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_CONTROL IN MICROBIOLOGICAL RESEARCH TECHNIQUE

The authors developed a sterilizable p_H measuring chain in which the
glass and the reference electrode (single-bar or single-member measuring chain)
were housed together in a compact /closed/ frame or mounting and in which the
overpressure can very easily be compensated for. The shock-resistant electrodes
reveal good properties with respect to absence of hysteresis, stability of
transconductance, and the position of the zero-point even after heat stress,
so that the electrodes can be sterilized along with the rest in the fermenter.
In this way we can make sure that the microbiological breeding systems will
be kept sterile. The article also includes the set up for a control loop for
automatic p_H control.

The hydrogen ion concentration heavily influences the metabolism of
microorganisms. This is why we will want to control this important magnitude
as much as possible in any microbiological reactions. The p_H value is normally
measured with a glass and calomel electrodes in a sample that has been taken
earlier. But such measurements require an additional expenditure and effort
in breeding dishes with sterile conditions and keeping the p_H constant -- this,
by the way, becomes particularly necessary in continual breeding -- calls for
setting up an automatic control circuit which can be adapted to the special
features of the various processes involved. Because of these special require-
ments, we cannot readily take over individual structural elements from such
a control circuit, that is to say, such as it is normally used in chemical
research and process engineering. First of all, the indicators, the final
control or correcting elements, and the space for the transport of acid and
lye must be reliably sterilizable and because of the long duration of continual
breeding, we have some very high requirements here with respect to the stability
of the measuring amplifier and the functional reliability of the entire con-
trol circuit or loop.

There is a considerable volume of literature available here on this
particular aspect of microbiological research technique and we do not want
to go into detail at this point. We will confine ourselves to some of the

more recent works which contain proposals for the solution of some of the partial aspects of these problems in terms of apparatus.

Experiments dealing with p_H control for microbiol.   al cultures were made as early as 1930; of course, the emphasis at that t   was on the actual measurement problems as such (for instance, 1, 2, 3). At   easurement technology and techniques improved, the other problems moved into the foreground and Kehoe (4) compiled all of these other problems in 1958 after a survey and study. This work illustrates the state of the art in the technical development at that time, when the studies described below were first started. The difficulties at that time stemmed mostly from the breakage of the glass or reference electrodes, the infections, the overgrowth covering the electrodes, and the electrode ducts. But above all, the electrode life was too short. However, further improvements were required in the manner of attachment, in making sure that the medium would not enter into the reference electrode, in the methods for keeping the entire assembly sterile, in protecting the electrodes against mechanical destruction, and in dimensioning the measurement indicators for small-scale fermenters. The transport and dosing of the acid or the lye generally seemed to cause less difficulties. The great extent to which microbial processes were at that time impaired by unsatisfactorily working devices is revealed by a comment made by Herbert (5); in his discussion on "fermentation technology," he expresses the hope that it would soon no longer be necessary to debate the equipment and control problems in fermenters on the occasion of microbiological symposia.

Requirements for Measurement System

For p_H measurements, two-bar measurement chains are in most cases either connected directly into the fermenter (6, 7, 8, 9, 10) or they are connected in a separate circuit (6, 9). Inserting the measuring chain into the breeding vessel itself offers more advantages (6, 9), but it requires sterilizable electrodes because any subsequent insertion or suspension of disinfected (11-13) or separately sterilized bars or rods (13, 14) can jeopardize the sterility of the setup. When large containers or vessels are used, it is necessary to attach the electrodes on the sides in order to prevent the entire structure from becoming too long; this causes very expensive alterations, for instance, in connection with the double-casing /double-lining/ version (6). Small-scale fermenters (11, 15) require special designs. An indicator for temperature compensation (14, 16) in most cases is not necessary and can be omitted because of the automatic temperature control over the content of the fermenter. The pressure at /on/ the reference electrode can be compensated for in various ways (6-9, 11, 14, 16-18), for instance, by placing the potassium-chloride storage vessel somewhat higher, by pressure-filling the electrode, or by means of a pressure-adjustment line or duct in the free fermenter space. In most cases, these measures are quite awkward or they create additional possibilities for infection.

The customary control valves cannot be used here as the adjusting members. To close off the acid and lye lines, we mostly use valves with an open-shut operation (11, 14, 17, 19). The neutralization media flow in either because of their gravity (11, 14, 17) or they are moved by means of compressed

air or with pumps (9, 10, 12, 13, 15, 18, 20, 21). Sometimes we can even use so-called titrators, that is to say, mechanical titrating devices (13, 18). In the case of small-scale fermenters, we must be able to dose the volume of lye or acid to be added very accurately (buret). In other words, the requirements for a perfectly operating p_H control system are quite manifold.

The measuring chain with the electrodes must be sterilizable when inserted in the fermenter, without its lifetime being considerably reduced as a result of this. Single-bar measuring chains (so-called combination electrodes) are particularly advantageous here. "Sterilization" outside the breeding vessels by means of preservation in formaldehyde, alcohol, or ethylene oxide -- which is tantamount to a mere disinfection -- is never anything more than an emergency solution to the problem (22). Of course, the electrodes must be able to resist heat and mechanical stress. The reference electrode must be provided with an easily operated device for pressure compensation and a rather longer-lasting potassium chloride supply. The efficient and effective mounting of the electrode is particularly important here; this frame or mounting must permit perfect sterilization and must make any infection impossible.

The stability of the measuring amplifier is decisive in the choice of the amplifier. In general, we might prefer AC amplifiers over the DC amplifiers. Since p_H amplifiers, regulators, and recording apparatuses are not built into one unit in most products, the entire setup may assume considerable proportions. In most cases however we can get along with an impulse intervalometer.

Another thing that is important for the final control member and for the addition of the neutralizing media is this: All possibilities of infection must be avoided and the final control member or element, the lines and ducts, as well as the storage vessels must be reliably sterilizable. The final control element can be designed for on-off or control operation. The on-off function in the smaller units provides more safety against overcontrol /overregulation/ (with a pause or interval timer connected in between) than the regulating or control function. Variably adjustable opening times and valve channels or paths give us good results also in connection with on-off controls. It should be possible in this connection to determine the acid and lye consumption during breeding.

Basically, an effort has been made to improve the electrodes and their frames and to arrange the neutralization agent input in such a way that it can be adapted to the requirements of the various individual processes.

Electrodes

For sterilization, the electrodes are heating for some time (for instance, 20 minutes, 121° C) and they must give us values that are repro cible in sustained operation whenever we breed at the breeding temperature is mostly between 20 and 40° C.

Glass electrodes are subject to wear and tear primarily because of

the pressure and sometimes, quite accidentally, through shock or impact. Under the usual working conditions, we can get maximum pressures of up to 2.5 ato. The electrodes should therefore be designed for 10-20 ato for safety reasons. They should also be able to withstand occasional blows or impacts (nonhomogeneity of the measuring solution, accidental bumping during installation). In electrodes with cylindrically designed "membranes" as the p_H-active glass part and, under certain circumstances, also in the case of spherical /cup-shaped/ forms, the impact resistance is great but the latter usually reveal unfavorable electrical properties. This kind of electrode can, for instance, penetrate the bottom of an upside down pyrex glass beaker when dropped from a height of 10 cm, without any damage (falling weight about 460 g).

In general, p_H-sensitive glasses /glass vessels/ do not stand up very well under hydrolysis. As the hydrolysis resistance increases and as the electrical resistance increases. the alkali errors mostly become smaller. We can say that the hydrolysis resistance is very good when the membrane surface and its function have practically not changed after 200 heat stresses or after half a year of use in the various breeding solutions.

The zero-point position of the electrode -- that is the p_H value at which the electrode chain gives us a voltage of 0 mV -- should remain constant at room temperature for a long time, even if the electrode was exposed to high temperatures in between. If this is not the case, then we must calibrate again. When we have electrodes that are inserted in a sterile state, this is undesirable even if subsequent calibration were possible by removing samples under sterile conditions.

The position of the zero-point depends primarily on the properties of the electrode glass, on the internal electrolyte, and on the deflector /leakage/ system of the electrodes. These facts were taken into consideration in the development of an electrode whose zero-point position remains unchanged even after heat stress. Figure 1 shows an excerpt from a p_H measuring strip between the 22nd and the 27th heating. Zero-point was adjusted after the first heating and was then no longer corrected; after the 94th heating, there was a deviation of 0.1 p_H units.
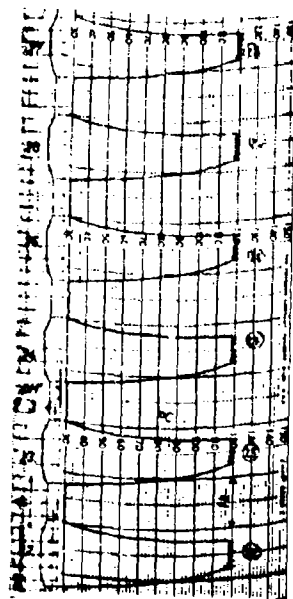
Figure 1. Behavior of a sterilizable measuring chain in a buffer solution (p_H 4) between the 22nd to the 27th sterilization cycle. Temperature 30° C, respectively, 120° C, without temperature compensation. Temperature cycle and pressure superposition /heterodyning/ controlled automatically.

Let us now look at an electrode with hysteresis phenomena; when we heat and then cool a solution to the same temperature, we do not get the same electrode potential at that electrode. The duration and the magnitude of this indication error depend on the extent of the hysteresis. If the initial potential is not achieved even after a long time, then this is tantamount to a shift in the zero-point, so that we will have to calibrate again.

Figure 1 shows us that the new electrode now works without any hysteresis. The temperature and p_H recording strips are shown here in a synchronised fashion, so that the adjustment of the potential balance and the temperature balance can be checked simultaneously. In the case of the electrode studied here, we observed practically no hysteresis effect after about 120 heating operations. The experiments were conducted in a fermenter with a volume of 10 liters and with automatic temperature control, in such a fashion that the heating, cooling, and temperature maintenance cycle at 30° C, respectively, 120° C, lasted only 2 hours. A pneumatic differential pressure relay, controlled

by the fermenter pressure, was used for automatic pressure compensation (0.1-0.2 ato).

The derivation /leakage, shunt/ system and the inside content of this electrode were developed earlier (25). The pH-active electrode glass could be determined by means of a selection from several hundred newly produced glass smelts.

The transconductance of the potential curve (with the potential plotted against the pH value) is a yardstick for the sensitivity of an electrode. In an ideal electrode, the potential difference at 20° C is 58.1 mV per pH unit; it changes as the temperature increases by +0.2mV/°C. Changes in the transconductance become noticeable as a result of a shift in the calibration position after longer use (temperature stress) (cf., Figure 1). We can expect that the newly developed electrodes will be able to withstand several dozen sterilizations without any noticeable change in their sensitivity.

The Reference Electrode

When the temperature is increased to more than 100° C during sterilization in the fermenter, the electrolyte temperature of the reference electrode (potassium chloride solution) will rise above its boiling point and we will also get an overpressure of 1 ato or more in the reaction vessel. In order to prevent the measuring solution (nutrient media) from penetrating into the interior of the electrode across the electrolyte bridge as a result of this overpressure and in order thus to prevent the electrolyte from becoming contaminated and to prevent the potassium chloride solution likewise from evaporating, we start with a higher pressure in the reference electrode (over-compensation). Here we mostly need about 0.1-0.2 ato. In older constructions, the glass and reference electrodes were separate. A combined design however is practically indispensable in the case of small fermenters for reasons of space. Although the first combination electrodes were placed on the market way back in 1947, they could not be introduced into microbiological research technique until a short time ago because their design did not meet the above mentioned criteria.

Figure 2 shows us a sterilizable measuring chain with combined glass and reference electrode. The reference electrode surrounds the glass electrode concentrically and is, in its upper portion, expanded into an abundantly dimensioned storage vessel for the potassium chloride solution. The electrolyte bridge (Diaphragms) has been improved to the point where the potassium chloride consumption is low even in case of heavy overcompensation of /for/ the reaction pressure. It is about 1 ml/24 hr at 2 ato. When the storage vessel has a volume of 30-35 ml, we would therefore, under these extreme conditions, require a single refill per month.
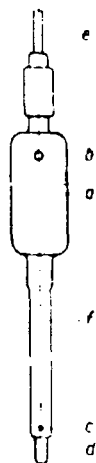
Figure 2. Sterilizable measurement chair with combined glass and reference electrode. a--storage vessel for KCl; b--feed opening; c--electrolyte bridge (Diaphragms); d--pH active part of glass electrode; e--screened /shielded/ cable; f--electrode shaft with variable structural length.

The electrode is connected to the amplifier with a teflon-insulated coaxial cable which permits us to provide for a perfect seal at the frame inlet.

Frame /Mounting/

Figures 3-5 show the newly developed frames for attaching the sterilizable electrodes in small or large containers. The packing element here consists of O-rings and this also applies to the sealing of the glass shaft in the mounting. During sterilization, the upper part of the electrode does not rise to the sterilization temperature but experience has indicated that the small volumes of escaping potassium chloride solution will not cause any infections. At any rate, the sterilization is guaranteed much more reliably in this fashion than by means of a mere disinfection of the electrode.

The frame can easily be used with a fast-locking device secured by a cap screw /clamping nut/. Wherever the standard counterpart cannot be attached to existing or currently available containers without an excessive effort, the version shown in Figure 3 is screwed onto the customarily used flange tubes.

Figures 4 and 5 show the mountings for the lateral insertion or installation of the electrodes in large containers or in the small fermenter. For pressure compensation, the electrodes can be attached either to a compressed-

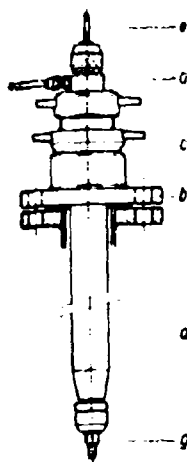air line or they can be placed under pressure with a compressed-air gun or
a bicycle pump.

Figure 3. Sterilizable p$_H$ measurement chain for installation in fermenters
of any desired size. a--immersion tube; b--intermediate flange; c--fast-
locking device; d--compressed air connection; e--electrical connection;
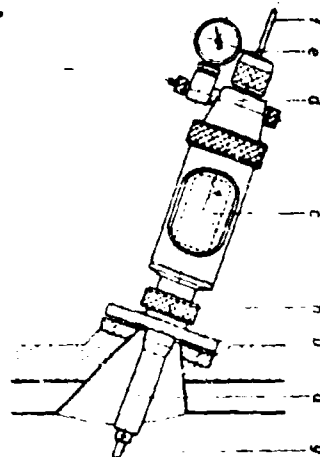g--p$_H$-active part, respectively, electrolyte bridge.

Figure 4. Sterilizable p$_H$ measurement chain for lateral insertion in double-
casing /double-wall/ fermenters. a--immersion tube; b--intermediate flange;
c--glass window; d--recoil valve; e--pressure gauge; f--electrical connection
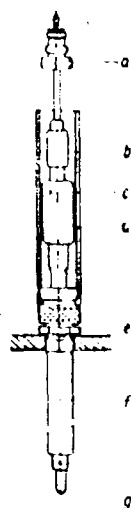(coaxial cable); g--p$_H$-active part; h--fast-locking device.

Figure 5. Sterilizable p_H measurement chain for insertion in small fermenter (volume 5-15 liters). a--coupling for coaxial cable; b--protective tube for upper part of electrode; c--feed opening for KCl; d--KCl storage container; e--screw-type lock, respectively, O-ring lock; f--protective tube for electrode shaft; g--p_H-active part of glass electrode.
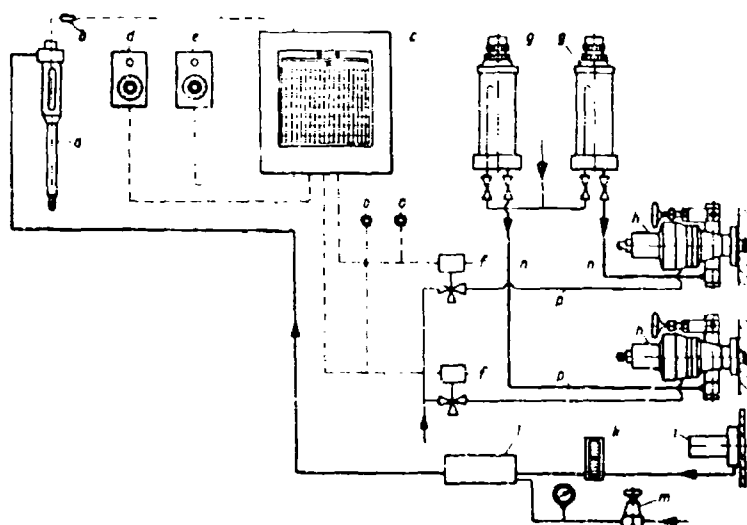

Figure 6. [Legend on following page]

Figure 6. Layout of automatic control circuit for p_H control in micro-
biological cultures. a--sterilizable p_H measurement chain with combined
glass and reference electrode; b--coupling to the coaxial cable; c--regulator
and recording unit with built-in p_H amplifier (Leeds & Northrup, Speedomax
H, amplifier model 7566); d--timer I (injection impulse); e--timer II (impulse
interrupter); f--magnetic valves; g--absorption bulbs /receivers/, 2.5 liters
for acid, respectively, lye; h--injection valves for acid or lye; i--pressure
transmitter, model Foxboro B 2713; k--pressure gauge for fermenter (model 50,
Foxboro); l--differential pressure relay (model 56, Foxboro); m--reduction
valve for adjustment of differential pressure (0.1-0.2 atm); n--acid or lye
pipeline; o--buttons for operating magnet valves; p--compressed-air line.

Layout of Control Circuit

Figure 6 shows the layout of a control circuit, such as it was used in
an experimental setup for the continual breeding of microorganisms (bacteria,
yeasts, molds) at the microbiological institute of the Swiss Technical College
at Zürich. The installation includes a fermenter with 10 liters of working
volume, before and after which we have two 150-liter agitator boilers as
storage and collection containers. The p_H measuring chain is attached to the
lid of the fermenter with standard screws and it is sterilized in the fer-
menter. The acid and lye storage containers (with a volume of 5 liters,
each) are connected to injection valves at the collar of the fermenter via
rust proof steel pipelines. The neutralization agents are forced into the
fermenter with sterile compressed air amounting to a maximum of 1.5 ato. The
storage containers and the lines are sterilized with steam in the empty state,
independently of the fermenter. A cleaning device, such as it has occasionally
been considered necessary, likewise proved to be unnecessary even in long-
lasting breeding experiments, provided the p_H-active part and the diaphragm
were forcefully washed by the fermenter content.

The amplifier, regulator, and recorder are designed in the form of a
combination unit and are placed in the housing of the Speedomax-H regulator
(Leeds & Northrup Ltd., Birmingham G.B., agent: Dr. Ness, Küsnacht, Zürich).
The impulses of the Speedomax regulator are transformed for the pneumatic
control of the final control elements and they are conducted to the magnetic
valves of the compressed-air line via two timers in a Flip-Flop connection,
whereby the first timer determines the opening time for the final control
element, while the second one determines the time of the pause or interval.
This arrangement quite reliably prevents any overregulation due to a certain
dead time. The on/off function of the final control element was selected
here for safety reasons because, when progressive control is used, the narrow
passageways in the valve seat cause clogging. The injection volume per im-
pulse can be influenced at three different points: First of all, by changing
the lift of the injection valve, then by pressure heterodyning at the acid
and lye container, and finally by varying the injection time.

The results obtained from this arrangement in terms of control engineer-
ing are very good; the various individual injections are no longer recognizable
on a recording strip with a width of 167 mm (p_H = 2-12) (Figure 7a); when

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necessary there is however a possibility of making them visible through the corresponding adjustment of the pause or injection timers (Figure 7b), so that, for instance, the occurrence of microbial activity can be pinned down in terms of time.
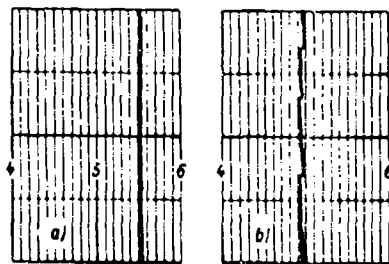

Figure 7a. Recording strips for a culture Saccharomyces cerevisiae with p_H control; continual breeding, required p_H value = 5.5.

The control circuit was so adjusted that the individual injections are no longer recognizable; feed rate 25.4 mm/hr; p_H of nutrient solution pumped in = 4.0.

Figure 7b. Recording strip for a culture of Aspergillus niger with p_H control. The control circuit was so adjusted that the various injections are recognizable so that we can determine the start of acid formation. Required value: 5.0; feed rate 25.4 mm/hr.

The circuit diagram of the control circuit as per Figure 6 can also be used for industrial microbiological installations and for laboratory equipment. On the basis of experience, the operational reliability of the elements used is so great that we need no longer expect any breakdowns from that aspect.

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