DIFFERENT LABORATORIUM-SCALE CONTINUOUS FERMENTATION SYSTEMS

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The extraordinarily rapid progress of fermentation technique as well as our deepening knowledge of the kinetics of fermentation processes enable us to replace old batch fermentation processes with continuous systems. The fundamental solution of the problem technically and theoretically is at our disposal; further experiences and theoretical data are to be obtained by intensive exploration work all over the world.

In our Department systematic research work in the field of continuous fermentation is in progress. At the initial phase of our work the aim was to construct fermentors that are suitable both for aerobic and anaerobic fermentations. In the following short paper some fermentor types are given, constructed in our fermentation department.

I. First the glass fermentor shown on Fig. 1. was constructed. The instrument, as seen on the figure, is an aerated continuous reactor without agitation. Aeration is supplied by an aquarium pump (1). Steady air current goes into a fibre-packed capillary flow meter, thereafter into the cottonpacked air filter (3) and then into the vessel (4) where the air is blown in bubbles through water, and thus becomes saturated by water vapour. This is important as otherwise dry air would involve considerable evaporation losses in the small laboratory-scale fermentor. This vapour-saturated jacketed glass vessel can be temperated by an ultrathermostate, and when necessary refilled with steril water from the vessel (5).

Steril, vapour-saturated air bubbles through glass filter No. Gl into the glass fermentor of 150 ml net volume (6), and into the fermentor body at the jacketed section. The top part of the fermentor joins the body by a ground glass joint. On the top part three pipe-stubs are to be seen: the first one provides junction to an anti-foam flask (7), the second one to the air outlet (8), and the third one to the medium feeder (10). The medium of the fermentation can be sterilized in a Deville-flask of suitable size (9), and from here it can be returned through the capillary feeder (10) into the fermentor. By means of an ultrathermostate, the capillary medium feeder can also be conditioned. Pipe marked by (11) is required for the compensation of the differential pressure between the

fermentor and the feeding system. Feeding rate is controlled by the level difference between the lower part of the pipe entering into the medium storage tank, and the outlet of the capillary feeder. Changing the level difference, feeding rate can be modified.

The fermented mash leaves the fermentor through an overflow pipe. On the upper part of this a hole inhibits suctioning of the medium from the fer-

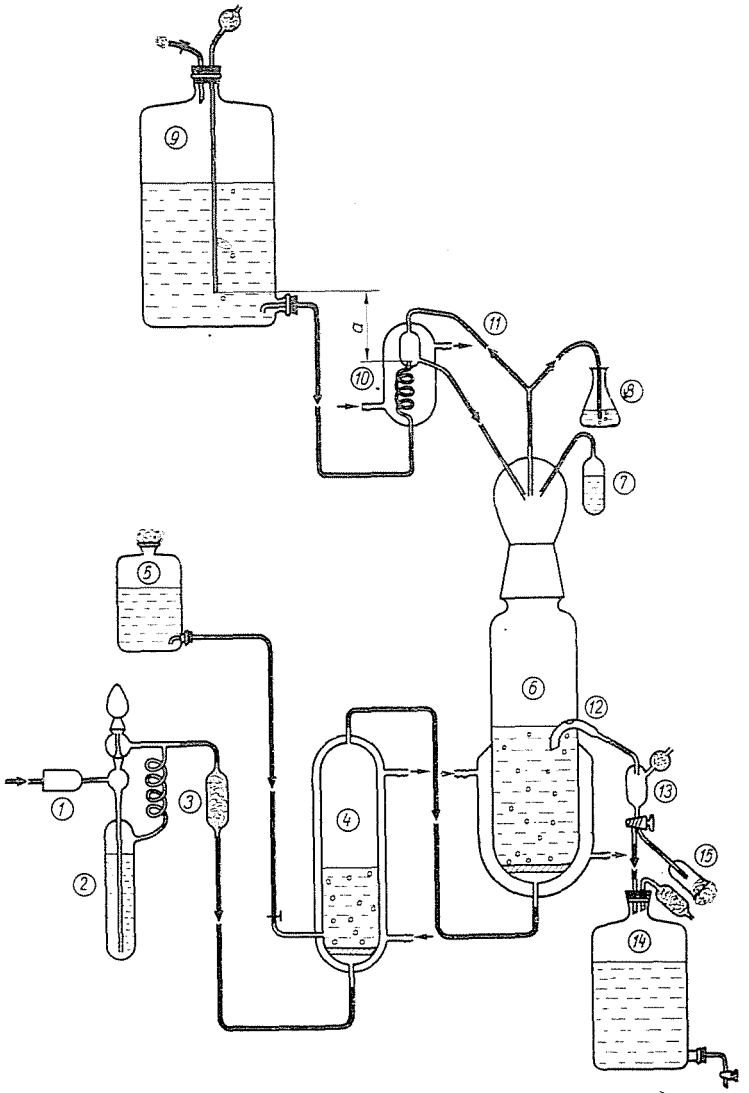

Fig. 1. Continuous aerobic fermentor without agitation. 1. Aquarium pump, 2. flow meter, 3. air filter, 4. tube for vapour saturation, 5. storing tank for sterile water, 6. fermentor, 7. feed flask for anti-foam agent, 8. air outlet, 9. storing tank for the medium, 10. capillary feeder of medium, 11. pressure compensation pipe, 12. overflow, 13. draining vessel, 14. storing tank, 15. sampler

mentor. Fermentation fluid goes from the fermentor into the draining vessel by (13), and from here through a double-bored valve either into the storing tank (14) or into the steril sampling stub (15). By the sampling, after removing the cotton stopper, a sterile test-tube is placed into the sampling stub, and the medium collected in vessel (13) pours into it.

We sterilized the apparatus in a completely assembled state, according to coupling represented in figure No. 1.

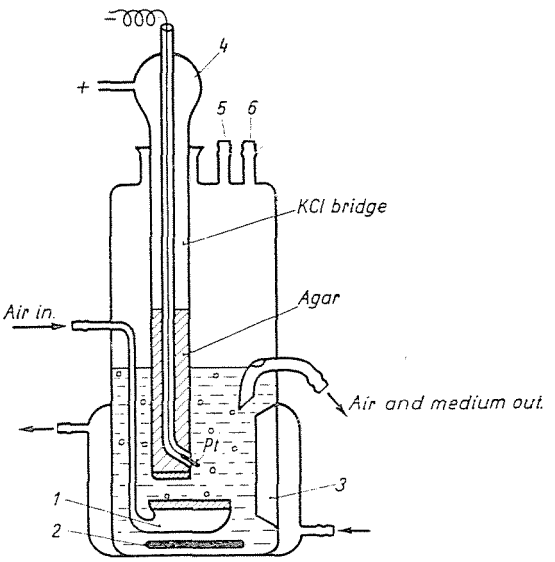

Fig. 2. Continuous aerobic fermentor with magnetic agitator. 1. Pipe for air inlet, 2. magnetic stirrer, 3. baffle, 4. Pt-calomel electrode, 5. stub for medium feeding, 6. stub for the inoculum and anti-foam agent

We ran sorbose "test" fermentation experiments in the small size fermentor described above. E.g., when we started feeding the fresh medium containing 10 % sorbitol with a dilution rate of D = 0.818/day, steady state set in after about 4 hours had elapsed, with 70% sorbose content. From our experiments we deduced that this type of fermentor primarily suits the investigation of the drug resistance of microorganisms, and genetical and bacterium physiological studies, but it is less suitable for the investigation of fermentation processes, in view of the fact that steady states are characterized by fairly low sorbose concentrations. It should be noted, however, that steady states occurring with low sorbose concentrations indicate an unsatisfactory oxygen level, due either to too rough feeding of the medium, or as a consequence of unsatisfactory aeration without agitation.

II. Designing the other small-size fermentor, we endeavoured to build our fermentor to be more similar to fermentor tanks used in industrial size (scale). In order to do this we constructed an aerated fermentor with agitation (Fig. 2).

In this type of fermentor, the feeder and the piping for the fermentation fluid as well as the part for sterile air supply are fully identical with the equipment described above. The fermentor body, similarly to the former one, can be conditioned by means of an ultrathermostate by its jacket. Aearation of the medium is provided by a glass filter (1), and agitation is supplied by a magnetic mixer (2). In order to provide horizontal fermentation level and turbulence-

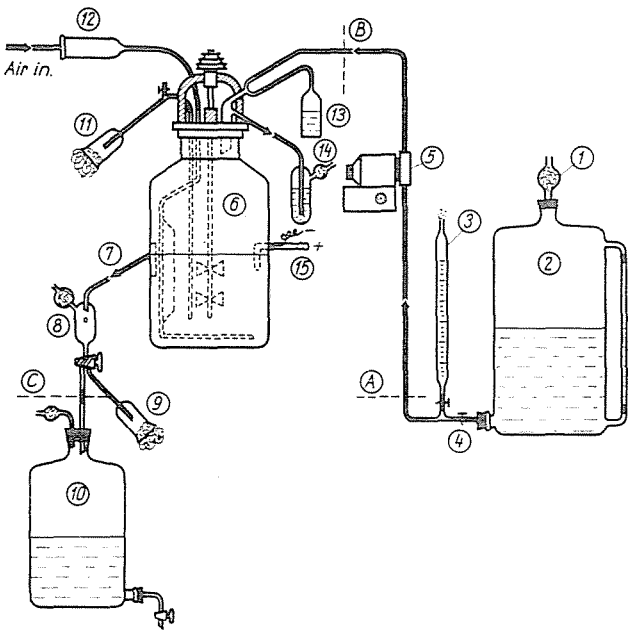

Fig. 3. Continuous aerobic fermentor of 10 l volume. 1. Cotton-filter, 2. storing tank for the medium, 3. burette, 4. tap, 5. precision feeding pump, 6. fermentor, 7. overflow, 8. draining vessel, 9. sampler, 10. storing tank, 11. sampler for batch operation, 12. air filter, 13. feeder for anti-foam agent, 14. air outlet, 15. Pt-calomel electrode

conditions, i.e. providing better aeration, baffles have been built into the fermentor body (3). For the determination of the redox relations and the efficiency of aeration of the fermentations, we hung a Pt-calomel electrode into the medium (4). It is only natural that we constructed the coupling of the electrode in a sterile proof manner, employing cotton packing (not to be seen on the figure). At the top of the fermentor, two pipe ends (5, 6) serve for the inoculation and for feeding the anti-foam agent and the medium. In our experience, this fermentor type is more suitable for studying steady states of fermentations than the former one with simple agitation.

III. A third fermentor of a larger size was built with 10 l net volume, the body of the fermentor itself constructed of stainless steel as well as all its fittings. This fermentor is to be regarded as a continuous aerobic fermentor

with aeration and agitation. Fig. 3 shows a rough sketch of the equipment and of the coupling.

Air goes into the substrate tank (2) through a cotton filter (1), replacing the departing medium. The substrate tank is constructed of stainless steel, with 13 l volume and is supplied with a level indicator. Sterile medium is fed by means of a precision chemical pump (5), as the capillary of previously employed feeders frequently plugged, and caused disturbances in feeding. Feeding rate of the pump has been checked with the burette (3) shown on the figure, by closing valve No. 4, and thus the pump furthered the medium from the burette (the top end of the tube being supplied with a cotton plug). On the cover of the stainless steel fermentor (6) the following fittings are mounted: sampling (11), cotton filter supplying sterile air, feeder of anti-foam agent (13), flask for air outlet (14), holes for the agitator and a pipe for substrate feeding. In case of continuous fermentation, the fermented fluid goes through an overflow (7) into the draining vessel (8), from which it could be drained into the sterile sampler (9) or into the storing tank (10).

Within the fermentor, the overflow-part is surrounded by a plate having a half-circle form which serves the purpose of preventing the immediate departure of the fresh substrate, so that it drains only after a certain retention time had elapsed. Through the hole on the side of the fermentor body a Pt-calomel electrode was immersed the construction of which was identical with the one represented in figure 2.

The equipment was sterilized taking it apart into four pieces, each piece being sterilized separately, dismantled at points A, B and C, and at the proper phases of the operation the medium tank, the pump unit, the fermentor and the storing tank were fitted together.

We checked the usefulness of our continuous fermentation system by a sorbose "test" fermentation. Fermentation was run on a medium containing 10% sorbitol and 0.5% corn steep liquor. After the batch operation cycle ended, fresh medium was fed into the system with 0.41 dilution rate (D = 0.41/day), and the sorbose content, bacterium number and pH value measured. The steady states of both the batch fermentation cycle and of the continuous fermentation are represented in Fig. 4.

As shown by the continuous sorbose fermentation experiments run in a stainless steel fermentor of 10 l net volume, this type of fermentor is suitable for continuous fermentation. Steady state could be maintained without any difficulty for 150—160 hours. At small dilution rate values a very good conversion was attained (93—96%), corresponding to the conversion run in batch operations.

In the following we wish to demonstrate the applicability of the Pt-electrode built into the fermentor. In the course of a batch fermentation we determined to increase the sorbose content, and the number of bacteria,

moreover the change of the pH value as well as that of the redox potential of the medium. From redox potential values (EO_2 and EN_2) we also established the values of dissolved oxygen concentration (C_L), employing the eval-

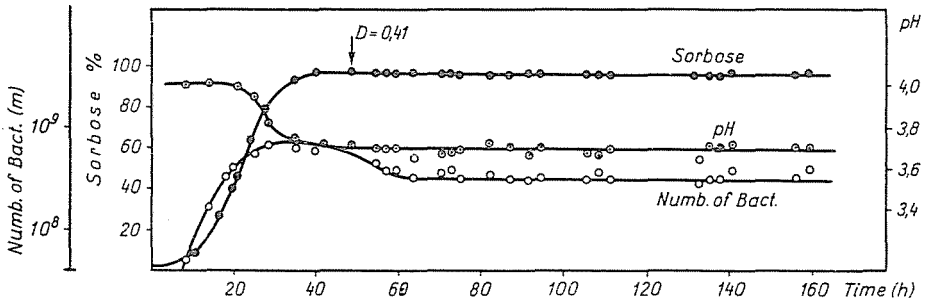

Fig. 4. Steady state of a continuous sorbose fermentation

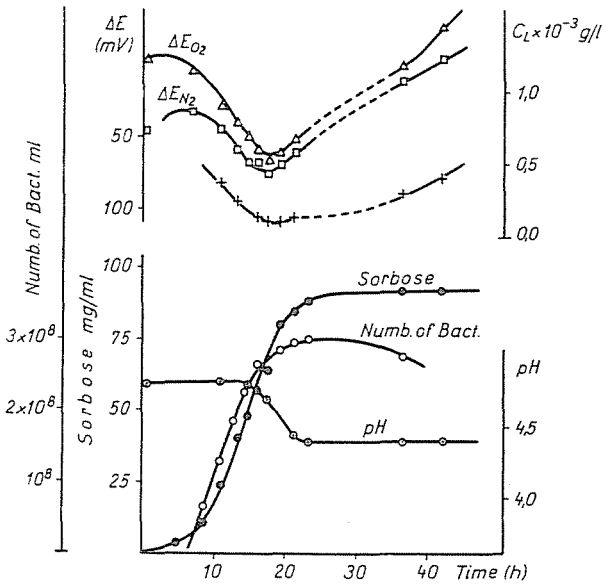

Fig. 5. Biochemical picture of a sorbose fermentation

uation method of TENGERDY [1]. The biochemical picture of a typical sorbose fermentation is demonstrated in Fig. 5. It became apparent that by means of the electrode, we could get more detailed information about fermentation processes. Thus e.g. as can be seen from the curves obtained, in case of sorbose fermentation not only redox potential curves possess a minimum in 70—75% sorbitol-sorbose conversion [2, 3] but there is a minimum at the very same point

on the oxygen concentration curve, too. Thus, it might be concluded that the speed of sorbose conversion has its maximum at this point, in consequence, the highest oxygen demand of the culture falls to this point.

IV. With certain fermentations it is more advantageous to work with two or even multistage fermentation systems, instead of a single-stage one. For this reason, we attempted to convert the single-stage system seen in Fig. 3.

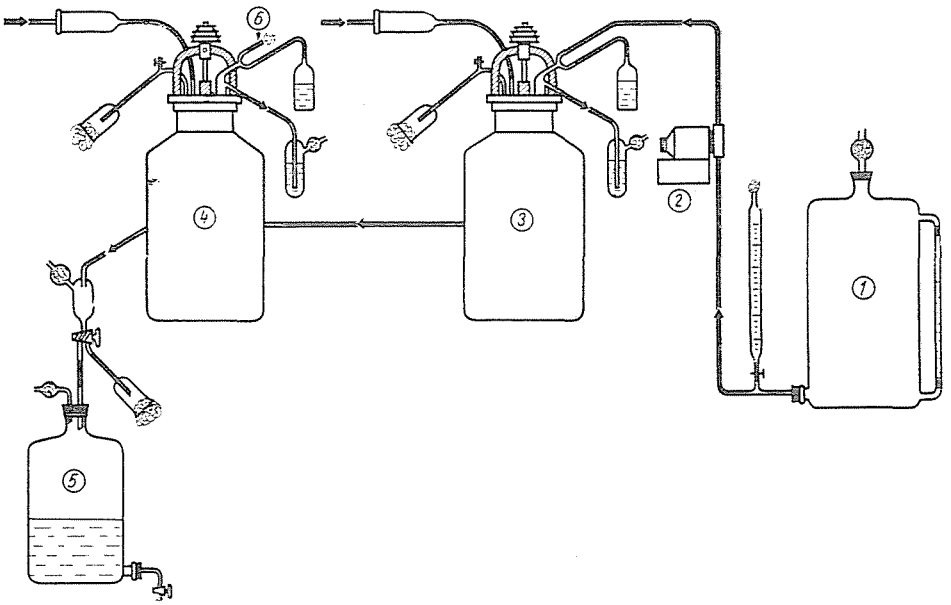

Fig. 6. Two-stage continuous aerobic fermentation system. 1. Storing tank for the medium, 2. precision feeding pump, 3. fermentor (first stage), 4. fermentor (second stage), 5. storing tank, 6. pipe end for feeding the precursor or the fresh substrate

into a system containing two fermentor elements (Fig. 6). The two connected fermentors are of identical construction with the fermentor represented on Fig. 3. Feeding of the second fermentor is provided by a simple overflow system of the first one. Both the overflow of the first fermentor as well as the inlet pipe and the overflow of the second one are supplied with the baffle described in the former equipment. Into the second fermentor unit fresh substrate or precursor can in the course of the fermentation be fed according to need through pipe end marked 6.

As evidenced by our experiments, this two-stage fermentation system is applicable for the study of continuous fermentations. We attained about 80% conversion with a dilution rate of 1.48 in sorbose fermentation experiments, employing a single-stage fermentor. With identical dilution rate, in case of a double-stage fermentation system this conversion amounts to 95%.

V. Fig. 7 represents an apparatus built for the continuous propagation of algae. Its feeding and discharging system are identical with the one disclosed in the former system. Tube fermentors are constructed of glass; stirring is provided by the CO_2-air mixture introduced through a glass filter. Tube fermentors were provided with the necessary fittings as gas outlet pipe (7), inocu-

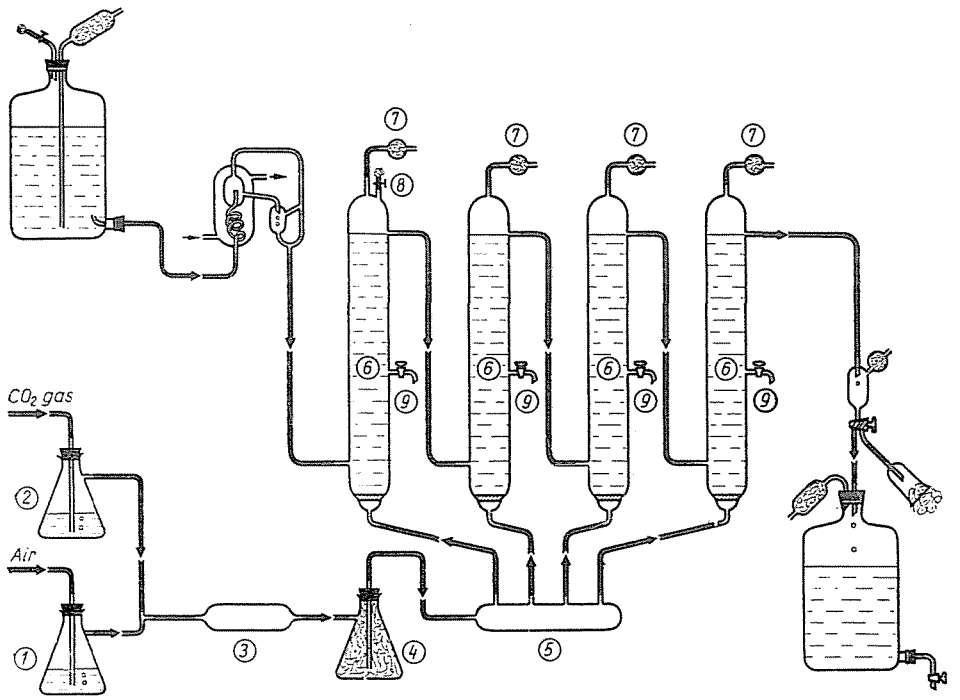

Fig. 7. Equipment for continuous alga-culture. 1. Air feeder, 2. CO_2 feeder, 3. mixing vessel for gases, 4. gas filter, 5. distribution tank, 6. fermentors, 7. gas outlet-pipe, 8. inoculum tube, 9. sampler cock

lation tube (8), sampler cock (9), inlet and outlet for the medium. Net volume of each fermentor was 1.5 l; they might be connected according to any number wanted.

The air-CO_2 proportion is adjusted in bottles 1 and 2, by means of the numbers of bubbling. Vessel No. 3 serves for the mixing of gases, No. 4 for the filtering of gas mixture and No. 5 for the distribution of the gas mixture. Tube fermentors were illuminated from the side with fluorescent light tubes.

Apart from alga cultivation, the apparatus is also suitable for different continuous fermentations.

VI. All fermentors described above may be conveniently employed for aerobic processes. As we had in view also the studying of some anaerobic processes, e. g. that of alcoholic fermentation, we constructed an anaerobic fer-

mentor tube on laboratory scale (Fig. 8). Both the inlet and the outlet of the fermentation system are fully identical with the systems described previously. Yeast propagation happens in the tube part of 1 liter net volume (2). The air goes by pressure through a cotton filter (1) into the part serving the propagation. Air dispersion is supplied by a glass filter built into the bottom of the

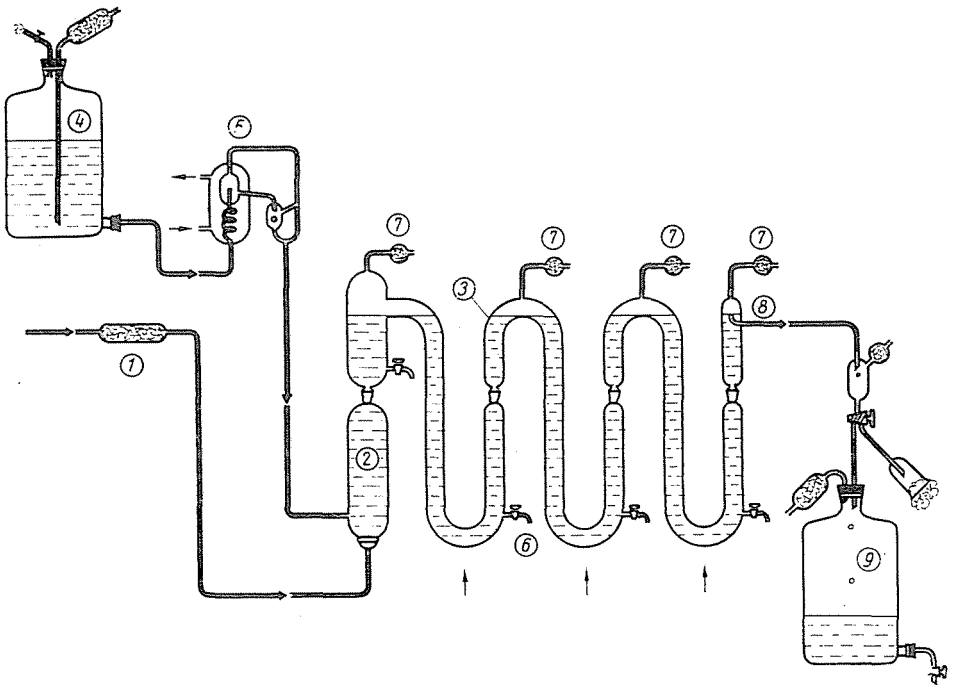

Fig. 8. Continuous anaerobic fermentation system. 1. Air filter, 2. culture tank, 3. tube fermentor, 4. storing tank for medium, 5. capillary feeder, 6. sampler cock, 7. tube for gas outlet, 8. overflow, 9. storing tank

tank No. 2. Fresh medium is fed through a capillary feeder (5) into the lower part of the propagator into the fermentor of tube construction, the individual units of which (3) are assembled with ground glass joints, in consequence the length of the system (thus the retention time) can be modified according to choice. Gas-metabolite-products produced by microorganisms discharged through a hole at the top of each tubing part, supplied with cotton plugs (7). The net volume of each tubing section amounts to 0.5 liters. Progress of fermentation may be traced by the analysis of the samples taken through the sampler cocks marked 6. Fermentation fluid is drained off from the last unit through a simple overflow marked 8.

When performing alcoholic fermentation experiments with the system described above, we experienced that yeast settled at the lower part of the U

bend. This may be avoided by placing a magnetic mixer at the places marked by arrowheads in each unit. It should be added, however, that this modification completely modifies the character of the whole fermentation system; the system that initially had been of a piston-flow one, is being converted into a series of mixed reactors. According to our experiments, this fermentation system can be conveniently employed for the investigation of alcoholic fermentation.

In our work done so far we aimed at developing the fermentation systems described above and for their construction. In the next future we wish to perform instrumentation and automation of our continuous fermentors.

Summary

The authors designed and built continuous laboratory fermentation systems of different volumes. All these different fermentation systems (only aerated, both stirred and aerated, single and multistage systems), may be conveniently applied for aerobic and anaerobic fermentations, i.e. for the propagation of different microorganisms and algae in a continuous manner.

References

1. TENGERDY, R.: Biochem. Microbiol. Technol. Eng. **3**, 241, 255 (1961).
2. KÁRPÁTI, S. I. and KRÁMLI, A.: Biológiai Közlemények **5**, 1 (1959).
3. KRÁMLI, A.: Biológiai Közlemények **2**, 7 (1954).

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