CHAPTER 4: OPERATION

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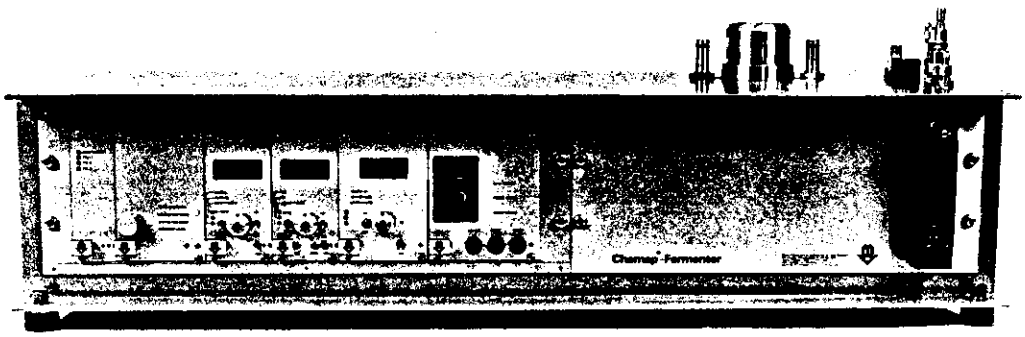
4.2.3.5. O2-Control via Rate of Aeration

4.2.3.6. O2-Control via Speed of Stirrer

4. DESCRIPTION OF THE VARIOUS MODULES AND THEIR PREPARATION

4.1. Basic Unit



power supply
Airflowmeter
speed control
weight control
temperature control
automatic sterilization
isolating amplifier

4.1.1. Rota Meter

Rota meter curve see next page

4.1.2. Power Supply

This slide-in contains the main switch, the switch for weight measurement, heat and lighting. (In Fermenter Type S, the cable is plugged into a three-prong socket, on the reverse side of the base cabinet.)

Microfuses

Heater	T 10 A
Pump	F 1 A
220 V ~	T 2 A
24 V ~	T 2 A

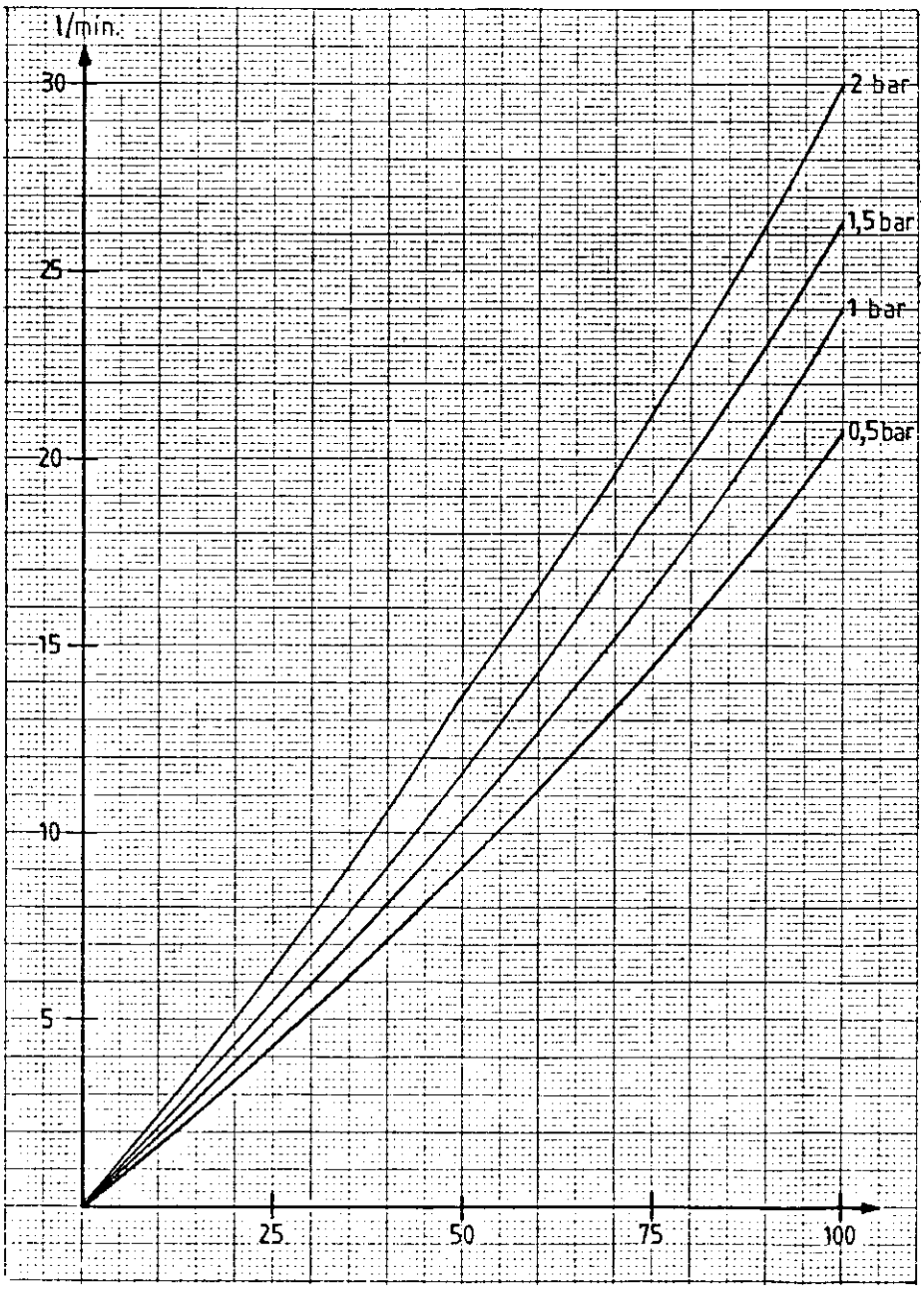
ROTAMETER DURCHFLUSSKURVE (bei 20°C in Abhängigkeit des Vordruckes)
CALIBRATION CURVE (at 20°C in relation to the pre-pressure)

4.1.1

Durchfluss in l/min. bei 20°C und drucklos.

Flowrate in l/min. at 20°C without pressure.

Rotameter Modell—Flow meter : EIC Purgemeter
Messrohr—Measuring tube : R-6-15-B-(CO) 195
Schwimmer—Float : SS 316



Überdruck Over-pressure

Überdruck Over-pressure

Überdruck Over-pressure

Überdruck Over-pressure

Ablesung auf Rotameter-Skala.
Indication on flow-meter scale.

Chemap AG
CH-8708 Männedorf
Switzerland
Telefon 01 922 11 01

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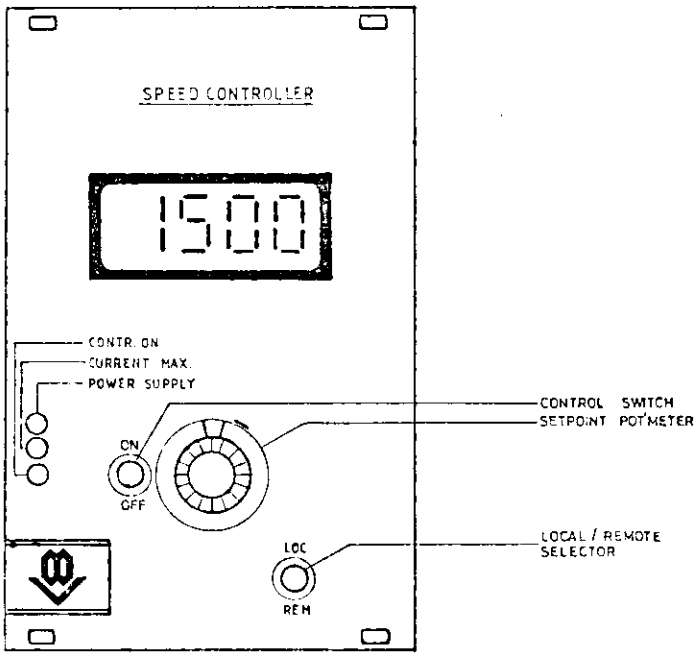
gepr.		4
ges.		

FO-2501

CN 4005 4 79

4.1.3. Rpm Speed Control



- digital read-out of agitator rotational speed

- control lights: control on (green)
  current max.* (red)
  power supply (red)

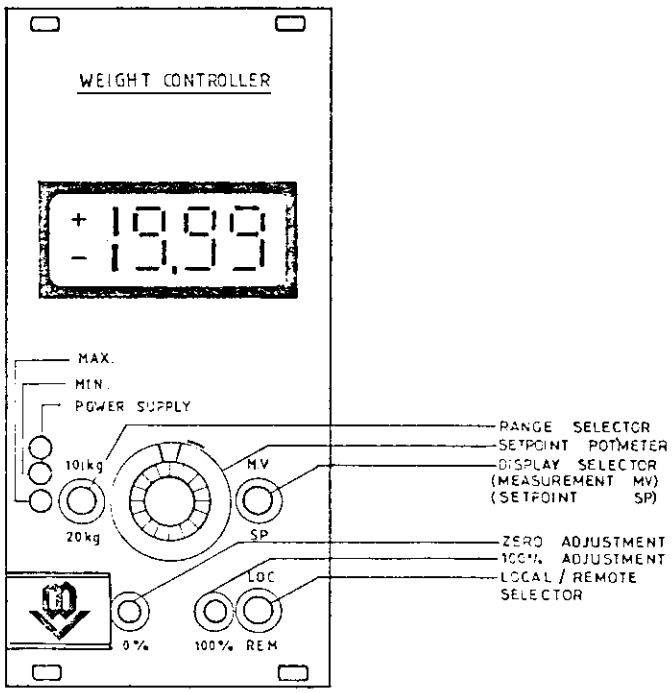
* turns on when rpm control encounters power limitation.
  In this case, reduce rpm.

- on and off toggle-switch

- local/remote toggle-switch

- rpm potentiometer: If possible, this should be returned to Zero before rpm control is switched off.

4.1.4. Weight Control



- 3 control lights: - power supply (red)
  - max. (green)
  - min. (yellow)
- range of switch 10 kg/20 kg (for recorder)
- toggle switch m.V./S.p. (actual value/set value)
- toggle switch loc/rem (local/remote)
- rotation potentiometer for set value setting
- calibration 100%
- Tare adjustment 0%

For continuous fermentation the weight control system load cell may be built into the CHEMAP®Laboratory Fermenter Type CF 3.5/7/14/20 (3.5/7/14 or 20 lit.).

The weight measuring module is mounted on the side wall of the drive module by means of four screws.

Before you start up the fermenter, put it on a stable, hard surface. Check with a spirit level whether the surface is truly horizontal. Whilst doing this, please note that supplied spacer discs can be inserted into the left rubber feet.

The FZ-cabinet may remain on the left side of the fermenter. This is even desirable, as objects are then less likely to be placed on the cover of the instrumentation control cabinet during fermentation, which would naturally affect weight measurement.

Calibration

Equip fermenter with all accessories necessary for fermentation, i.e. probes, FUNDAFOM®, FUNDA®LUX and appropriate transfer needles.

All feed points to probes, as well as all tubes to the necessary pumps should be by the shortest route. They should be free of stress. Best of all, the layout should be on a stand, so that they cannot, at a later time, in an uncontrolled manner affect the weight of the fermenter.

Now prestress the equilibrating spring with a hex-key, as far as it will go, to top position. Back off a little, so that the fermenter swings freely when you tap it lightly, without it however coming to a stop at the upper balance limit. You can best observe this by looking beneath the weight control module and watch the path of the transmission rod above the support ball.

The right potentiometer (100%) is placed with a watch-maker's screw driver in the middle range (total range 18 turns).
Middle: 9 turns after initial stop.
Then set with left potentiometer (0%) tare at 0.000 kg.
After filling the fermenter vessel with 5 lit of water, set with the 100% potentiometer to 5,000 kg.

Now check whether the weigth module does not come to a stop at the lower limit of the transmission rod. Should this happen, then the equilibration spring has to be retightened with a hex key. Rebalancing must be completely repeated.

Utilisation of calibration weights makes calibration easier. The time-consuming filling and emptying of the fermenter vessel is no longer applicable. Take care, however, that the calibration weigths are placed in the middle of the fermenter cover.

If later, during fermentation, an additional needle is used, the increased weight of the tare can be equalized to zero % on the potentiometer.

For the recording of the weight pre-run, on the 6-point-recorder on the small Anphenol plug on the left side of the base unit, poles 4 and 7 +an (same plug used for temperature recording, as well as on the drive as on the control cabinet).

Max. load: 500 ohm

On the left side of the base unit is a power socket for 220 V, which can be utilised for the operation of the harvesting pump.

Caution: This socket is always live.

For the US execution, the DIN outlet is eliminated.

You must use a pump which can be turned on and off via a potential free contact. This contact is on a DIN socket, also on the left side of the base unit (max. contact load 24 V. 1. Amp. at ohm's load).

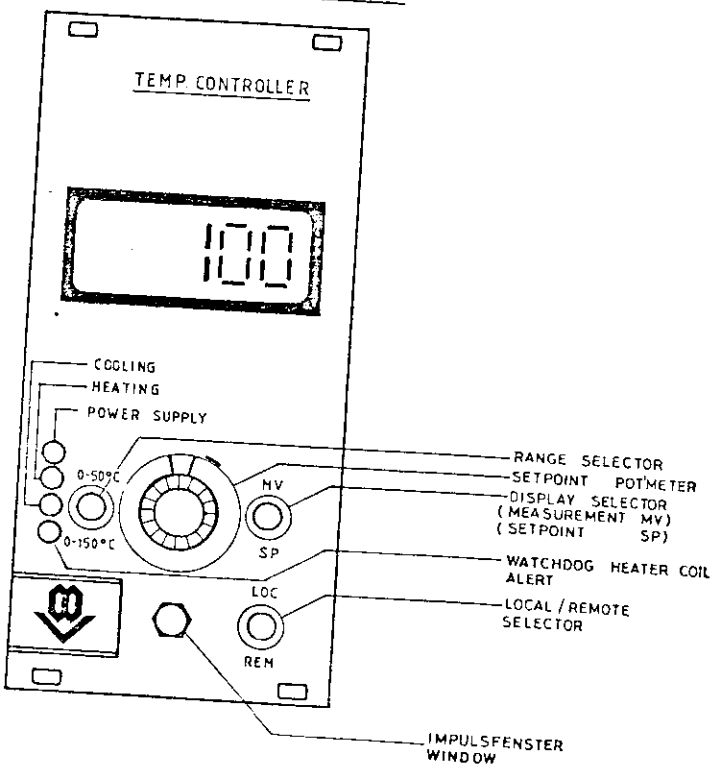
Execution for US: No DIN socket, instead pump can be directly plugged into the contact.

Max. load: 110 V, 1 A

Check of Functions

After finished equilibration, the harvest pump is set into motion and the set value of the potentiometer is set at 4,000 kg. The harvest pump must now pump approx. 1 lit of water out of the fermenter, which can be calibrated with a graduated cylinder.

4.1.5. Temperature Controller



Caution: The temperature probe must be connected at the rear of the base unit!!

3 control lights:
- power supply (red)
- heating (green)
- cooling (yellow)
- the fourth light lights up when the heating rod is without power (see chapter 9.1.5.)

- Range Switch for 0 - 50°C or 0 - 150°C
- digital temperature read-out (actual value/set value), depending upon whether toggle switch is on m.v. = measured value or sp = set point
- set value potentiometer
- toggle switch for local or remote

Caution: If you change from the first position (heat by steam/sterilization by steam) to another combination, you must first fill the heating system with water. Turn the button on position "fill system". After approx. 2 minutes you may change over to any other position.

Adjusting the Width of the Window opening

This potentiometer serves for adjusting the temperature controller for the different Fermenter vessels.

Proceed as follows:

1. Set set-value temperature on 37.0°C.
2. Should the actual temperature be 37.0°C or higher, set the mode switch on "Fill System" so that the temperature falls below 35°C.
3. Position the mode selector to the desired program.
4. Now the Fermenter is heating up. At the temperature listed in the table below the green light should begin to blink. If it does not, then adjust by turning potentiometer "window".
5. Now turn on the automatic sterilization until the set temperature rises to above 39°C and reset this again. The red light should start to blink according to the temperature listed below.
   If necessary, re-adjust.

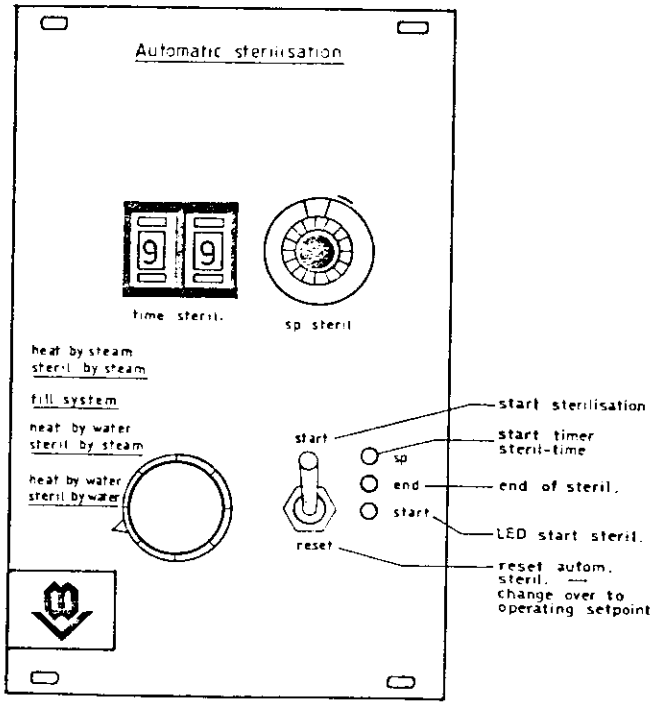
Fermenter size	heating up (green light)	cooling down (red light)
3,5 Liter	35,8	38,2
7 Liter	36,2	37,8
14 Liter	36,2	37,8
20 Liter	36,6	37,4

Note:

To reduce the window opening: Turn potentiometer counter-clockwise

To enlarge the window opening: Turn potentiometer clockwise

4.1.6. Automatic Sterilization



3 control lights: - sp (red) means sterilization temperature reached
- end (green) means sterilization time expired
- start (yellow) means heating up process step starts

Sterilization duration (in minutes) can be set by means of the two-digit digital potentiometer with push buttons. Sterilization temperature is set by means of the sp-potentiometer. The toggle switch is set on position "start". On the temperature control, the toggle switch must be set to sp. By turning the sp-potentiometer (slide-in printed circuit for sterilization), the sterilization temperature can be set. Reading takes place off the digital display on the temperature control.

Caution: After setting has been made, put toggle switch on "reset". Otherwise, sterilization will run out.

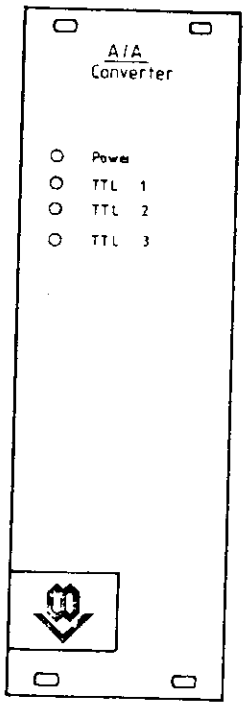
For sterilization or heating, three combinations can be chosen:

- heat by steam / sterilization by steam
- heat by water / sterilization by steam
- heat by water / sterilization by water

Caution: If you want to switch from the first position (heat by steam) to one of the other combinations, the heating system must first be filled with water. This is done by turning the button to position "fill system". After waiting for about 2 minutes, you can set to any other position.

4.1.7. Separation of Potential

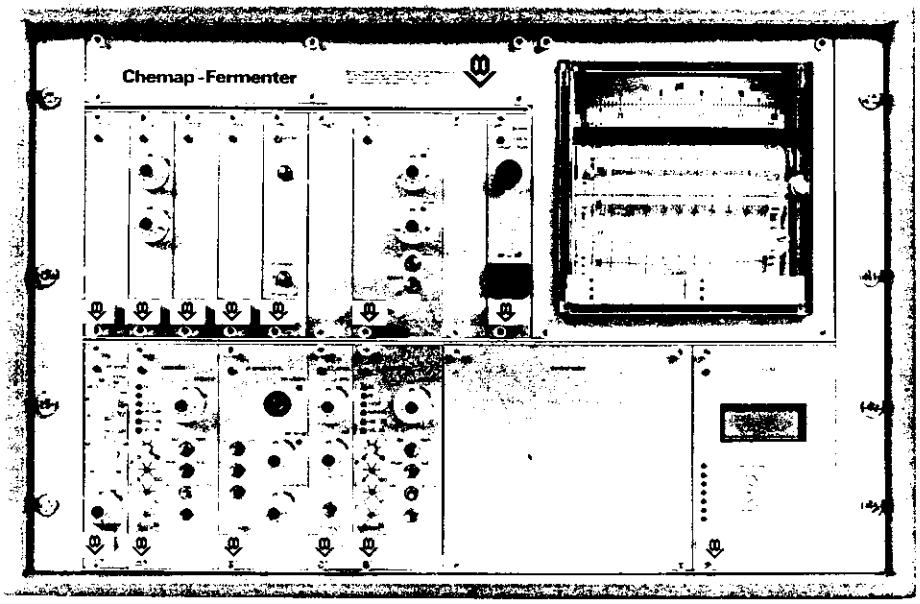
A measured value coming from a controller is led to the computer as a potential free contact by means of a separation potential.



4.2. Control Cabinet

4.2.1. pH Measurement and Control Device

4.2.1.1. pH Amplifier and Controller



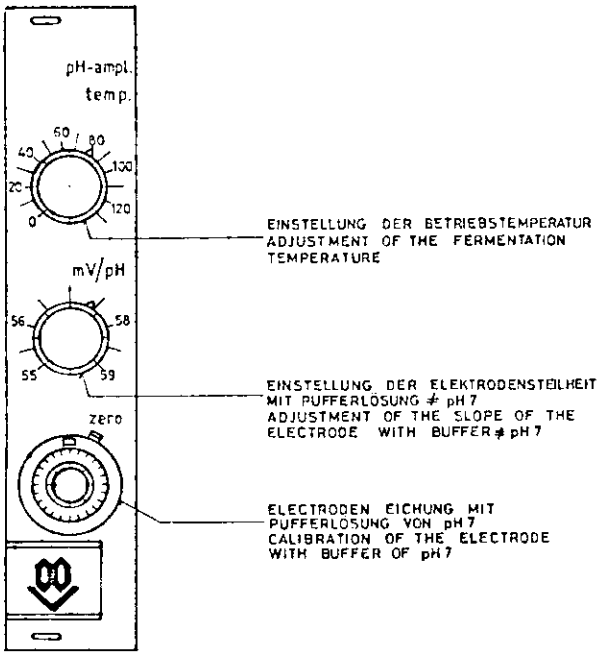
The pH Amplifier contains:

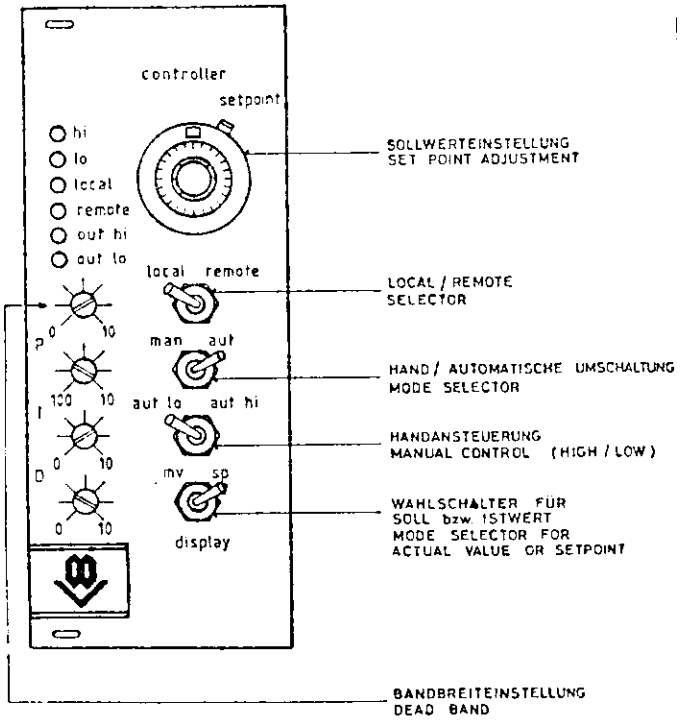
- Temperature compensation 0 - 130°C
- Electrode slope mV/pH
- Zero point equilibration

The controller comprises:

6 control lights:

- actual value is higher than set value (hi)
- actual value is lower than set value (lo)
- local setting (local)
- remote setting (remote)
- relay high (rel hi)
- relay low (rel lo)


pH-ampl.
temp.
0
20
40
60
80
100
120
mV/pH
56
58
55
59
zero
EINSTELLUNG DER BETRIEBSTEMPERATUR
ADJUSTMENT OF THE FERMENTATION
TEMPERATURE
EINSTELLUNG DER ELEKTRODENSTEILHEIT
MIT PUFFERLÖSUNG ≠ pH 7
ADJUSTMENT OF THE SLOPE OF THE
ELECTRODE WITH BUFFER ≠ pH 7
ELECTRODEN EICHUNG MIT
PUFFERLÖSUNG VON pH 7
CALIBRATION OF THE ELECTRODE
WITH BUFFER OF pH 7


controller
setpoint
hi
lo
local
remote
out hi
out lo
local remote
man aut
aut lo aut hi
mv sp
display
P
I
D
0
10
100
SOLLWERTEINSTELLUNG
SET POINT ADJUSTMENT
LOCAL / REMOTE
SELECTOR
HAND / AUTOMATISCHE UMSCHALTUNG
MODE SELECTOR
HANDANSTEUERUNG
MANUAL CONTROL (HIGH / LOW)
WAHLSCHALTER FÜR
SOLL bzw. ISTWERT
MODE SELECTOR FOR
ACTUAL VALUE OR SETPOINT
BANDBREITEINSTELLUNG
DEAD BAND

- set value-potentiometer	(sp)
- potentiometer for dead zone	0 100
- P-Band	proportional band xp
- I-Band	readjustment time Tn
- D-Band	preadjustment time Tv
4 toggle switches:	- local/remote
	- man./aut.
	- rel. low/rel. high
	- m.V/sp

The DIN plug for the probe connection is at the rear of the FZ-cabinet.

In order to protect the amplifier, it is mandatory that the measurement entry be always occupied, either by a pH-probe, or with a shielded plug.

4.2.1.2. pH-Electrode

The pH-electrode probe, equipped with a combined glass electrode Type 465-K1, is usually supplied assembled. Before putting into operation, this is to be taken apart, as described under 1, below. Electrode checks must then be carried out, as under 2. All the settings mentioned in instructions below, are to be found on drawing No. 2780.

1. Disassembly of the Electrode

a. Loosen the nut (764-31.62) and the valve carrier (764-31.66) with the pressure gauge (Mano 2 or Mano 6) from the cross bore of the upper part (764-31.51). This is only necessary when O-rings R 5a or the pressure gauge must be replaced.

b. Loosen the nut (552-16.4). Remove the cable screw parts (552.16.3 and 552.16.2) from the cable.

c. Loosen the cap nut (764.31.52) and remove the head piece (764-31.51).

d. Turn the glass cylinder (764-32.1) until it comes loose from the lower gasket. Lift the cylinder out, together with the upper gasket (764-32.2). There is a gasket at both ends of the cylinder (764-32.2).

e. Unscrew the outer metal cylinder (764-31.41) from the shaft (764-31.11).

f. Grab the electrode at the KCL chamber, initially make a short rotary motion along the vertical axis, until the electrode comes loose from the O-ring seal. Now pull out the electrode.

2. Checking the Electrode

a. The reference electrolyte: The combination glass electrode Type 465 is filled with VISCOLYT-B (Order No. 209816250). VISCOLYT is a 3 molar potassichloride solution, which contains an additive increasing viscosity.

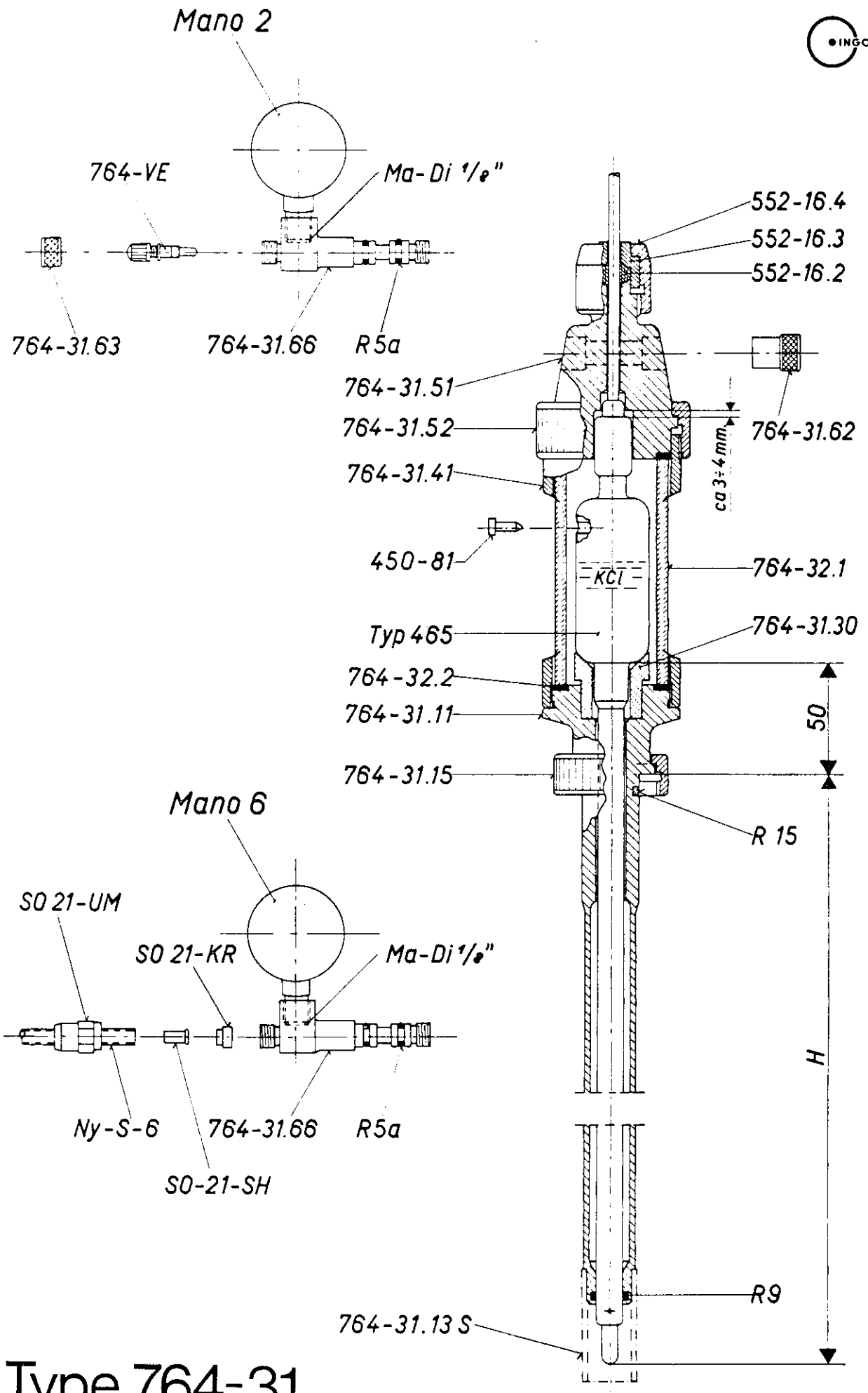
The liquid level of a properly filled electrode is approx. 1-2 cm below the feed nozzle. For security reasons this is closed during transport with the T-stopper 450 81.
If necessary, the reference electrode must be refilled with the pipette. The filling volume of the electrode is approx. 30 ml.

b. Removal of air bubbles inside the electrode membrane: Check that there is no air bubble inside the tip of the electrode. The conducting element of the glass electrode must immerse free of bubbles. This conducting element is visible in the middle of the lower shaft of the electrode. Air, which has collected there during transport will disappear as the electrode is slowly placed in a vertical position.

c. Feed opening for the reference electrolyte: The T-shaped rubber stopper 450 81 must be removed before the electrode is built in. This also goes for any KCI-solution which might be in the feed tube (remove with pippette 00 365 2000 or with wick made of cotton wool). Insertion of electrode into the probe and assembly as described under 3.

3. Assembly of Probe, in accordance with drawing 2780

a. Place the gasket 764-32.2, which had been removed before, on to the tube (764-31.11). Any new gasket ring must be lightly lubricated with silicon grease of medium viscosity.



Type 764-31

2780

b. Introduce electrode, without plug, into the tube (764-31.11) until it is upright on the teflon saddle (764-31.30).

c. Remove rubber stopper (450-81), if this has not yet been done.

d. Insert metal cylinder (764-32.1) and center accurately (cylinder must not touch the sides).

f. Place upper gasket (764-32.2) on upper rim of glass cylinder.

g. Insert the head screw (764-31.51) and the screw cap (764-31.52). Tighten by hand, moderately.

h. Place the parts of the cable screw on the cable and tighten nut moderately by hand.

i. Insert valve shaft (764-31.66) and Manometer into the cross drill in the head piece, and tighten nut moderately by hand.

k. Mount electrode plug (part S-962 of the Ingold coaxialcoupling 962 or 964) according to Instructions to Mounting Ingold-Coaxialcoupling 962-02.

l. For calibration of electrode and pH meter, see 4.2.1.3.

m. Insert electrode into nozzle and tighten nut 764-31.15 by hand, moderately (compare para. 5).

Caution: An assembled electrode probe must not be turned upside down during transport.

4. Insertion of the pH-Electrode

a. When inserting at a slant (i.e. at 15° toward the horizontal), one must be careful that the KCL-feed nozzle does not, under any circumstances, get immersed in the KCl solution of storage container. Should this happen, the KCl solution would then travel, at a pressure drop, into the outer pressure chamber. The reference electrode is thereby short-circuited against ground. If this does happen, the probe must be washed carefully with distilled water and must be thoroughly dried. To avoid above, be sure that the end of the feed nozzle always remains in air space above the KCl level . You achieve this simply by rotating the pH electrode into the above described position.

5. Pressurising the Electrode

A bicycle pump is used to create pressure of 0-2 bar (0-32 psig). For fermentations operating under standard pressure, you create an over-pressure in the electrode housing of 0,5 bar (7,5 psig).

During sterilization the over-pressure must be kept at 1,1 barg(16 psig).

4.2.1.3. Calibrating the pH Amplifier

- The temperature compensation button is turned to the temperature for the buffer solution.
- The pH electrode is immersed in a buffer solution of pH7.
- The zero potentiometer is positioned to pH7. (on the controller, the toggle switch must be in position m.v. i.e. actual value. The read-out is made at the digital indicator on position pH.
- Rinse electrode tip with distilled water. Any adhering drops must be removed with blotting paper. Do not rub.
- Immerse electrode in buffer solution pH4 or 9. The decision depends on whether the fermentation will proceed in the acid or alkaline range.
- With the button mV/pH the electrode slope is positioned at pH4 or respectively 9.
- Rinse electrode and repeat calibration with buffer pH7.
- Adjust temperature compensation button to the temperature of the fermenter.
- Insert pH electrode into the fermenter vessel.

4.2.1.4. Maintenance and Cleaning of the Electrode

a. After every operation cycle, the electrode tip and diaphragm must be thoroughly cleaned with distilled water. Under no circumstances are you allowed to store the pH electrode dirty. This is most important.

b. When you store fully mounted electrodes, the electrode tips and diaphragms must be immersed in 3M KCl.

c. Diaphragm: It can happen during the fermentation that a furry coating will form on the diaphragm. Various strains of mycelia can develop a predeliction for K (potassium), which causes this phenomenon. This film can be mechanically removed with sand paper. The formation of this coating can be prevented by adding approx. 2% formaldehyde to the 3M KCl solution. You can spot the phenomenon of furry coating: the pH reading in a stirred fermenter will differ from the pH reading when the agitator is turned off.

d. Rapid Rise of Resistance in the Reference Electrode:

Rapid rise of the resistance in the reference electrode (up to 1 m ohm) can occasionally be observed when measurements are made in substrates of high protein enrichment. Therefore protein coated diaphragms must be treated with a protein remover (Ingold Item 20 9891 250). This reagent must be allowed to act for several hours.

e. Black Diaphragm: Fermentation solutions of a high sulphide content can induce a black discoloration of the diaphragm. This phenomenon will also show up as a rise in resistance. Such blackened diaphragms must be cleaned with diaphragm cleaner (order no. Ingold 20 9892 250) until discoloration.

Time required: several hours.

The reference electrolyte must be drained completely before above treatment. This draining can be circumvented in the following manner: You fill partially the water retention rubber cap with diaphragm solution and slide it over the electrode shaft, so that the diaphragm is covered by the cap. You now turn the electrode upside down and allow the reaction to proceed.

For the prevention of blackened diaphragm caused by high sulphide content in the fermentation broth, we recommend the use of a combined electrode with an electrolyte bridge (Ingold type 465-90, 465-35-90 or 465-38-90), always according to type of electrode used .

The bridge electrolyte, which must be changed at least once a week, must not contain Ag+. We recommend VISCOLYT-B (order no. Ingold 20 9816 250) 3M KCl without AgCl (order no. Ingold 20 9823 250).

f. Check of Reference Electrode

This applies to single reference electrodes, as well as to the reference electrodes on a combined electrode.

Procedure:
The test-reference electrode (373) is plugged into the reference electrode socket of the pH meter. The reference electrode to be tested is connected with the high Ohm entry on the pH meter. Since the reference electrode potential is taken off via the cable shield on all combined electrodes, you must conduct this potential at the end of the cable or at the branch provided for this purpose. If you choose the two former alternatives, you use a short braided cable with a crocodile clamp.

Potential in buffer pH 4 = ... mV
Potential in buffer pH 7 = ... mV
Potential in saturated KCl solution = ... mV

Evaluation:
- These three potentials must not diviate more than 2 mV from one another.
- In so far as the test reference electrode and the reference electrode being checked have the same lead off system (the proposed type 373 has an Ag/AgCl lead-off system), all three potentials must be within a range of -10 to +10 mV.

Measures for Corrections:
- When the potentials deviate more than 10 mV from the set value, you must renew the reference electrolyte.
- When the potentials deviate from one another, dramatically, the diaphragm is dirty (see 4.2.1.4.)

g. Checking the Measuring Electrode

Procedure:
Measure potential E against the built in reference electrode in buffers pH4 and pH7, at a temperature of 20 to 25°C.

Evaluation:
E (in buffer pH4) minus E (in buffer pH7) should be between 172 and 176 mV.

Measures of Correction:
Should the difference in potential be < 172 mV: attempt regeneration with HF (1% solution, item Ingold No. 9895), immerse membrane for 1-2 minutes and gently agitate. Rinse after this treatment and leave it in water overnight. Should the potential difference be < 150 mV: the membrane electrode must be replaced; it connot be regenerated.

h. Response Time of Membrane Electrode

Procedure:

- Immerse electrode into buffer pH4 and measure potential against reference electrode.
- Rinse electrode with KCl solution or buffer pH7.
- Dab electrode with blotting paper.
- Immerse electrode in buffer pH7 and read potential at 60 seconds and 120 seconds.

Evaluation:
The difference in potential between value after 1 minute and value after 2 minutes must not exceed 3 mV.

Measures for Correction:
If response time is too slow, treat with HF.

i. General Remarks about the Treatment of Electrodes:

- The membrane of the electrode should never be dried by rubbing. This causes static charges.
- It is immaterial whether the membrane electrode is stored in KCl or in AqDist. The optimum method for storing combined electrodes is to store them in reference electrolyte.
- Once an electrode has been put into operation, it must never be stored dry.

4.2.1.5. pH-Control
(see also 2.1.)

- The acid - respectively alkaline pump, must be plugged into the correct outlet on the reverse side of the FZ panel box. In addition, you must plug the control cable into the socket "control" in the pump and plug the other end of the control cable into the FZ control panel beneath the power plug of the acid, respectively, alkaline pump outlet.
- The toggle switch on the pump beneath the socket control must be positioned on "remote", whereas the other toggle switch, beneath the socket "0-20 mA" must be positioned on "local".
- Fix the tube in the pump. Turn on power switch on the pump. Select direction of rotation and pump velocity.
- On the controller slide-in card the sp/mV toggle switch is positioned on sp (set point). The set value must now be adjusted by means of the set point potentiometer. The read-out is made on the digital voltmeter.
- The dead zone is adjusted as follows:

1. Immerse electrode in solution of known pH value.
2. Put switch on set-point.
3. Adjust set-point of 1/2 of the desired dead zone higher than the value of the buffer solution.
4. Turn switch dead zone (0-10) until the light barely flimmers.

Example: We want to select a dead zone of Δ pH = 1. The lower limit value should be pH 6, the upper one, pH 7. Our buffer solution has the pH 4.

Procedure:

- Set point at 4.5 (0.5 = 1/2 of the desired dead zone). Equilibrate dead zone with potentiometer. Adjust set value to 6.5. The controller now operates at pH 6 and pH 7.

- PID controller: It is recommended to operate initially with P controller:

  I : (resp. Tn) = oo (all the way to right)
  D : (resp. Tv) = 0 (all the way to left)

- Toggle switch man/autom. to be put on automatic. Please note that the feed volume of the pump and the concentration of the acid, resp. alkaline, also effect the quality of the control.

- Setting the Controller for Continuous Operation:
  You switch to continuous operation in order to transfer inoculum into the fermenter, or, in order to fill the tubes for pumping acid or alkaline.

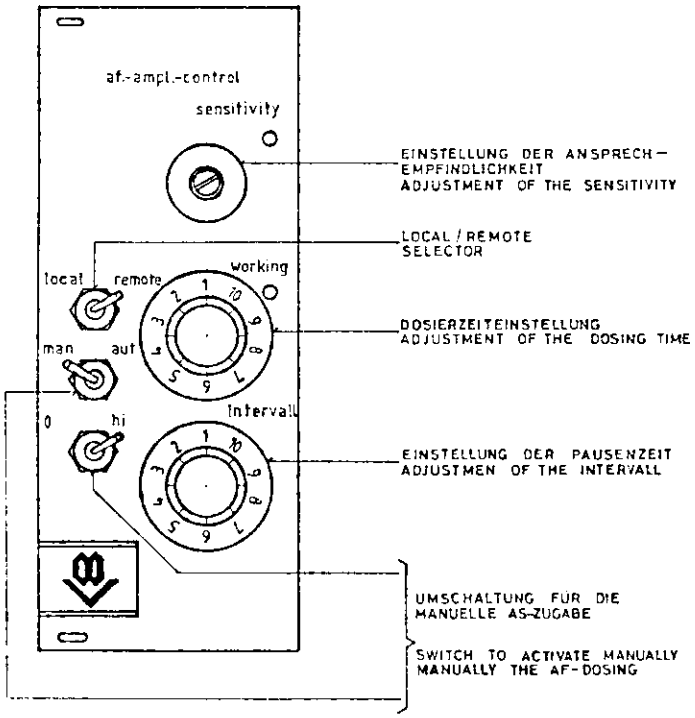
  Example: tubing must be filled with acid:
  1. Start pump
  2. Put toggle switch man./autom. on manual
  3. Put toggle switch rel.Lo/rel.hi on rel.hi

  Caution: As soon as the tubing is filled, you must turn off the pump.

  In order to fill the tubing with alkaline, simply put toggle switch rel.Lo/rel.hi on rel.Lo. Otherwise proceed as described above. Finally, put toggle switch on neutral and toggle switch man./autom. on automatic.

4.2.2. Chemical Foam Control

4.2.2.1. Anti-Foam-Amplifier/Controller



3 toggle switches loc/rem
                   man./autom.
                   0/hi in position hi, the peristaltic pump is on continuous operation

- adjustment of sensitivity
- potentiometer for working time
- potentiometer for interval time

The connection for the anti-foam probe is on the reverse side of the control cabinet.

- Preparation of anti-foam probe:
  The fermenter cover must be equipped with an anti-foam probe socket. See 3.2.1. "anti-foam probe".

  The protector tube and the probe are now mounted through the cover from the outside. For sterilization, the probe should reach into the fermenter space, as far as possible.

  After sterilization the probe is withdrawn to the desired height. Obviously it cannot be lowered again (sterility).

- Adjustment of sensitivity:
  The degree of sensitivity can be adjusted with a screw driver.
  Right-turn stop: maximum sensitivity
  Left-turn stop: minimum sensitivity

  The correct adjustment depends on the conductivity of the foam. We recommend the following method of adjustment:

  Adjustment screw must be turned to the right until end stop. Then back off about 1/3 of the range.

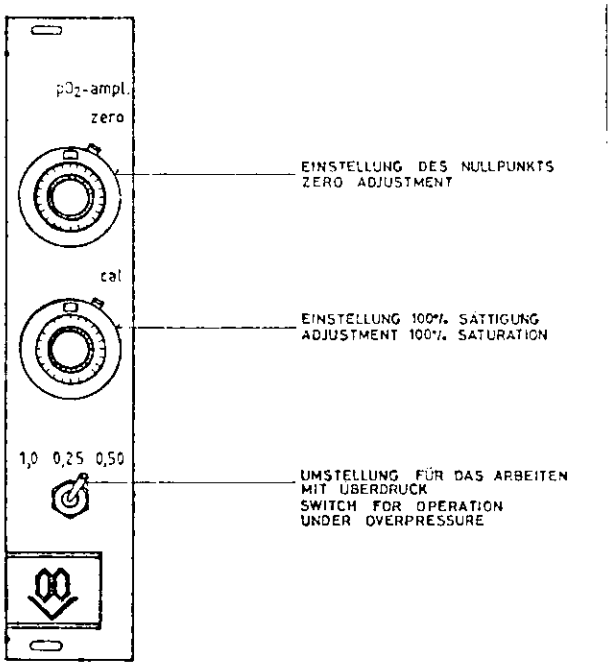
- Anti-foam control:
  The peristaltic pump is connected to the control cabinet in the manner described under 3.6.

  On both potentiometers the on- and interval times are set as follows:
  Interval time: min approx. 10 sec. max approx. 25 min.
  On-time : min approx. 1 sec. max approx. 10 min.
  Toggle switch: man/autom. on automatic

- Continuous operation:
  In order to fill the tubing quickly with anti-foam reagent, you turn the pump on continuous operation.
  Toggle switch: man/autom. on manual
  Toggle switch: 0/hi on high

4.2.3. pO2-Measurement and Control

4.2.3.1. pO2-Amplifier



1,0 = DRUCKLOS / PRESSURE / pO_2 0-0,25 bar

0,5 = BIS 1bar ÜBERDRUCK / UP TO 1bar OVERPRESSURE / pO_2 0-0,5 bar

0,25 = DRUCKLOS MIT REINEN O_2 / PRESSURELESS WITH PURE O_2 / pO_2 0-1 bar

- Zero-Potentiometer
- Calibration-Potentiometer
- Toggle switch: Saturation (1; 0.25; 0.5)

  Range 1 : 100% correspond to fully saturated water at atmospheric pressure

  Range 0.5 : 100% correspond to fully saturated water at 2 bar abs. pressure

  Range 0.25 : 100% correspond to fully saturated water at 4 bar abs. pressure

The outlet for the probe is on the reverse side of the FZ cabinet.

In order to protect the amplifier, it is necessary to keep the signal entry always in use, either with a pO2 probe or the pO2 simulator.

4.2.3.2. pO2-Electrode

At the beginning of each fermentation, the membrane body should be inspected for damage or growth of micro-organisms. For renewing the electrolyte see Ingold-Instructions, 10.

4.2.3.3. Calibration

- Amplifier - zero point

  . plug pO2 simulator in at signal entry

  . put simulator on position 0

  . adjust to 0 point with zero potentiometer

  Caution: Zero point equilibration is good when the read-out shows a range between +0.5 and +1.5.

- Avoid negative read-out

  . put simulator on position M

  . put saturation switch on range 1

  . put cal.-potentiometer on 100%. When you switch to range 0.5 and/or 0.25, you should get a reading of 50%, respectively 25%.

- Electrode zero point

  The electrode must be polarized before calibration can start: plug pO2 electrode into FZ control cabinet. Switch on control cabinet. Calibration can start after 2-3 hours.

- Immerse electrode in water saturated with N2. Slowly bubble N2 through the water. After 5 minutes, the zero current can be read. If there is only a small deviation from the amplifier, the zero point can be put on 0 by means of the zero potentiometer (avoid negative read-out). If there is a big deviation, see Ingold Manual 6.4.

- Electrode slope

The calibration of the electrode slope can only be done after sterilization, since the membranes undergo changes during sterilization. It is essential that calibration takes place before inoculation. After cool-down the fermenter is aerated through the air-in filter. Once saturation has been reached, the 100% value is set by means of the cal. potentiometer, i.e. saturation switch on range 1.

Since temperature and pressure changes affect the pO2 value, it is usual to set the saturation value at 90 to 95%.

The calibration should be repeated after each break in the operation (i.e. when probe is separated from the amplifier), as when you switch off the probe, small, irreversible changes can take place.

4.2.3.4. Maintenance

- For change of membrane, see Ingold Manual chapter 10

- Removal of coatings on the membrane:
  clean carefully and mechanically with damp soft rag

- Prevention of caotings of the membrane:
  the addition of a few drops of 30% formaldehyde per 100 ml electrolyte can retard or prevent biological growth on the membrane.

- Removal of the black silver sulphide deposits from silver anode:
  remove by means of polishing paper, or with a solution of thio-urate.

4.2.4. Redox (ORP)
(see Ingold mode of operation)

The Redox electrode is connected on the reverse side of the control cabinet. If this electrode is not connected, you must protect the amplifier with a shielded blind plug.

4.2.5. Press Button Print (Power Supply)

This slide-in contains the main fuse (MO, 4/250 C). (lag 0.4 A) and the main switch on/off.

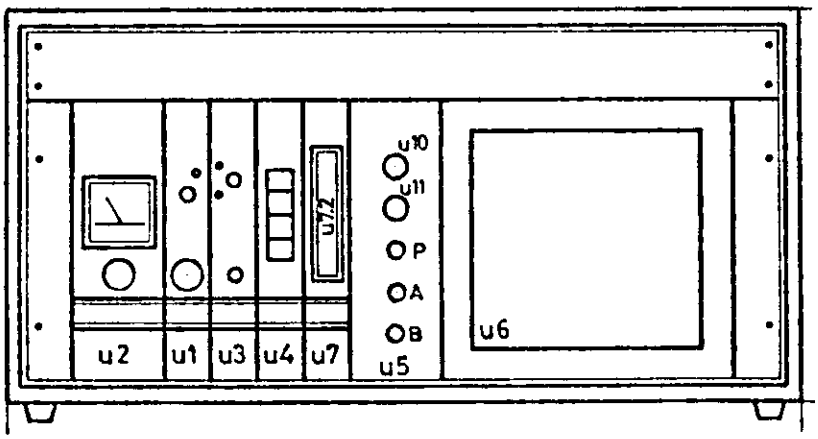
4.2.6. Digital Voltmeter

Digital read-out and rotary switch of the measured parameters. The channels are normally taken as follows:

Channel	Parameter
1	pH
2	pO2
3	Redox (ORP)
4	CO2
5	Air-in valve, opening 0-100%
6	FUNDA®LUX, external entry

4.2.10. FUNDA®LUX (FZ 004)

4.2.10.1. Description of the racks



1. Stabilizing Print
2. Amplifier
3. Pulse Generator
4. Plug Print
5. Indicator Print with Limiting Contact Device
6. Terminal Block
7. Printer

1. Stabilizing Print

The stabilizing print contains the voltage stabilizer (± 15 V) and the light intensity control. At the front side you find the socket for the light- and electronic part as well as the µA indication for the light intensity control. The setting value is indicated on the test record (inside the FZ 004 cabinet). If the indication shifts to higher values, the light must be changed.

2. Amplifier

The red light indicates that a sample is inside the gauge head and the degassing time has expired. The indication can be changed only if the light at the amplifier is illuminated. A sample and a hold amplifier holds the measured value until the following cycle is terminated (Ejection of the sample, sucking-up and degassing of the following sample).
The range of extinction may be choosen between 0.2/1/2 by the switch.

The zero-potentiometer is used for setting and suppressing the zero-point. For that use the range plug must be set on range 0.2 or 1. On range 2 are measured only absolute values.

3. Pulse Generator

The degassing time is adjusted by the pulse generator:

left side limit: minimum degassing time (sterilization!)

right side limit: maximum degassing time

The optimum degassing time is adjusted as follows:

- adjust the wanted stirrer speed
- adjust the wanted aeration rate
- mount the light part on the measuring head
- switch-on "sample in/out" and "control"
- pulse generator at right side limits
- shield by hand the lateral photo resistance (light illuminates)

- observe the illuminated photo gauge:
  Determination of the degassing time by a chronometer. This degassing time is set on the impulse generator.
  Check: the illuminated light on the amplifier indicates the executed measurement. No more gas bubbles are rising inside the sample.
- The degassing time should be limited to a minimum, so that no sedimentation can effect the measurement.

4. Plug Print

- Button "sample in/out": sucking in and ejecting of the samples in the automatic run

- Button "control": switch on the light mounted on the measuring head
- Button "sample out": emptying by hand the sample of the measuring gauge
- Button "recorder": on/out button for printing out of the FUNDA®LUX curve

5. Indicator Print with Limiting Contact Device

This plug-in is for the actual reading of the FUNDA®LUX-measuring value as well as for the set-point for continuous fermentation (see 4.2.10.8.).

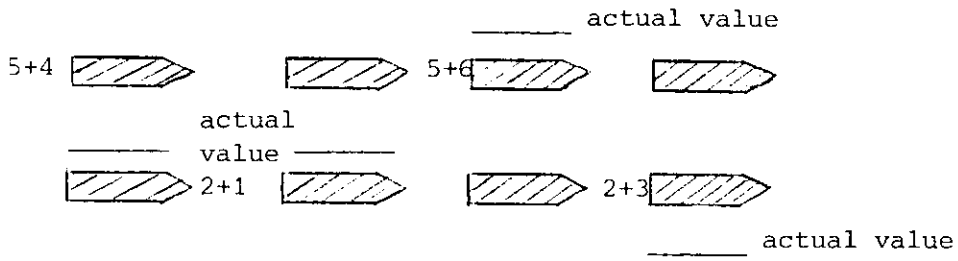
6. Terminal Block

The adjustable limiting contacts of the contact device are potentialfree connected with a 6-pole plug. The media pump is switched on or out via those contacts.

Drive:

upper limiting contact: actual value > setting value → 5+6 have contact
actual value < setting value → 5+4 have contact

lower limiting contact: actual value > setting value → 2+1 have contact
actual value < setting value → 2+3 have contact



Normally in the workshop the device is so adjusted, that if the actual value is greater than the upper limiting contact, the media pump is turned-on (5+6 are in contact).

7. Recorder (not contained in standard version)

The recorder serves to register the FUNDA®LUX growth curve.

For later assembly: see 6.2.1.

For general information: see chapter 10.

4.2.10.2. Description of FUNDA®LUX measuring head (see chapter 9.5.10.)

The FUNDA®LUX measuring probe consists of the measuring chamber, the suction device (2) the electronic part and the light part.

By means of a pneumatically activated Teflon belows a sample of approx. 50 ml is sucked into the measuring chamber. The resulting underpressure causes a rapid gasification of the sample. The operating time of this pneumatic cylinder can be adjusted at the chokes, which are located opposite the air connections.

Two highly sensitive cadmiumselenide photo resistances measure the brightness of the light. The central photoresistance measures the extinction, whilst the excentrical one compensates the temperature coefficient and fluctuations of the light source.

For optimal adjustment to the various media and organisms an interference filter of approx. 515 mm was chosen. It is known empirically to give the best results. You can read off from the test record the width of the band of transmitted light. This recording you find inside the cabinet.

4.2.10.3. Sterilization

The measuring probe is mounted at the Fermenter, which has been filled with medium (standard socket with FUNDA®LUX suction tube). For the sterilization the electronic- and light-part must be removed! Press key "Sample in/out" and set the potentiometer on the pulse generator for the shortest degasing time, (turn fully to the left). During the sterilization of the Fermenter vessel the FUNDA®LUX is automatically sterilized too.

4.2.10.4. Adjustment for the Fermentation

After sterilization and cool-down of the media mount the electronic- and lamp-part at the measuring probe and adjust the optimal pulse time as described under 4.2.10.1.(3). Then set the measured FUNDA®LUX value at zero:

- Put zero-potentiometer on zero
- Put range switch on 0,2 or 1. (No calibration possible on range 2)
- When the red checking light at the amplifier lights up, the FUNDA®-LUX value can be put on zero by means of the zero-potentiometer.
- The addition of the inoculum will cause the FUNDA®LUX value to jump.

Should during the fermentation the FUNDA®LUX value show almost 100 % you can change over to the next less sensitive range or you can suppress the actual value with the zero-potentiometer. (Possible only in range 0,2 and 1).

4.2.10.5. Printing of the Calibration Curve

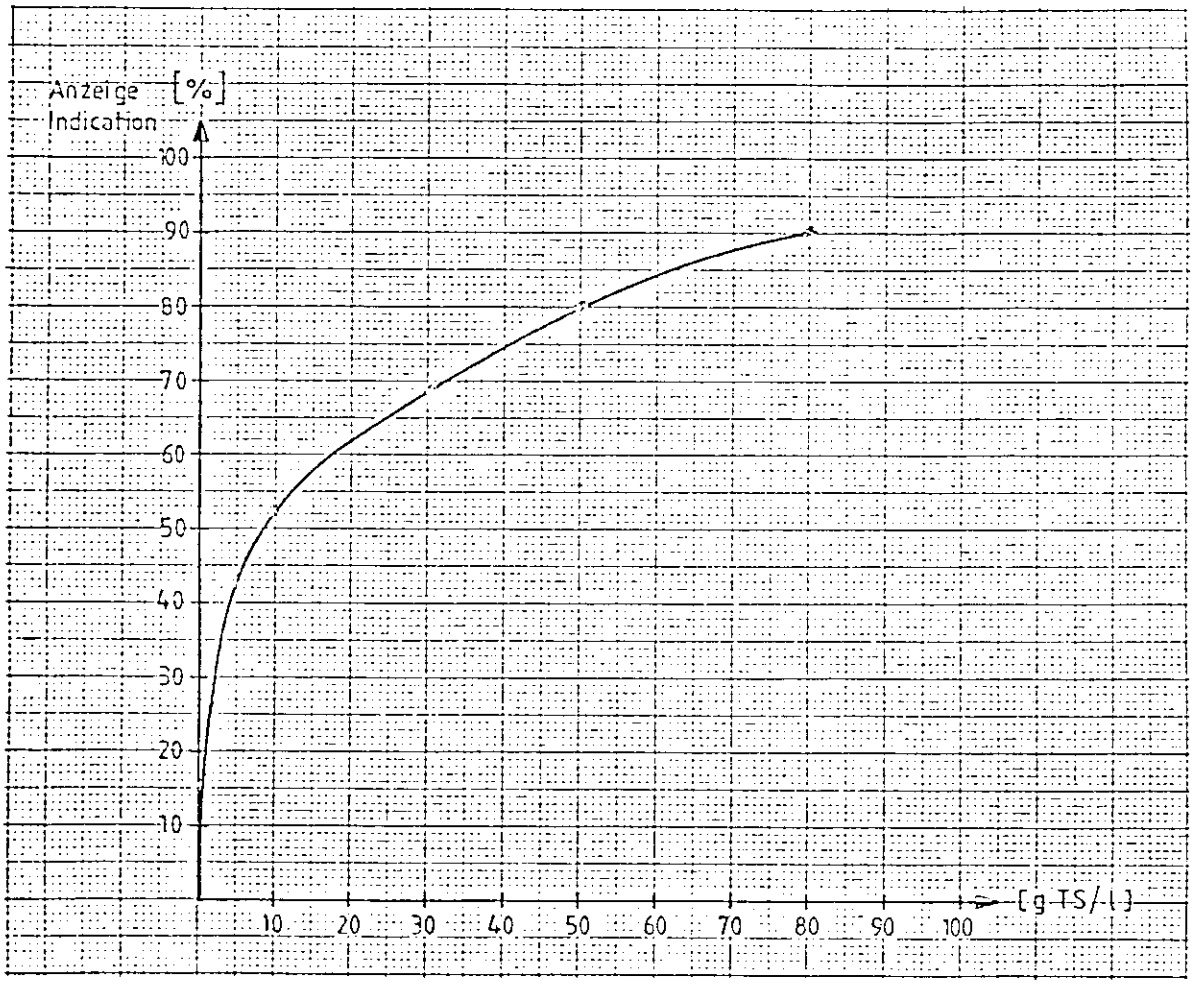
In order to establish a calibration curve samples are taken out during the fermentation at certain intervals of time, the actual FUNDA®LUX values are noted and the dry weights of the biomass ascertained.
The outcome can be plotted graphically.

ERSTELLEN DER EICHKURVE
PRINTING OF THE CALIBRATION CURVE

4.2.10.5

Beispiel für Backhefe (FUNDALUX Bereich 2)

Example for Baker's Yeast (FUNDA®LUX Range 2)



Chemap AG
CH-8603 Männedorf
Switzerland
Telefon 01 922 11 01

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4.2.10.6. Theory

The working principle of the FUNDA®LUX is based on the Beer-Lambert's law:

$$E = \log \frac{I_.}{I} = - \log \frac{I}{I_.} = - \log T$$

E = Extinction

I = Intensity of the light prior to the sample

I = Intensity of the light after the sample

T = Transmission

The FUNDA®LUX indication is given in the inverse logarithm and multiplied by 100

$$E = \frac{\text{F®LUX in \%}}{100\ \%} \times \text{range}$$

The range equals the negative logarithm.

Range 0,2

$0,2 = -\log.10^{-0,2} = -\log.\ 0,63$

63% of the light meets the photo resistance (37% get absorbed).

FUNDA®LUX indication : 100%

$$E = \frac{100\%}{100\%} \ .\ 0,2 = 0,2$$

$$T = 10^{-0,2} \ .\ 100\% = 63\%$$

Range 1:

$1 = -\log\ 10^{-1} = -\log\ 0,1$

10% of the light meets the photo resistance, (90% get absorbed).

FUNDA®LUX indication : 100%

$$E = \frac{100\%}{100\%} \ .\ 1 = 1$$

$$T = 10^{-1} \ .\ 100\% = 10\%$$

Range 2:

$2 = - \log 10^{-2} = - \log 0{,}01$

$\longrightarrow$ 1 % of the light meets the photo resistance (99 % get absorbed)

FUNDA®LUX reading 100 %

$$E = \frac{100\ \%}{100\ \%} \cdot 2 = 2$$

$$T = 10^{-2} \cdot 100\ \% = 1\ \%$$

Calculating the Extinction and Transmission according to the FUNDA®LUX Values

Example:

FUNDA®LUX indication: 47 %, range 0,2

$$E = \frac{47\ \%}{100\ \%} \cdot 0{,}2 = 0{,}094$$

$$T_{\%} = 10^{-0{,}094} \cdot 100\ \% = 80{,}5\ \%$$

4.2.10.6.1. Theoretical Evaluation based on Example 4.2.10.5.

By means of the Beer Lambert's law one can determine the concentration of a light-absorbing material. ($E = \varepsilon \cdot x \cdot d$)

E = Extinction

ε = molar decade coefficient of the extinction

x = concentration in g/l

d = thickness of the layer in the measuring cell

In diluted solutions and at a certain wave length ε is a constant. In concentrated solutions or, as is the case here, in suspensions, the Beer-Lambert's law does no longer apply.

A certain portion of the diffused light is added to the absorbed light.
This portion can be determined only by a more complex law which takes into account the size and form of the suspended particles.
Therefore x = f (E) is best determined as a calibration curve and for easy calculation replaced by a polynom.

$$x = a_o + a_1E + a_2E^2 + a_3E^3 + \ldots + a_hE^h \text{ approximated}$$

x = (g/l)	E (% Funda®Lux)
80	90
40	75
20	62
10	52
5	43
2	27
1	17
0,5	11
0,25	9

In our case the coefficients are:

$a_o = - 5.6845$
$a_1 = + 1.6547$
$a_2 = - 1.8854 \cdot 10^{-1}$
$a_3 = + 1.1812 \cdot 10^{-2}$
$a_4 = - 4.2082 \cdot 10^{-4}$
$a_5 = + 8.7188 \cdot 10^{-6}$
$a_6 = - 1.0242 \cdot 10^{-7}$
$a_7 = + 6.3303 \cdot 10^{-10}$
$a_8 = - 1.5949 \cdot 10^{-12}$

Example:

FUNDA®LUX read out = 57 %
Cell density calculated by above polynom 14,4 g/l
see diagram ========

The computer program for this polynom is available from Chemap AG.

4.2.10.7. Cleaning

Whilst cleaning the Fermenter (see chapter 4.3.) the FUNDA®LUX is set for the shortest interval time. Remove light-and electronic-part. If the probe is dirty, a through cleaning is necessary: Disassemble the probe as per chapter 6.1.6.

4.2.10.8. Supervision and Control for continuous Fermentation "Turbidostat"

In order to retain a constant biomass-concentration in a fermentation the limit contact switch is employed. (See also 4.2.10.1.(5./6.).

The upper limit contact is taken as set-value on the desired FUNDA®LUX value, (desired biomass-concentration).
When the actual value rises above the set-value a media pump is switched on. It pumps medium into the Fermenter until the actual value ≤ set-value. Obviously then the weight control comes into action (see 4.1.4) to prevent overfilling the Fermenter.

You can use the FUNDA®LUX also in other areas, e.g. for supervising the turbidity in centrifuges etc.

4.3. Cleaning the Fermenter

4.3.1. Fermenter without FUNDAFOM®

- Remove all needles, electrodes, air-in and exhaust filters.
- Close all connections, excepting 2, with new membranes and blind plugs.
- Close the bottom valve.
- Fill fermenter up to the cover with water containing a non-foaming detergent.
- Set stirrer on 300 rpm.
- If the fermenter is equipped with FUNDA®LUX:
  Remove electronic part and housing of the lamp of the FUNDA®LUX and set on shortest interval time.
- Set temperature control (either with steam or water heating) for 70°C.
- When that temperature is reached close off the remaining 2 connections and increase rpm to 1000. (Spin-stirrer 500 rpm).
- As soon as all dirt is removed turn the heating off, reduce rpm to 300, open 2 sockets and empty the fermenter.
- Rinse the fermenter twice with fresh water.
- Switch the stirrer off
- Switch FUNDA®LUX off.
- Should the fermenter not be used immediately again for another fermentation, fill the fermenter vessel with approx. 3 cm of water to prevent encrustations on the mechanical seal.

4.3.2. Fermenter with FUNDAFOM®

- Remove all needles, electrodes, air-in and exhaust filters.
- Close all ports, except one, with new membranes and blind plugs.
- Close bottom valve.
- If the FUNDAFOM® is connected laterally, install a needle with tubing and connect the end of the tubing to the water tap.
- Fill detergent solution through the open port.
- Fill fermenter up to the cover with water.
- Set stirrer at 300 rpm.
- If the fermenter is equipped with FUNDA®LUX:

  Remove electronic part and housing of the lamp and set to shortest interval time.
- Set temperature control (either with steam- or water heating) on 70°C.
- When that temperature is reached, close the port, remove the end of the tubing from the water tap and insert into the drain.
- Switch the FUNDAFOM® on until all dirt has come off.
- Switch off FUNDAFOM® and heating and empty the fermenter.
- Connect the end of the tubing again to the water tap.
- Open one port.
- Rinse the fermenter twice with water.
- Switch stirrer off.
- Switch FUNDA®LUX off.
- Should the fermenter not be used immediately again for another fermentation, the fermenter vessel should be filled with approx. 3 cm of water to prevent encrustations on the mechanical seal.

4.4. Preparations and Sterilization of the Fermenter Vessel

4.4.1. Preparing the Fermenter

- If the fermenter is equipped with a weight measure you must calibrate before sterilization. (See chapter 4.1.4.)
- Replace all pierced membranes by new ones. Easiest tested by squeezing the sides between thumb and forefinger, (see picture).

- Mount exhaust air filter. (Fermenter with FUNDAFOM®:onto the FUNDAFOM® socket).
- Open the knurled screw at the pressure adjustment unit and install this unit on the exhaust air filter.
- Prepare the probes you need:
  a) pH probe: see 4.2.1.3. (calibrating).
  Do not forget to give overpressure of 1.1 bar on the measuring device!

b) Antifoam probe see 4.2.2.1.

c) pO2-probe see 4.2.3.2. (preparation)
4.2.3.3. (calibrating 0-point)

d) Redox-electrode

e) CO2-electrode: see 4.2.8.3.

- Install the electrodes and probes you need in the fermenter.
- Mount FUNDA®LUX, connect to compressed air, (see 4.2.10.2. and 4.2.10.3.) and remove electronic- and light part.
- Fill in the medium.
- Put new membranes and blind plugs on all still open ports.
- Set FUNDA®LUX for shortest interval time.
- Mount protection hood to the glass-fermenter!
- Set stirrers to 600 rpm.
- The fermenter is now ready for sterilization.

4.4.2. Sterilization of the Fermenter

(See also chapter 4.1.6.)

- Select fermentation temperature. (Toggle switch on sp. Adjust the fermenter temperature with the potentiometer).
- Set sterilization duration in minutes. (Begins when sterilization temperature has been reached).
- Choose heating system.
- Adjustment on the printed circuit "autom. sterilization".
- Start sterilization with the toggle switch: first put switch on "reset" then on "start".

- Choosing the sterilization temperature:

  Toggle-switch at the temperature controller must be set on sp. By turning this potentiometer the sterilization temperature can be selected. Reasing takes place at the digital display of the temperature controller.

- Put toggle switch at the temperature controller back on m.V.

- Switch FUNDAFOM® on.

- When 100°C has been reached, close the pressure adjustment unit by turning the knurled screw.
  Caution: At 121°C the pressure is 1,1 bar. Steam must escape!

- Open steam valve at the bottom valve. (20 min.)

- When sterilization time has elapsed, the contents of the fermenter are automatically cooled down to the fermentation temperature selected at the temperature controller. The vacuum which results from cooling down is automatically compensated by the pressure adjusting unit.

- Remove protecting hood.

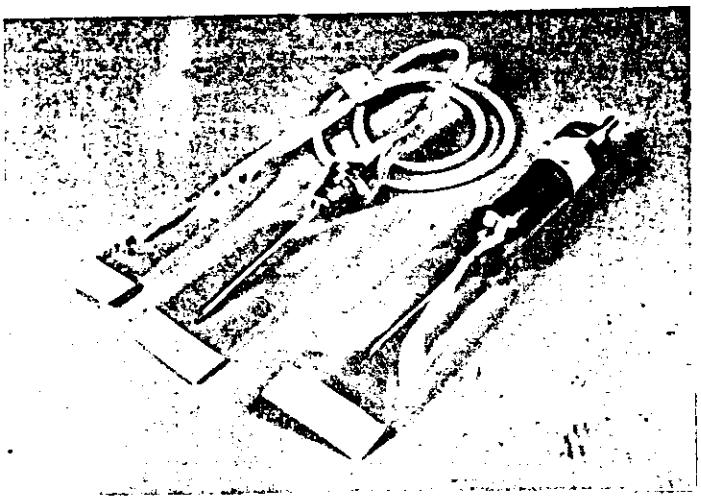
- Reduce pressure on pH probe to 0,5 bar.

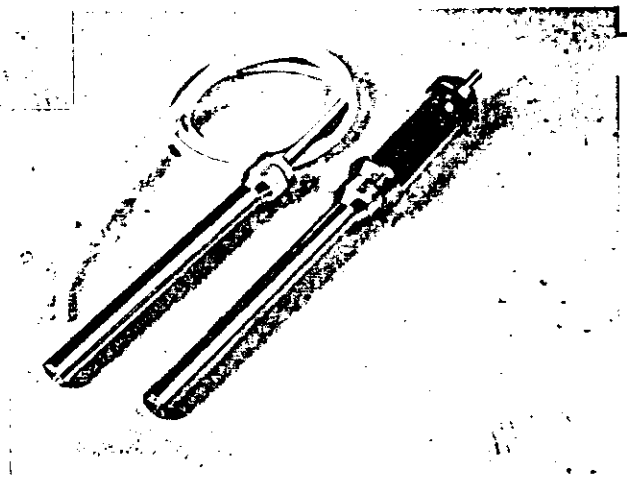
- Turn the knurled screw at the pressure adjusting unit out completely.

4.4.3. Preparation and Sterilization of Accessories

- Cut pieces of tubing (5/8 mm) to the correct lengths, cover one end of a tube with a spring and fix this end to one of the needles.

- Pack needle and the free end of the tubing into the sterilization tube. For the needles the sterilization ferrules can also be used.





- Necessary accessories:

  - Needle and tubing for adding acids
  - Needle and tubing for adding alkalines
  - Needle and tubing for addition of medium if continuous culture is planned.
  - Needle and tubing for harvest if continuous culture is planned.
  - Needle and tubing for additives such as methanol, sugar solution etc.

  You inoculate by means of a tube which afterwards is used for pH control. Fix a glass tube to the end of the tubing and wrap into sterilization tubing.

- Air inlet filter
  Insert lower portion of the sterilization tube (see photo), or employ the sterilization ferrule.

Sterilize the prepared accessories for 25 min. at 121°C (autoclave).

4.4.4. Preparation of the Fermenter

In case your fermenter is equipped with a FUNDA®LUX:

Mount the light- and electronic part, select optimal degasing time and set at zero. (see 4.2.10.2.)

After sterilization the pO2-electrode must be calibrated.

- At every socket to which the aeration pipe leads remove the blind plug and put a few drops of ethanol on the membrane. Iflame the ethanol.
- Unwrap the sterilized air inlet filter. Do not touch the needle! Heat the whole length of the needle over the flame of a Bunsen burner. Pierce the membrane. Be sure to keep the filter level.
- Screw filter on.
- Through the air inlet filter blow air into the stirred fermenter.
- Set the slope of the electrode (100%) of the pO2 probe. (see 4.2.3.3.).
- Switch off air-in.

Now the necessary needles are pierced as described for the air inlet filter. Before taking the needle out of the sterile packing insert the tube into the pump and clamp it tight with a hose clip.

The fermenter is now ready for inoculation.

4.4.5. Inoculation of the Fermenter

- Insert tube into the pump.
- Remove hose clip.
- With a gas flame heat that end of the tubing which has the glass tube attached and immerse it into the inoculum under sterile conditions.
- Set pump on local (on back side) (see 4.2.1.5.) and pump the inoculum into the fermenter.

- Switch pump off, cut off the tubing above the glass tube, heat shortly and attach it to the acid- or alkaline bottle. Fill tube with acid, (resp.with alkaline) and switch over to pH-control by setting the switch at the back side of the pump again to local.