

Standardized Procedure Marubishi MPF-U -bioreactor

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1 Purpose of use

The Marubishi MRF-U Bioreactor is intended for microbial cultivation on a small pilot scale (recommended volume up to 100 l).

2 Person in charge

The equipment manager of the Marubishi MPF-U bioreactor is Tero Eerikäinen.

3 Operation instructions

WARNING!

- Before starting the mixing, always make sure that either steam or pressurized water is fed into the mixing pass seals and that the bioreactor is not completely empty! Otherwise, the agitator gasket may be damaged!
- Before starting the heater, make sure there is water in the heat exchanger! (eg by opening valve 49 and looking that water is coming)

3.1 Preliminaries

- Turn on the Steam-Elmo steam generator. Close the outgoing lines from the steam generator, turn on the main switch, open the water tap from the sink. Set the steam generator at half power first, but at full power during bioreactor heating.
- Install the required electrodes (eg pH and oxygen) in the bioreactor. Calibrate the pH electrode outside the bioreactor before sterilization (see, for example, Iwaki pH control pump instruction)

- Close all bioreactor valves
- Check that the ends of the aeration ring are secured. Use pliers.
- Close the lower door (window) with a torque wrench. The correct torque is 15 Nm.
- Make sure that all Power switches (push buttons: mixer, heaters, etc.) on the front panel are in the off position.
- Turn on the main switch of the switchboard.
- Make sure that the Auto Fuses on the rear panel of the control panel are all in the on position (top). NOTE! The wires inside the panel have high voltage!
- Turn on the Bioprocess Controller and make sure it is not in the Cultivation position. (The cultivation position can be switched off by pressing the "cul" key.)
- Open the main taps for air, water and steam lines. (air and water next to the wall, steam on Steam Elmo)
- Empty (if not already emptied) reactor jacket: Valves 44, 3 and 41 open and closed

3.2 Filling the bioreactor

- Fill the bioreactor with water or broth through the top window.
- Make sure there is water in the mixer lubricant. If necessary, add water through the water(-grease) container cap.
- Pressurize lubrication system. Open valves 4, 8, 9, 10, 11, 50 and 54. Make sure that the lubrication pressure has risen above 0.5 bar.
- Turn on the mixer from the front panel red Agit-On push button and manually adjust the speed using the Bioprocess Controller (slow-fast) eg 200 rpm (mixing speeds up and slows down with a delay). SV or set value obeys commands only in the cultivation + Auto position. Auto setting = Auto + enter, Enter Auto + CLR to return to the manual position.
- Add any solid materials you may need to the substrate. Adjust the pH of the substrate.
- When homogenous, stop mixing: turn off the mixer and close the valves (4, 8, 9, 10, 11, 50 and 54) opened above and the top window of the bioreactor with torque wrench. The correct torque is 15 Nm.

3.3 Sterilization

Sterilization is carried out in six steps, which are heating₁, heating₂, sterilization, cooling 1, cooling 2 and finishing. For each step, see General Information below and enclosed lists with adjustable valves and order of adjustment.

Symbols used in the checklist:

- O Open the valve
- X Close the valve
- S Steer the valve
- O- -X Open during emptying and then close

HEATING 1:

In Heating 1, the medium is heated to boiling by passing steam into the jacket. At the same time, most of the air in the reactor is removed and the waste lines are sterilized. Monitor the temperature on the screen (the thermometer on the side operates with a delay).

HEATING 2:

The heating 2 step is started when the solution is about 95-98 °C. At this stage, steam is introduced into the bioreactor from all passages. At the same time, the temperature and pressure rise to the desired sterilization values inside the reactor. The sleeve safety valve opens when the sleeve is pressurized to 2 bar.

STERILIZATION:

This step can be started when the solution temperature is 110 °C. All incoming steam lines are throttled and the exhaust air valve opened if necessary. Excessive opening of the exhaust air valve drops the pressure and causes the solution to boil out of the reactor. The exhaust air valve should be adjusted carefully (however, the valve cock may be turned quite a lot before the line opens; yes, the air flow is noticeable). The safety valve located in the exhaust air line opens at 2 bar. When the temperature drops, steam may be released into the sleeve for about 20 seconds, otherwise the sleeve will be steamed during the sterilization phase. The duration of the sterilization step is 7 to 8 minutes shorter than the total sterilization time. If the pressure in the mixer rises above 2 bar during sterilization, adjust the pressure with valve 53. Also the water in the mixer lubricant will be drained, so at the end of steaming, briefly set off the mixer and add water to the lubricant vessel..

COOLING 1:

During cooling 1 first close all lines entering the bioreactor. Thereafter, all lines from which the steam escapes from the reactor are immediately closed. After adjusting all the necessary valves, wait 5-10 minutes for the air filter to dry and the temperature to fall below 120 ° C. Thereafter, move to step of Cooling 2 (a slight overpressure must be maintained throughout the reactor cooling).

COOLING 2:

This takes rather long time. Cooling 1 first closes all lines entering the bioreactor. Thereafter, all lines from which the steam escapes from the reactor are immediately closed. After all necessary valves have been adjusted, wait 5-10 minutes for the air filter to dry and the temperature to fall below 120 ° C. Thereafter, move to Step 2 of Cooling (A slight overpressure must be maintained in the reactor throughout the cooling).

FINISHING OF STERILIZATION:

The cooling of the bioreactor is stopped and the automatic temperature control is switched on. Setting the temperature control: In addition to setting the setpoints (temperature: auto-enter-cul-> adjust SV = set value), the jacket pump and the heating resistors are pressed. (Depending on the desired temperature, either one or two heat resistors are used).

The air is directed through the flow meter to the bioreactor. Once the "Finishing of Sterilization" is completed, the acid, alkali and antifoam containers are filled.

NB! The acid, base and antifoam must be sterilized separately in an autoclave. The filler tanks are filled with bunsen flame and the tank caps are kept in ethanol during the filling. The pressure valves in the tanks are closed during filling (18, 21 and 24).

Other desired setpoint settings are set in the Bioprocess Controller. At the same time you select which quantities you want to adjust automatically and manually (auto = auto + enter, manual = auto + CLR). Be sure to set the Cultivation setting. So, for example pH adjustment: auto-enter-cul -> adjust SV as desired.

For dissolved oxygen, see Table 1. Note! The use of mixing in DO control should be considered on separately for each cultivation (mixing rises only / up to 500 rpm).

Table 1. Various options for controlling dissolved oxygen

Quantity	Mode	DO-control
DO	Auto	DO control is achieved by both mixing speed and aeration
Agit	Man	
Air	Man	
DO	Auto	DO control is done only by the mixing speed
Agit	Man	
Air	Auto	
DO	Auto	DO control is done only by aeration
Agit	Auto	
Air	Man	
DO	Man	No DO control

Once the desired temperature has been reached, adjust the reactor to a slight overpressure by adjusting the exhaust air and aeration (14 and 25).

3.4 Inoculation

- Connect the precultivation bioreactor bottom valve with hose to the inoculation valve 38.
- Sterilize the inoculation line towards the precultivation bioreactor by first closing valve 37 and then opening valves 36, 39, 38B and slightly valve 40 (steam drying). After a while, open valve 38. Steam the desired time. Close the open valves and change the direction of sterilization.

- Change sterilization direction: sterilize the inoculation line toward the 200-liter bioreactor: Open the bottom steam of the precultivation bioreactor and open the valves 38 and slightly the valves 40 from the 200-liter bioreactor. Sterilize as long as needed and close the valves..
- Close the aeration during inoculation.
- Transfer the bioreactor by first increasing the precultivation bioreactor pressure to about 1 bar and then opening valves 38, 38B and 35 and finally the precultivation bioreactor bottom valve.
- Sterilize the inoculation line towards the 200-liter bioreactor again (close the passage to the 200-liter bioreactor)

Alternatively:

- Autoclave the inlet hose• Steam 10 L reactor bottom valve and 200 L reactor inlet
- Attach the inoculation hose and steam it through a 10 L reactor and allow to cool.
- Stop the 10 L reactor cultivation (put settings off) and leave stirring on
- Stop aeration if on
- Close the 10 L reactor exhaust air line to raise the pressure
- Carefully raise the pressure to the 10 L reactor with low aeration rate, open the precultivation reactor bottom valve and the 200 L reactor three inlet valves.

3.5 Reactor sampling

- Sterilization of sampling line: Close air line (valve 30). Open valves 29 and 28 (Steam valve 1 must be open and steam generator has to be on). At the end of steaming, close the valves.
- Take a sample after the tubes have cooled slightly by opening the valves 27 and 28. The sample comes out by the pressure of the bioreactor.
- Blow the remaining sample back into the bioreactor by briefly opening valves 30 and 27.
- Steam the line again.

3.6 Stopping the cultivation

- Decrease mixing and aeration rates.
- Change settings from automatic to manual. Close the water cycle.
- Open the exhaust air valve (25) completely to avoid vacuum in the bioreactor.
- Open the bottom valves and remove the growth from the bioreactor (biomass killing, eg by acidification or heat treatment).
- Close the aeration valve (14) completely when the reactor is empty.
- Open the top door (window).
- Remove the electrodes.
- Rinse the inside of the bioreactor with warm water. Most dirt will be removed if rinsing is done immediately. Also remember the backside of the baffle plates and the aeration ring (ie loosen the screws).
- Open the solenoid valves on the filling vessels to empty the containers. Let warm water through the solenoid valves of the addition vessels.

- Fill the reactor with 100 liters of 15-20 °C water. Set the aeration rate at about 10 L / min. The mixing can be e.g. 50 rpm. Add a bottle or 750 ml of Chlorine Hypochlorite Solution (less than 5% sodium hypochlorite) or equivalent. Leave on for about 15 minutes (top door open), empty the reactor and rinse the inside surfaces very carefully. Close the aeration when the reactor is empty.
- If necessary, sterilize the bioreactor.
- Turn off all power switches and leave both top and bottom doors open.

4 Appendices

HEATING 1

Set Steam-Elmo to half power, make sure that there is enough water in the steam generator tank. Close all valves, open main air, water and steam valves (not in reactor).

Order	Valve nr.	Valve or function	Valve position
1	01	Steam in	O
2	04	Air in	O
3	05	Water in	O
4	02	steam trap	O
5	03	Drying of steam	O- -X
6	11	Air pressure meter	O
7	16	Base control	O
8	19	Acid control	O
9	22	Antifoam control	O
10	18	Base pressurization	O
11	21	Acid pressurization	O
12	24	Antifoam pressurization	O
13	alkl	Base solenoid valve	Open from control cabin
14	acid	Acid solenoid valve	Open from control cabin
15	af	Antifoam solenoid valve	Open from control cabin
16	57	Exhaust air out	X (is already?)
17	58	Exhaust air to steam pipe	O
18	25	Exhaust air control	O
19	29	Sample, steam	O
20	28	Sample, out	S
21	33	Bioreactor drain line steam	O
22	32	Bioreactor drain valve 2	S
23	36	Inoculation, steam	O
24	39	Inoculation, air/steam input	O
25	38	Inoculation, input 1	S
26	40	Inoculation, out	S
27	42	Steam pressure meter	O
28	43	Sleeve steam trap	O (noise)
29	41	Sleeve, Steam in (increase temperature to about 95 °C)	O (noise) -> S
30	44	Sleeve drying	O (steam) -> X
31	51	Mixer sealing, steam	O
32	54	Mixer sealing pressure meter	O
33		Mixer start	

HEATING 2

A busy step. Set Steam-Elmo to full power. NB! Monitor the temperature on the control unit display. Start this step when the temperature is 95... 98 °C according to the bioreactor control unit.

Order	Valve nr.	Valve or function	Valve position
1	12	Filter, steam in	O
2	13	Filter, steam trap	O
3	25	Exhaust air	X
4	14	Aeration	O
5	15	Aeration, steam trap	O
6	28	Sample, out	X
7	30	Sample, air	O
8	27	Sample	O
9	32	Bioreactor drain valve 1	X
10	34	Bioreaktorin drain air	O
11	31	Bioreactor drain valve 2	O
12	35	Inoculation	O
13	37	Inoculation, air	O
14	38 ja 38 B	Inoculation, input 1	X
15	40	Inoculation, out	X
16	50	Mixer sealing, air	O
17	53	Mixer sealing, out	S (1 bar), open so that steam flows
18	56	Mixer sealing water vessel drain	O- -X
19	41	Sleeve, steam in	X (until temperatue is almost 110 C)

STERILIZATION

A busy step. Set Steam-Elmo to half power. Start this step when the temperature is around 110 °C.

Order	Valve nr.	Valve or function	Valve position
1	25	Exhaust air (open slowly, but almost fully open)	S ↑
2	14	Aeration (almost closed)	S control the pressure
3	27	Sample	S n. 1.2 bar?
4	31	Bioreactor drain valve 1	S ↓ (keep under 2 bar)
5	35	Inoculation	S
6	17	Base, cap	S
7	20	Acid, cap	S
8	23	Antifoam, cap	S
9	55	Mixer sealing cap	S

Start timer when the temperature rises to 121°C. Keep this temperature for the desired time, eg 10 minutes. NB! When the pressure inside the reactor or jacket rises to 2 bar overpressure, the safety valve opens (often occurs).

If the temperature rises above 121 °C, adjust to more closed 12 (steam in), 14, 31, 27 and 35, and adjust to more open 25 (exhaust air).

During sterilization, watch for agitator pressure and open valve 53 if it rises above 2 bar.

COOLING 1

Start when the desired sterilization time has elapsed (normally 15-20 minutes at 121 °C). The onset of cooling (121 → 120°C) takes about 10 minutes, then cools more rapidly, ie if the desired sterilization time is 20 minutes, start this step when about 10 minutes have passed.

Order	Valve nr.	Valve or function	Valve position
1	14	Aeration	X
2	31	Bioreactor drain valve 1	X
3	27	Sample	X
4	35	Inoculation	X
5	25	Exhaust air	X
6	17	Base, cap	X
7	20	Acid, cap	X
8	23	Antifoam, cap	X
9	12	Filter, steam in	X
10	08	Air flow meter bypass	O
11	09	Rotameter input	S (quite open)
12	10	Rotameter output	S (quite open)
13	29	Sample, steam	X
14	28	Sample, out	S
15	36	Inoculation, steam	X
16	40	Inoculation, out	S
17	38, 38B	Inoculation, input 1	O
18	33	Drain, steam	X
19	53	Mixer sealing, out	S *
20	50	Mixer sealing, air	X
21	52	Mixer sealing, condense water	O
22	55	Mixer sealing, water vessel, cap	O- -X
23	alkl	Base solenoid valve	X
24	acid	Acid solenoid valve	X
25	af	Antifoam solenoid valve	X

Now calibrate the pO₂ electrode zero point if necessary.
 Wait 5-10 minutes, then proceed to cooling step 2.

* Make sure the water is flowing in lubrication vessel, if the vessel fills up and the pressure rises, empty via valve 56.

COOLING 2

This step takes a relatively long time.

Order	Valve nr.	Valve or function	Valve position
1	53	Mixer sealing, out	X
2	55	Mixer sealing, water vessel, cap	S--X
3	13	Filter, steam trap	X
4	14	Aeration	O
5	Alkl	Base solenoid valve	O
6	Acid	Acid solenoid valve	O
7	Af	Antifoam solenoid valve	O
8	25	Exhaust air	S
9	28	Sample, out	X
10	38	Inoculation, input 1	X
11	40	Inoculation, out	X
12	45	Sleeve, water in	O
13	43	Sleeve steam trap	X
14	46	Water circulation	O
15	47	Cooling water, bypass	O
16	51	Mixer sealing, steam	X
17	50	Mixer sealing, air	O
18	52	Mixer sealing, condense water	X

To add more water to the condenser mix, first close valve 50 and open valve 51 and 52. (The condenser converts the steam to water). If there is not enough water, gently open valve 50, however, be careful not to raise the mixer pressure too much. If necessary, switch off the mixing!

FINISHING OF STERIZATION

Start when the temperature is about five degrees below the desired cultivation temperature.

Order	Valve nr.	Valve or function	Valve position
1	06	Air flow meter, input	X (aerobi O)
2	07	Air flow meter, output	X (aerobi O; air open etupaneelista)
3	08	Air flow meter, bypass	O (aerobi X)
4	09	Rotameter input	O
5	15	Aeration, steam trap	X
6	Alkl	Base solenoid valve	X
7	Acid	Acid solenoid valve	X
8	Af	Antifoam solenoid valve	X
9	16	Base control	X
10	19	Acid control	X
11	22	Antifoam control	X
12	26	Exhaus air condenser water	O
13	Jacket pump	Circulating pump	O (check that water flows)
14	49	Air removal from heater	O—X
15	47	Cooling water, bypass	X
16	14	Aeration	X (aerobic cultivation O)

Some notes and reminders:

Fill acid, alkali and antifoam containers if you are going to use them.

The desired setpoint settings are set in the Bioprocess Controller. Be sure to set the **cul**-setting and the desired variables to **auto** (T, pH, mixer). So, for example pH adjustment: auto-enter-cul -> adjust SV (set value) as desired.

Cool the broth to about 5°C below the desired cultivation temperature and then set the automatic temperature control. (If the temperature is above the setpoint, the control cabinet may beep, then use the CLR key to make a correction).

Setting the temperature control: In addition to setting the setpoint, the jacket pump and the heat resistors are pressed on. (Depending on the desired temperature, either one or two heat resistors are used).

Heater on! (Heat buttons in control cabinet)

Adjust with valves 14 and 25 a small overpressure in the bioreactor.

It is advisable to tighten the cooling water valves slightly, because when fully open, the cooling effect is too strong leading to large temperature fluctuations.