open ..." button opens a window that allows you to review and export the log. The "Delete" button deletes the log files selected from PC. "Download current log" button to download the log of the last cultivation performed, or the current one.

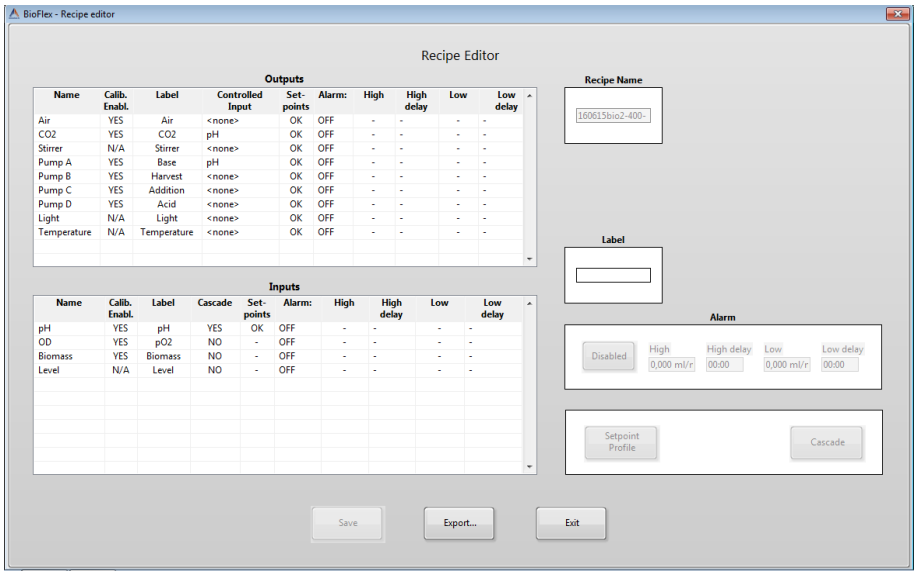


Click on Open button will open the review data window; this window is composed of two pages, named Graphs and Events.

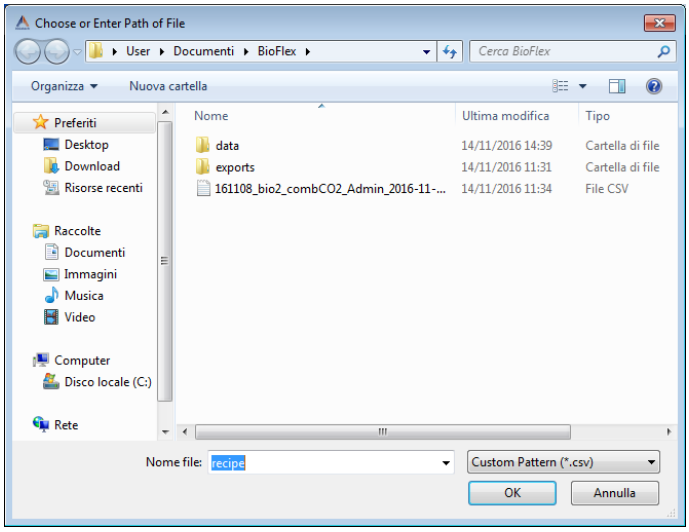
The Graphs page reports graphs of the selected cultivation; it is possible to operate on the graph look, in many ways. For detail, see the graph Section paragraph.



The Events page reports list of all the events occurred during the selected cultivation: time, event, and detail for each record is reported.



View recipe is a shortcut to Recipe Tab; click on the button will open the recipe of the selected cultivation, opening the window on the left. In this window the user can view the recipe, but not modify it.



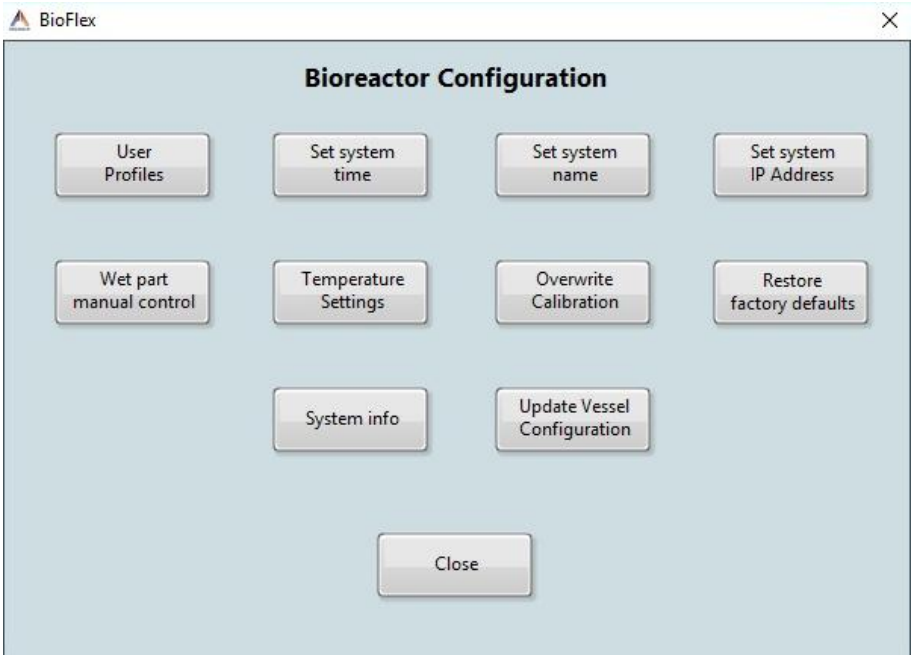
The user can export the data of acquisitions, events, and graphs by click on “Export acquisition”, “export data” and “Export graph image” buttons. Both will open a window where the user can choose name and folder to save data.

4.5. System configuration:

The “System configuration” tab allows you to access the advanced configuration of the bioreactor by under sections:

- User profile
- Set system time
- Set system name
- Set system IP address
- Wet part manual control – inactivated until “Manual” button is pressed
- Temperature settings
- Overwrite Calibration
- Restore factory defaults

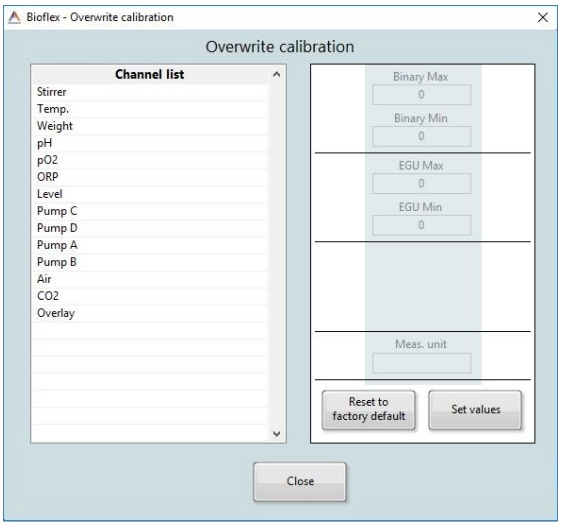
- Update Vessel Configuration
- System info



4.5.1. Overwrite Calibration

This function is available only for Administrator, and is used to overwrite calibration values. By this window the administrator is able to set minimum and maximum values for each utility, and the appropriate Measurement Unit. Click on the value to open a keyboard where user can enter the values. Normal operation do not require modifications of these values, but during calibration procedure it requires to be changed.

"Max" and "Min" fields represents minimum and maximum calibrated values. These values depends on the specific input or output. For inputs have to be set with "1" and "0", for outputs can be set with "1" and "-1" values (i.e. for Pumps, indicating two different rotation directions).



EGU means "Expected General Unit", and is referred to the measurement unit of the specific channel. "Max EGU" and "Min EGU" have to be set with maximum and minimum values of the device, in relation to the measurement unit ("Meas unit" field). For example, for Stirrer set the minimum rotation value (i.e. 5 RPM) and the maximum rotation value (i.e. 1200 RPM) for device purchased. For Pumps the values have to be set both positive and negative, indicating two rotation directions: for example, if the

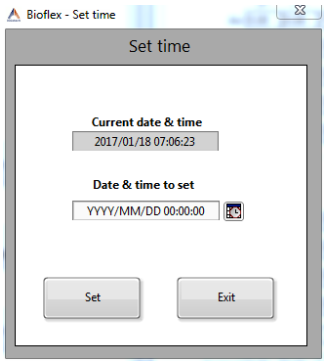
maximum value is 100 ml/min (to be set in “Max EGU” field), set -100 ml/min in “Min EGU” field.

“Max 2 out” and “Min 2 out” fields are referred only to Mass Flow Controller. If these devices are present, these fields have to be set with “1” and “0” values.

In this window, it is possible to invert the rotation direction of peristaltic pumps; to invert rotation, select the pump in the list, then change the “Max EGU” and “Min EGU” values into each other.

When values for a channel are modified, press on “Set values” to save the changes done. Press “reset to factory default” button to set to default conditions.

4.5.2. Set System Time

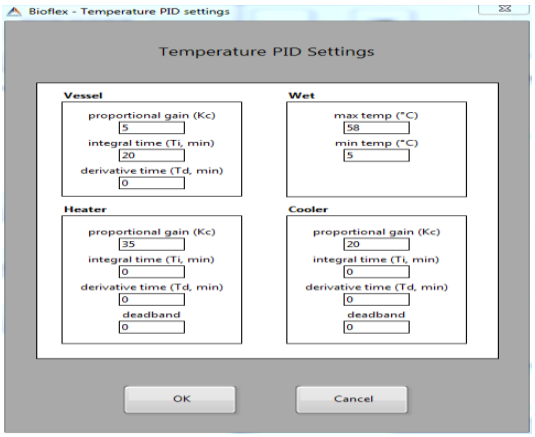


By this window the user can view the current date and time, and eventually change it. A click on Date & time will open a keyboard where the user can define the correct date and hour. Click on “Set” to confirm the date and time entered, click on exit to discard the changes and close the window.

4.5.3. Set System IP address

In this window, the user can define the new system IP address to the bioflex software. The existing IP addresses can be seen through the connected systems tab which was shown in the section 3. The user have to change the IP address, only when there is another system connected in the same network with the same IP address. The default IP address will be 192.168.111.33 or 34. If the user decides to change it, because there is another system with the same IP address. He/ she can modify it as 192.168.111. (0-255). This window can be accessed through system configuration tab.

4.5.4. Temperature PID settings

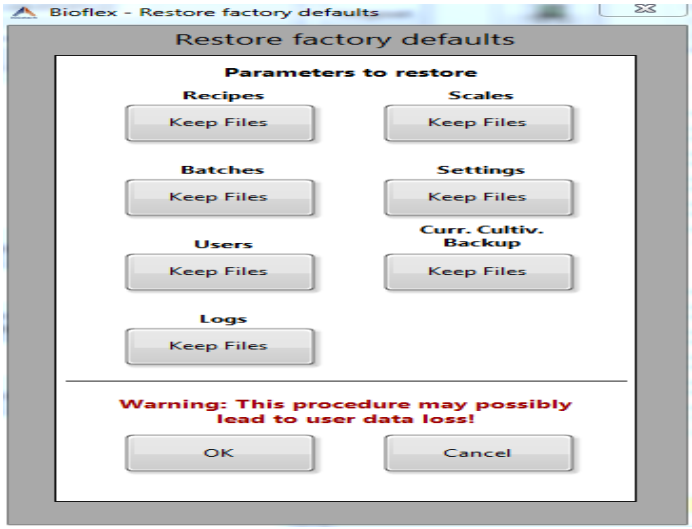


In this window, it is possible to set the values of PID, so that the response of the system can be within the regulation of parameters. User can set the values of proportional gain Kc, integral time (Ti) and derivative time (Td) for Vessel, Wet, Heater and Cooler. Modification of these values will lead to a different speed of responses from the instrument. However, it is not recommended to modify these values.

4.5.5. Restore Factory Defaults

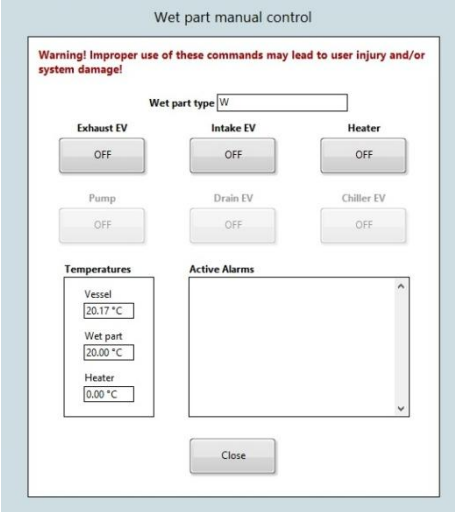
By this window, it is possible to restore the default parameters of the system; the user can choose to maintain some of the files, while resetting the rest. Click on Ok will restore the factory parameters, with the exception of the selected file by the user.

Use this function when a new vessel configuration is updated on Bioflex. After updating, restore all factory defaults, for a proper working of the new configuration.



4.5.6. Wet part manual control

The temperature control circuit can be manually controlled in this window “Wet part manual control”. By this window the user can manage the exhaust cooler valve manually, Intake valve, Drain valve, optional Chiller valve, Recirculation Pump and Heater.

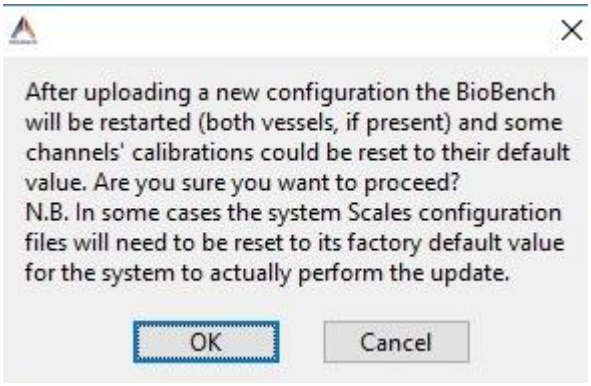


Pressing the button of each utility will turn it on and off. Press “Exit” to close the window.

Use this function to fill the temperature control circuit before starting cultivation. It is important that the circuit is correctly set up as explained in Bio-bench Manual; check all the hoses are correctly disposed.

Open the Intake valve will fill the circuit with water from supply line. Open also drain, and start recirculation pump, to fill completely the circuit and avoid formation of air bubbles. Once the circuit is full of water, close the Drain valve. It is important to test heating system only with full-circuit; turn on heater without water in the circuit will led to serious damage of the heating system.

4.5.7. Update Vessel Configuration



A click on Update vessel configuration button will open the window on the left: this function is used to upload to Bioflex a proper configuration file. If the user desire to proceed, a click on OK will open a window where the user can search on his computer the configuration file to be uploaded. Configuration file is a text file with .ini extension.

It is possible to configure Biobench system in many different ways, adding or removing modules connected through ETH cables; changing system configuration requires a change in Vessel configuration, and a proper configuration file. In order to configure, it is necessary to contact Solida Biotech for a configuration license.

Once the file is uploaded, Bioflex will log out; wait few seconds until the indicator turns green, then log in again to the newly configured Bioflex.

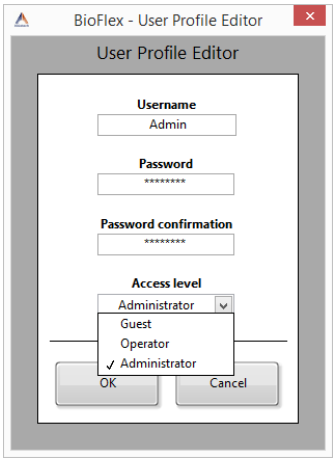
4.5.8. System info

Pressing the System info button will open the window, where the HMI, RT versions and serial number and part number of the current Bioreactor is reported.

4.5.9. User profiles tab

The "User profiles" tab allows you to configure a list of user profiles, which allows you to make a new user or delete the old one, as shown in the figure below.The system provides three level of access, based on privileges:

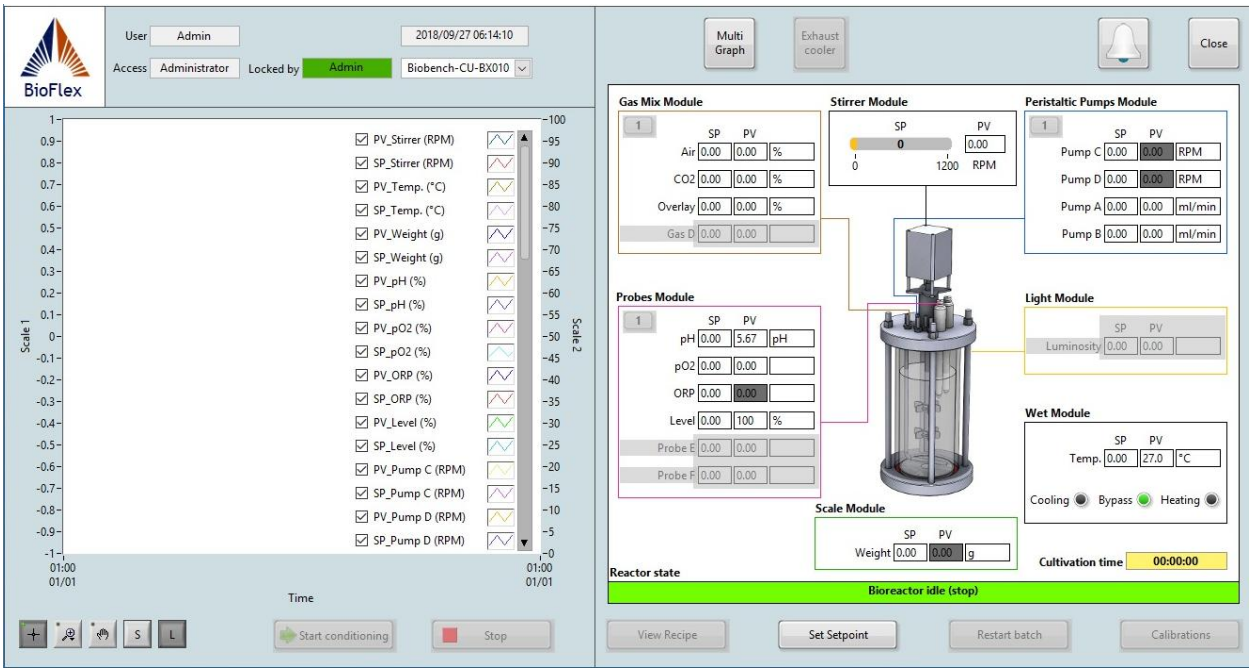
- Guest – basic access. Guest cannot create or modify recipes, they just export data. Can start a cultivation by a batch, but cannot modify it. Guest cannot operate manually on parameters.
- Operator – intermediate access. Operator can set up and modify recipes, batches, and operate manually on parameters and also on Wet. The operator can also set up calibrations.
- Administrator – full access. Administrator have full access to all functions of Bioflex, including creating new users.



It is possible to create a new user, by the button “New…”, and delete user from the list, by the button “Delete”. The default User “Admin” cannot be deleted.

The button “New…” will open the “User Profile Editor” window, as shown in the image on the left. In this window, it is possible to set the new user profile, in terms of Username (minimum length 5 characters, only alphanumeric characters and dots are allowed), and Password (minimum allowed size is 8 characters), that must be confirmed below. It is necessary to specify the new user Access level (Guest, Operator, or Administrator). Press “OK” to confirm or “Cancel” to abort the new user creation and to close the window.

5. Synoptic section



In the right half of the screen, displayed the synoptic of the bioreactor, in which the set point (SP) and the present value (PV) of each of the devices of the system were displayed.

On the top right are four buttons, from left to right:

- Multi-Graph: allows an operator the graphic view of the process (One, Two and four vessel).
- Exhaust cooler: allows operator to enable and disable the recirculation of the cooling water of condenser
- Events Viewer (bell): opens the screen cultivation events including alarms.
- Close: is used to close the program (alternative to the (X) windows button at the top right)

On the bottom right are displayed four buttons, that allow the user to interact with the devices, when authorized, even during cultivation. From left to right:

- View Recipe: is a shortcut to Recipe Editor; is used to review the recipe set, of the current cultivation
- Show messages/Set Setpoint: To view an error message during the cultivation/ To set the values to the certain devices.
- Restart batch: Allows the user to restart the batch from the beginning. It can be used even during the cultivation.
- Calibrations: allows the user to perform the calibration of the devices during the standby or when the cultivation is stopped

In the middle of the window a scheme of the Vessel is shown, and Set Point Value and Present Value for each utility are reported.

The modules are the following:

- Gas Mix Module – is the module that manage the intake and out take of gases
- Probes Module – user can view the values for the set probes
- Stirrer Module – user can view the speed set point and present value of speed of the stirrer
- Peristaltic Pumps Module – user can view set point and present value of the set pumps
- Light Module – user can view set point and present value of the light in the vessel
- Wet Module – if a Wet is present, user can view set point and present value of temperature of the water, and if the system is cooling, heating or bypassing the vessel
- Scale module – if a scale is present under the vessel, user can view set point and present value of the weight of the vessel

Synoptic Section is the main interface for monitoring parameters and operate on them during cultivation. A click on each utility SP/PV, will open a keyboard where the user can define the Set point Value to monitor the Present Value. Some of the Modules or single utilities may not be active, depending on the utilities present in purchased system and on the configuration updated. If an utility is not connected, PV window background is dark grey; after connecting an utility, should turn white after one minute. If not activates after a minute, put the system in block and restart it. For oxygen probe, wait (2-24 hours)until polarization automatic procedure to complete.

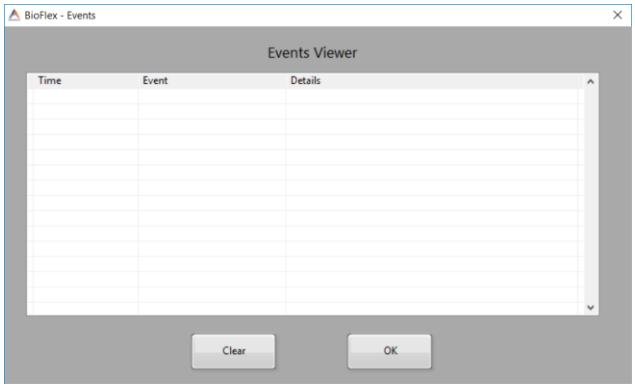
Gas Mix Module:

In Gas mix module, based on the configuration asked by the customer, we at kbiotech design gas mix module either Rotameter or Mass flow controller (MFC). The below table shows the flow rate for both rotameter and MFC's.

Gas	Flow rate (Rotameter)
AIR	500 – 10000 CCM
O2	100 – 1500 CCM
N2	100 – 1500 CCM
CO2	100 – 1500 CCM

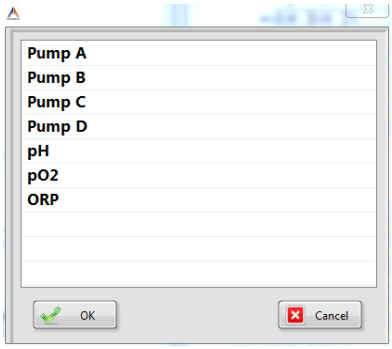
Gas	Flow rate (MFC)
AIR	20 F.S. – 1000 CCM
O2	20 F.S. – 1000 CCM
N2	20 F.S. – 1000 CCM
CO2	20 F.S. – 1000 CCM

5.1. Events viewer



Pressing the Events Viewer button(Bell icon) will open the window on the left, where the user can see all the events occurred during a cultivation. Press Clear button to delete the events.

5.2. Calibrations

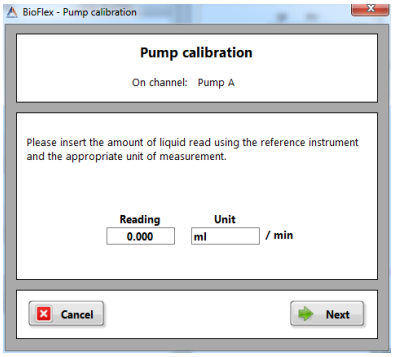


This section is used to set the calibration for the pumps, and for pH, and pO2 probes. It can be calibrated also for level and ORP probes. Calibration of biomass probe is set through Fogale Software (read “Additional Software chapter).

Calibration is necessary for the instrument to work properly; it is recommended to perform at least a calibration of probes before starting cultivation and a calibration for the pumps when the hoses are changed.

A click on “Calibrations” button will open the window on the left. Here the user can choose the device to be calibrated. Select the device to calibrate and press OK. This will start the calibration procedure.

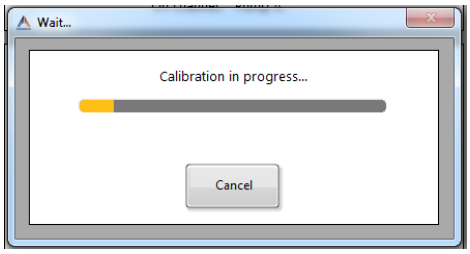
5.2.1. Pumps calibration



Before starting, connect the tube (3X8 mm or 4X8mm)to the pump, put the rear of the tube in a container with water and the tube head in an empty graded becher.

At first, the system will ask whether the direction of rotation of the peristaltic pump have to be inverted or not. Once selected, the direction of rotation, click on “start pump” to start the pump, and let the pump working until the hose is totally filled; this step avoids errors in calibration due to presence of air bubbles in the tube.

When the hose is totally filled stop the pump, by clicking on “Stop pump”, and proceed to the second step by clicking on “Next”.



Click on “start calibration” will start the pump for a minute and after the pump will stop automatically. Once the pump has stopped, enter the value which was measured by the use of graduate flask and weighing scale in the reading tab and by clicking “Next” will end the calibration.

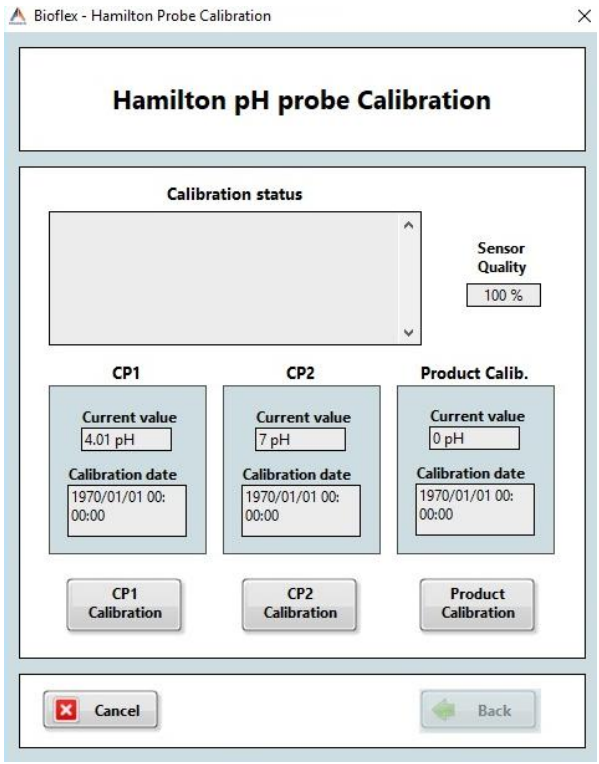
Tube	Flow rate	
	MIN.	MAX.
3X8	2.5 ml/min	50 ml/min
4X8	5 ml/min	85 ml/min

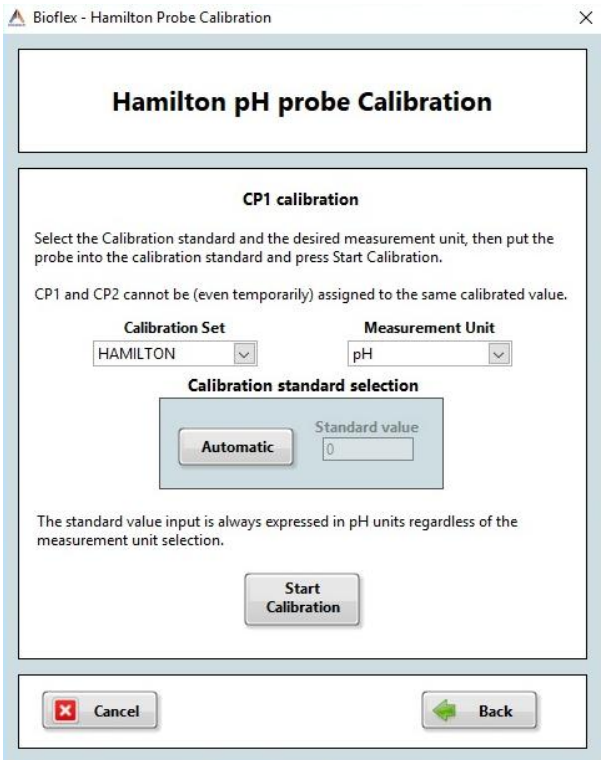
5.2.2. pH calibration:

Calibration of pH probe is made at two values, e.g. an acid and a basic value or an acid and a neutral value. To calibrate pH user needs standard solutions with known pH values, or a already calibrated pH meter.

First select the measurement unit, in this situation, “pH”; click on next.

Insert the probe to be calibrated in the acid solution. In the “Probe reading” field a signal form the probe will appear, in form of number three decimals (e.g. 0,123); wait until the value is stable.

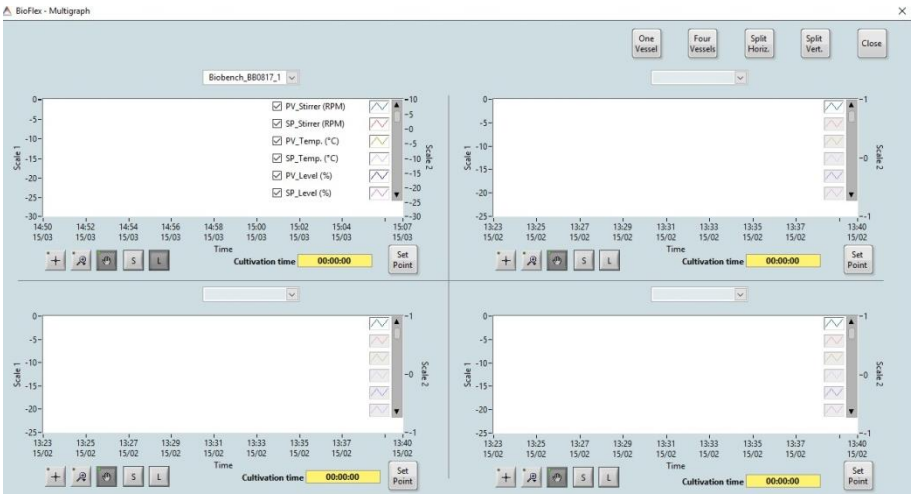




Click on the “Calibrated value” field will open a keyboard where enter the pH value of the solution in which the probe is inserted, or the value red on the calibrating device. Once inserted close the keyboard and click on “Next”.

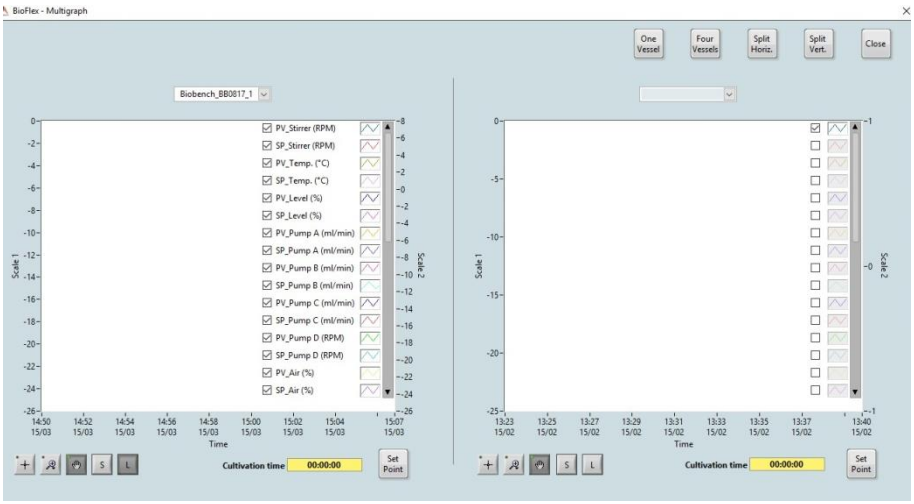
Repeat the procedure done with a second solution with a different pH value, and inserting correct calibrated value. Once completed this second step, click on “Next” to end the calibration.

6. Multi-Graph section



It is possible to go to graph section by pressing the graph icon, in the main interface, over the synoptic.

The 2 vessel configuration graph is shown in the following figure:



The graph section allows the user to view the variation of parameters during the time of current cultivation. For this reason are reported all the parameters, both current and the set point.

By default, on the right of the graph area, legend of the parameters is reported. Below the graphs area are the buttons:

- Cursor graph
- Enlargements graph
- Drag the graph
- Setting up scales of the graph
- Graph legend (enabled by default)
- Button "start conditioning" / "start cultivation": the first press starts the conditioning, second press starts the cultivation
- "Stop" button: Stop current cultivation

Cursor graph is activated when the icon is selected: with this tool the user can move and select small areas on the graph.

A click on the enlargements graph icon will open another window, with different zoom options for the graph area.

Drag the graph is activated when the icon is selected: with this tool the user can move the whole graph.

The graph plots option is activated by click on the icon with “S”. Click on the icon opens a window where the user

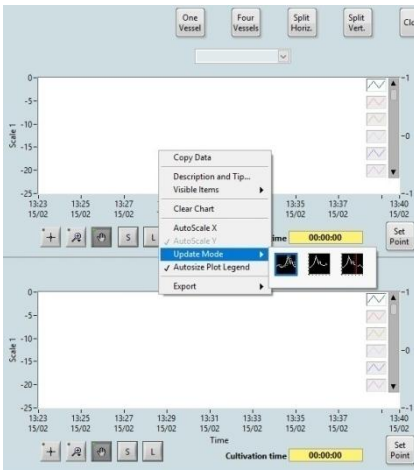
The first option (Plot Visible) is used to select the variables to be displayed in the graph; is possible to select the variables to display even from the graph area, making a thick on the desired variables.

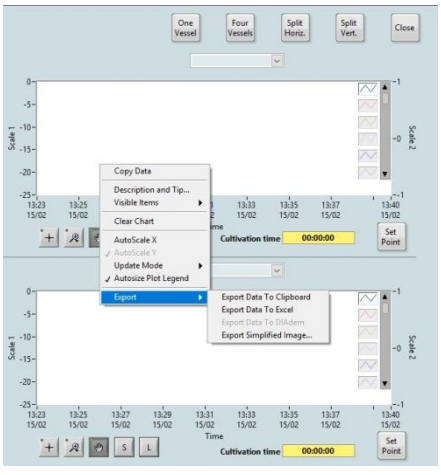
Click on “Common Plots” will open a window that lets the user choose different styles of plots, as shown in the image on the right.

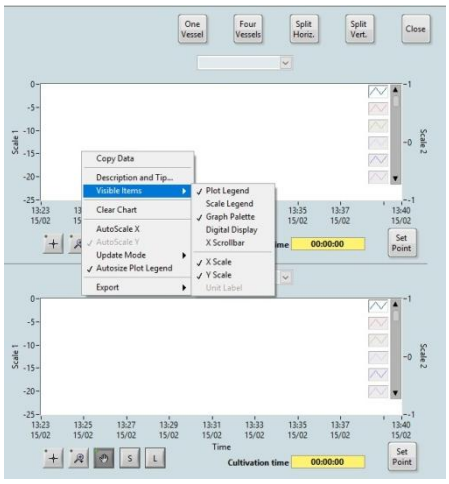
The line can be personalized even with the “Line style” option and “Line Width” option, that let the user choose the style and width of the line of the selected parameter. If the user does not modify these parameters, default color, width and style will be applied.

The “Anti- Aliased” option, if selected, enhance the quality of the line when high zooms are performed.

The “Fill Base Line” option is used to choose the style of coloration of the graph under and above the line: user can choose to color all the graph area above the line, or under the line, or between line and zero.







The “X Scale” and “Y Scale” options are used to determine the axis on which the parameter is represented. It is not possible to modify the X scale, which is always the Time. The user can choose, the selected parameter, if the values have to be reported to the first or the second Y axis.

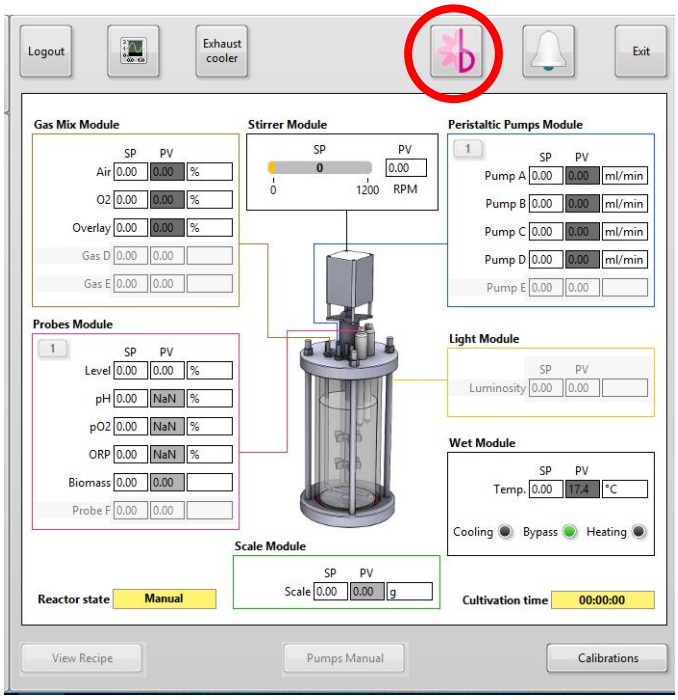
It is possible to export the data to clipboard or to Excel by selecting the “Export” option.

7. Additional software required

Bioflex software, as shown in the previous chapters, is used to manage the Biobench system. This software is given to the user in a CD-Rom, ready for installation on user computer, or already built, in the Biobench with integrated touch screen.

To help the user with technical assistance, your Biobench system can be remotely controlled from a PC via supervisory software. For this reason, it is highly recommended for the user to install on the computer associated with the Biobench, the free version of TeamViewer (www.teamviewer.com/Download).

7.1. Foggle Software and Cell density probe calibration



If a Cell density Probe is purchased, Installation of Foggle Software is required. Hamilton Density probe can be used with Foggle interface, without connecting it to Biobench, or through Bioflex interface, connecting it to Biobench Control Unit Module (refer to Biobench Manual). Once installed, Foggle icon will appear on Bioflex Synoptic section, as shown in the image on the left.

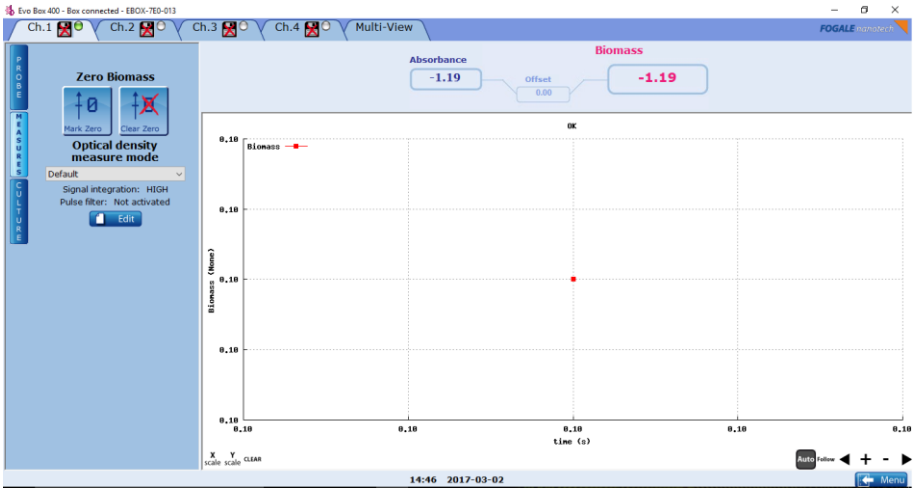
By this link, user can enter in Foggle interface. Keep the Foggle software open when operating with a Biomass probe; however values red by probe will appear in Synoptic Probes Module section.

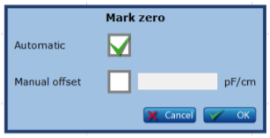
This software is used also for calibration of Biomass probe. Calibration procedure is reported, for further detail, please read device manual.

Open Foggle Software clicking on the icon. Check the Ch. 1 indicator on top left of the window is green, indicating the probe is correctly connected and ready. For connection procedure refer to Biobench Manual.

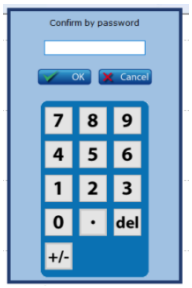
If probe has just been connected, wait a minute until the system recognize the probe.

Equilibrate the Incyte Sensor in culture medium, for at least 30 minutes prior to product calibration and inoculation. Verify on the Main Graph that the cell density measurement is stable.





Go to the Measure Settings Menu and press the Mark Zero Button to perform a product calibration.



Enter the User password (14147125) if one has been defined. Press OK.Select automatic. The cell density measurement is now compensated for an offset shown in the measurement values above the Main Graph on the Run Menu.

8. Contacts

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