ABSTRACTS OF PAPERS

FIFTH INTERNATIONAL FERMENTATION SYMPOSIUM

BERLIN 1976

EDITED BY H. DELLWEG

ABSTRACTS OF PAPERS

FIFTH INTERNATIONAL FERMENTATION SYMPOSIUM

FOURTH INTERNATIONAL SPECIALIZED SYMPOSIUM ON YEASTS

BERLIN 1976

EDITED BY H. DELLWEG

VERLAG VERSUCHS- UND LEHRANSTALT FÜR SPIRITUSFABRIKATION
UND FERMENTATIONSTECHNOLOGIE IM INSTITUT FÜR GÄRUNGSGEWERBE UND BIOTECHNOLOGIE
ZU BERLIN

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PREFACE

Optimal transmitting of information in science and technology implies that it will be quick and without delay and that it is confined to substantial facts. It is known by experience that it would take at least 9 to 12 months to collect the manuscripts of a congress to make them up to proceedings and to mail them to all participants, notwithstanding the fact, that in our case it would not have been possible to comprise all reports of this Symposium in one book. We made it our aim to supply all members at the beginning of the Symposium with satisfying informations on the scientific contents and for this reason to renounce later proceedings. The speakers were asked to send extensive abstracts of their reports in an extent of about 800 words 4 months before the Symposium. We thank warmly all speakers for the well-timed and exemplary manuscripts which are partly supplied with instructive graphs and figures so that this book with the preprints of all lectures could be finished in due time.

At the final meeting of the Fourth International Fermentation Symposium in Kyoto, 1972, the Institut für Gärungsgewerbe und Biotechnologie had invited officially to Berlin. We are glad that our invitation has been accepted by the International Union of Pure and Applied Chemistry (IUPAC). The large organizing committee has done its utmost to help the Fifth International Fermentation Symposium to success.

During the preparations we were asked by the International Association of Microbiological Societies (IAMS) to undertake a specialized symposium on yeats, entitled "Yeast for Industrial Use" within the frame-work of the Symposium. Thereupon Prof. Dr. S. Windisch compiled 37 lectures which will be given within the four Y-sessions.
The "International Symposium on Microbial Growth on C_1-Compounds" took place on the 5th of September 1974 in Tokyo. There it had been decided to plan likewise a separate session under this important theme at the Berlin Symposium. Accordingly 16 lectures have been selected which will be given in session 21.

We should like to express our thanks to the Bundesminister für Forschung und Technologie and to the Senat von Berlin for making available the financial basis of the Symposium. Furthermore we thank for the generous gifts coming from the industry.

About 1200 Active Members and Students together with 140 accompanying ladies from 40 countries have already announced their participation in the Symposium.

We hope that the 430 lectures, presented within the next days, and further fruitful discussions will serve to deepen our basic knowledge of microbial processes and to improve existing technical procedures. They should likewise contribute to an improved availability to mankind of vital commidities, to optimal exploitation of available raw materials and energy sources as well as to an improved human environment.

Berlin, June 1976

Hanswerner Dellweg
Chairman
of the Fifth International
Fermentation Symposium

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SESSION 1

FERMENTER OPERATIONS

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H. Taguchi
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(Frankfurt/M. GFR)

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SCP FROM METHANOL AND ETHANOL IN BUBBLE COLUMN FERMENTER

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The aim of the present project is to investigate the applicability of bubble columns as bioreactors for synthetic protein production. A fine steel bubble column fermentor of 50 l volume was used, which can be equipped by porous plate as well as by perforated plate gas distributors. The present investigations were carried out by porous plate gas distributor in concurrent operation of air and medium. The medium was feed back to the bottom of the column. The natural circulation of the medium was maintained by the bouoyancy forces of the bubbles and by the density differences of bubble column and gasfree medium in the feed back tube.

The yeast, Candida boidinii, supplied by the GMBF, Stöckheim, was cultivated in extended culture at substrate concentrations of 0.4 to 0.5 %.

The systematic investigation of the oxygen transfer rates in different media indicated the great disadvantage of the application of antifoam agents which promote coalescence and by that diminish the specific interfacial area between the gas and liquid phases. To avoid this undesired effect, the fermentation was carried out without antifoam agents, but with mechanical foam destroyer.

During the fermentation following process variables were measured:
temperature, pH, cell concentration (by optical as well as by gravimetric methods), partial pressure of oxygen in the medium at six longitudinal positions in the column, concentrations of substrate, oxygen and CO_2 in exhaust air, liquid and gas flow rates and bubble size distribution (by optical method). The results of these investigations indicate that bubble column fermentors are suited to aerobic fermentations especially to synthetic protein production, because of the high specific interfacial area and/or oxygen transfer rate at low energy requirement and high final cell concentration attainable in such fermentors. A comparison of methanol (M) and ethanol (E) as substrate indicates the superiority of the second with regard to the volumetric mass transfer coefficients k_La = 0.28 (E) resp. 0.13 (M) s^{-1} and/or oxygen transfer rates: 6.56 (E) resp. 3.25 (M) g/lh, as well as for μ_{max} = 0.20 (E) resp. 0.11 (M) h^{-1} and yields: 0.68 (E) resp. 0.42 - 0.45 (M).

Figure 1:
Growth of Candida boidinii on methanol. Substrate concentration 0.4 % CH_3OH, aeration rate 0.8 - 1.1 vvm, max. productivity 1.56 g BTM/l/h, max. yield 0.42 g BTM/g CH_3OH
(1) biomass concentration BTM (g/l)
(2) O_2-consumption g/l/h, (3) CO_2-production [g/l/h] - as function of fermentation time t [h].

Figure 2:
Comparison of growth rates of Candida boidinii on methanol and ethanol
BTM [g/l] as function of the fermentation time t [h]

Figure 3:
Mean bubble diameter d_B mm as function of the biomass concentration BTM [g/l] during the fermentation of Candida boidinii on methanol and ethanol. Gasdistributor: porous plate.
NMP: standard salt medium
(d) Beginning of the fermentation.

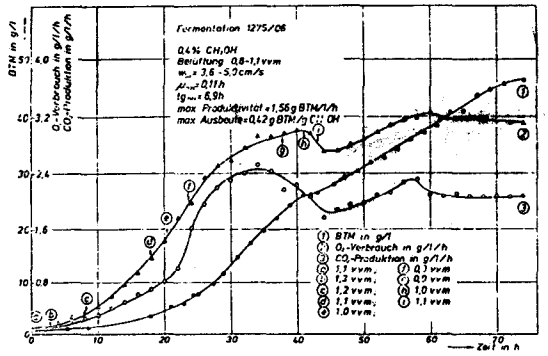

Figure 1

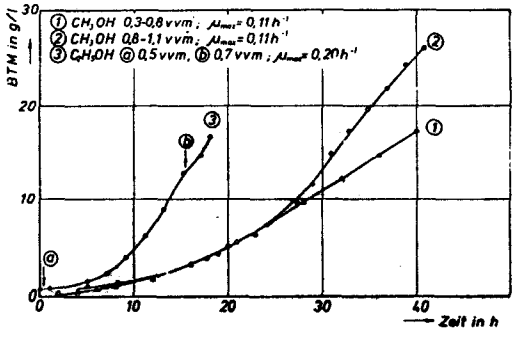

Figure 2

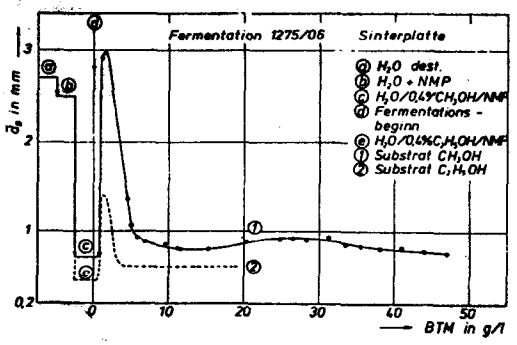

Figure 3

PERFORMANCE OF A NEW TUBULAR FERMENTOR

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The well-known theories of reactor calculation show that in continuous operation, the performance of plug flow fermentors is superior to that of infinetely mixed fermentors. In aerobic culture, this kind of fermentor is difficult to realise, especially for biomass production which requires a high energy input for agitation-aeration. We developped a tubular fermentor characterized by a system in which the required energy for dissolving of oxygen is supplied by the pulsation of a liquid in a perforated plate column.

This reactor of a volume of seventeen useful litres and diameter of 100 mm is composed of a perforated plate column (21 plates pierced with 366 holes of 2.5 mm diameter) ensuring a vacuum coefficient of 0.23, with the plate 50 mm apart.

At the bottom of the column, a crankshaft and connecting rod are fixed to a piston which pulses the liquid in the column. The amplitude and the frequency of the pulsation are variable. At four places in the reactors there are modules where the temperature and the pH are regulated and where we can sample or make an addition.

1) Hydrodynamic performance of the fermentor.

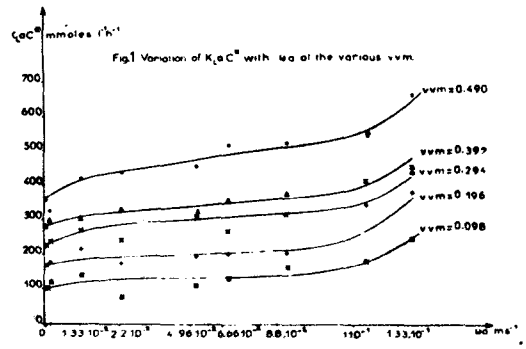
a) Energy requirement for mixing. The energy is introduced by the pulsation and by the gas dispersion. An estimation of the energy of pulsation is given by the equation : $P = (\rho g \pi \frac{D^2}{4}) H_1 \omega a$

with : ρ = density, g = acceleration of gravity, D = diameter of the piston, H_1 = height of the liquid, a = amplitude of the pulsation, ω = frequency of the pulsations, so, ωa characterizes the pulse velocity. The energy dissipated by the dispersion of the air is far less than the energy of the pulsations.

b) The variation of the hold-up is given by : $H = \frac{V_g}{V_u}$ (V_u is the volume of the air-water emulsion and V_g is the volume of the air in the column) and is estimated for various values of pulse velocity (ωa) and for various air flows : Q. The observation and the statistical analysis of the results leads to a semi-empirical equation : $\frac{H}{1-H} = \beta \ (\omega a)^{\alpha} . Q$

Contrary to other authors we have found that α and β vary according to the air flow. The experimental results agree with our theoretical values.

c) Aeration capacity (sulphite method). This is given by the rate of oxygen dissolution (mmoles $0_2\ l^{-1} . h^{-1} . atm^{-1}$) when the fermentor is gassed by air at normal temperature and pressure. The experimental results can be found in figure 1 for various air flows (vvm)



and values of the pulse velocity ωa.

Under the borderling operating conditions ωa=0,133 ms^{-1} (P/V = 1 Wl^{-1}), at vvm = 0.5, 600 mmoles $0_2\ l^{-1}\ h^{-1}$ at^{-1} can be dissolved. Potentialy, 19.2 g of oxygen can be dissolved for an input of energy of 1.1Wl^{-1} which is better than results published by other authors.

2) Fermentation. The growth rate of our strain follows Andrew's model :

$$\mu = 0.7 \ \frac{1}{1 + \frac{0.1}{S} + \frac{S^2}{8}}$$

If we call D = Q/V, the dilution rate (h^{-1}), F the feed rate of carbon substrate ($gl^{-1}h^{-1}$) X, the outlet substrate concentration (gl^{-1}), DX the productivity ($gl^{-1}h^{-1}$) ; R the effective yield, the preliminary results we got during the steady state are for example : with D = 0.156 h^{-1}, F = 0.917 $gl^{-1}h^{-1}$; X = 1.6 gl^{-1} ; DX = 0.25 $gl^{-1}h^{-1}$; R = 0.484.

If we compare these results to those we got with the same strain cultivated in a chemostat, for equivalent conditions the tubular reactor gives a better productivity and yield.

During the fermentations we observe a significant sedimentation of cells (biomass concentration gradients inside the reactor) creating an internal recycling of the biomass which is a possible explanation for the increase of the productivity rate. The better conversion rate is linked, according to the maintenance concept, to the fact that the growth rate is higher (near μ_m) in a tubular reactor than in a chemostat.

In conclusion our pulsed reactor shows a better efficiency of the rate of dissolution of oxygen for a given energy input. The plug flow fermentor offers a better technology in biomass production than a well mixed fermentor

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1.03

STAGE OF DEVELOPMENT OF HIGH EFFICIENCY FERMENTERS.

K. Schreier
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Nowadays, the possibilities to construct large-size fermenters with high specific performance are not the subject of theoretical reflexions any more, but there exist already practical operation experiences.

The new fermenter system is distinguished in principle from conventional systems by the fact that the hitherto non-homogeneous gas-liquid system is replaced by a homogeneous gas-liquid two-phases mixture. This homogeneous two-phases mixture can be determined fully on physical basis, on the other hand there is the possibility to apply new principles for apparatus designs based on the physical data.

Central parts of the new fermenter are special two-phases pumps which are able to transport a homogeneous two-phases gas-liquid mixture with a specific gravity of γ = 0.4 - 0.95.

The parameters of this system, as oxygen transfer up to 12 kg per ton and hour, specific energy demand of 0.35 to 0.5 kWh per kg D.M.S. and the further biological data of this system are described in detail. The IZ fermenter system has originally been developed by Ingenieurtechnisches Zentralbüro Böhlen, DDR and adapted by Vogelbusch for the fermentation technique in general. The experiences gained up to now with this system on the following substrates and organisms combinations are given in detail:

molasses - saccharomyces
molasses - torula
whey - saccharomyces fragilis
sulfite liquor - torula (fir, birch-tree)
paraffin - candida lipolytica
slops - mixture of bacteria
nightsoil - bacteria
waste water - bacteria
antibiotica nutrient broth - streptomyces
bacteria nutrient broth - E.coli

The new fermenter type is distinguished from conventional systems not only by its defined physical characteristics and its large application spectrum, but also by its simple technical conception. Fig. I shows the system and its functions:

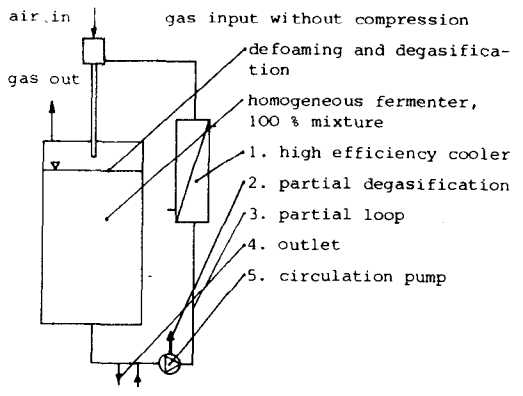


Fig. I

The functions circulation, mixture, aeration and degasification, cooling and defoaming are executed by only one single standard mechanical equipment.

The present construction principle allows the following technical and economical solutions:

- optimization of fermenters by moduls
- far-reaching standardization
- construction of large-size fermenters (multiple arrangements)
- multistage fermenters

Fig. II shows a large-size fermenter with multiple arrangement:

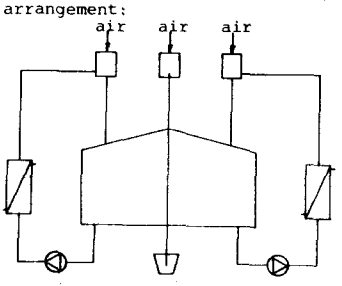


Fig. II

Fig. III shows a two-stage multiple arrangement. This solution, although more expensive, brings a further reduction of the specific energy consumption.

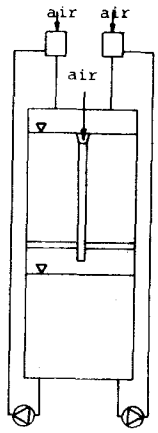
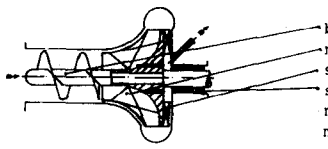


Fig. III

The special VB-IZ pump is shown in Fig. IV:



booster stage
main stage
separation stage
sealing, bearing
medium: 2-phases mixture of liquid and air γ = 0.4-0.95
efficiency η = 0.7

Fig. IV

Standard pumps are available for the following performances:

type	hight m w.c.	capacity m3/h	motor kW
10 MN	up to 25	1.200	37
12 MN	up to 25	1.500	55
16 MN	up to 40	3.000	132
20 MN	up to 30	4.500	250
24 MN	up to 40	7.000	400

The overflow shafts of various constructions enable air aspiration rates of $O_F:O_L$ = 1:0.9-1.2.

Some characteristic data of large-size fermenters are shown below:

fermenter m3	200	400	1.000	2.000
filling net t	80	130	400	1.000
total transp. cap. t/h	2.500	3.500	44.000	70.000
O_2 transfer kg/h	280	410	4.800	8.000
air quantity Nm3/h	3.500	5.000	60.000	100.000
v.v.m.	1:0.7	1:0.5	1:2.5	1:1.7
spec. electr. energy consumpt. kW/kg O_2	0.7	0.65	0.55	0.45

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THE USE OF COMMON CHEMICAL REACTORS IN FERMENTATION PROCESSES

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Dr. U. Faust Fr. Uhde GmbH

1) From a chemical engineering point of view a fermenter is a reactor which brings into contact gas and liquid phases, providing adequate mixing and heat exchange and sufficient mass transfer in the gas liquid interface and to and from the microorganisms.

In the field of industrial fermentation the aerated stirred tank is looked upon as the standard reactor. The necessary agitator power input of 5 KW/m^3, the cooling capacity of 10.000 kcal/hm^3 and 30 to 60 Nm^3/h·m^3 of fresh air lead to accordingly high running costs. The rising price of energy sources is an incentive to develop energy-saving reactors.

A research group of the Hoechst AG looked at this task and applied their knowledge of common chemical reactors to fermentation processes, concentrating on the manner in which high energy input affects fermentation. Although all known process parameters have been satisfactory, according to industrial experience a further increase of energy input leads to a further increase of antibiotic yield.

The actual fermentation reactor is the microorganism itself. The technical apparatus provides only nutrition and environmental conditions. To be effective any change of fermentation parameters must induce a change in the cell -surroundings. This means that high energy input to the fermenter will have to influence the flow pattern near each cell. Accordingly we concentrated on phase reactors that are likely to distribute hydrodynamic energy as homogenously as possible in the liquid contents and which would create shear effects in the cell-surrounding medium.

The oxygen transfer coefficient k_l·a was chosen as criterium.

From a number of possible reactor-types a surface system and a submerged system were tested thoroughly.

The characteristic feature of the surface-system is the application of a packing that forms vertical channels. The culture medium is recycled to the top and is so distributed that it runs down the vertical walls as a liquid film. From the wall to the gas surface the liquid velocity increases thus inducing an extra shear rate, or rotation, on the suspended organisms. The gas-liquid interface "a" in the falling film reactor does not depend essentially on the air-flow. Apart from the pressure drop of the air flow caused by the friction in the packing no additional gas bound energy is required. The mass-transfer experiment indicates the strong influence of superficial air-velocity on the oxygen transfer coefficient in the packing. This makes it possible to apply an economic recirculation of process air. It is only necessary to add the ammount of air required to keep the oxygen concentration constant. Scale-up for a falling-film-fermenter is simple because of the constant pressure conditions.

A forced-circulation reactor was chosen for submerged processes. The essential characteristic incorporated was the addition of up to 10 % of inert solid particles of 1,4 g/cc and approximate weight of 25 mg per particle. The upflowing liquid keeps them in a suspended oscillating state. Each particle causes a velocity-drop in the surrounding fluid, which accelerates the mass transfer to and from the cells. When the particles collide with the rising air bubbles they give them a favourable impact. The oxygen-transfer-rates were as much as 20 % above those measured without particles.

A comparison of fermentation data of the abovementioned circulated fermenter with a stirred tank shows a fall of about 60 % in agitation power demand.

A CONTRIBUTION TO THE BIOMASS FORMATION DURING BATCH AND CONTINUOUS ALCOHOLIC FERMENTATION.

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The aim of all industrial fermentations is to reach the highest required productivity with respect to the product formed and to the minimization of substrate losses in an outflow stream at the maximum dilution rate. The continucus alcoholic fermentations were mostly carried out in heterogeneous multistage systems (e.g. MALCHENKO 1960, YAROVENKO 1972). The main factor for glycolysis regulation is a feedback inhibition of phosphofructokinase which is either performed by the end product, i.e. ATP (BROWN et al. 1971) or by citric and isocitric acids (SALAS 1965, PERNER 1974). An increase in their concentrations is connected with the decrease of pyruvic acid, inorganic phosphate, fructose-1,6-diphosphate and with the increase of acetaldehyde which can be regarded as an important idicator of the alcoholic fermentation process. Under certain circumstances, i.e. in presence of high sugar concentration and in the aerobic conditions Pasteur effect is likely to reduce (DEKÉN 1966) and degradation of glucose takes place by means of a fermentation. Crabtree effect (CRABTREE 1939) can be reckoned as the significant factor of the physiology of Saccharomyces cerevisiae. Our experimental work was mainly concentrated on the possibility of the process regulation, i.e. the glycolysis and the biomass formation by changing the main external conditions such as: the dilution rate and the substrate concentration in a feed stream. Prior to the continuous experiments a series of batch experiments has been carried out under different conditions of aeration and mixing in order to evaluate some kinetic parameters by using KONO and ASAI mathematical model (1969, GRÉGR et al. 1971). In good accord with LAFFERTY et al. (1974) we have found out that the ethanol production during aerobic fermentation is probably associated with the yeast growth rate, whereas in anaerobic conditions the ethanol is apparenly formed even when the yeasts no more grow. Having analysed the results of the batch cultivations we took up the approach of three following variants: 1. A continuous method in a series of three fermenters. The diluted molasses medium (3 °Bg) was entered into the first vessel (aerated) while the concentrated medium was only fed into the second anaerobic stage. Results here obtained were not satisfying enough, for the aerobically propagated yeasts in first stage had been of worse activity and physiological state due to the influence of lower substrate concentrations and higher degree of aerobiosis. Remarkable differences between physiological states of microorganisms in both fermenters were observed. 2. A continuous method in a series of three fermenters where the strong molasses medium was fed only into the first stage. All fermenters were operated under anaerobic conditions. At dilution rates higher than 0.07 h^{-1} (for one fermenter) and with the sugar concentration in feed of 180 g/l the washout has been achieved. From this reason, i.e. small productivity of the whole system, we suggested to alter the anaerobic conditions in first stage for aerobic ones in order that the first stage could partly work as an inoculation vessel. 3. When changing k_La^X (sulphite number) in the first fermenter up to only 85 mmol O_2/l.h the higher dilution rate could be used. In our work a number of experiments, with changing D (0.1-0.3 h^{-1}) and feed sugar concentration until 220 g/l has been carried out. The further two fermenters were performed under anaerobic conditions ("homogenous system"). It was found out that the highest biomass productivity, the highest biomass concentration resp. were reached in first vessel at D=0.3 h^{-1} when the feed sugar concentration of about 80 g/l was strictly maintained. This steady-state concentration corresponds to the "optimum cell number" with the object of attaining the high alcohol productivity. The first stage is very sensitive to variations in feed substrate concentration. If its concentration increased from 80 to 120 g/l at the same D, the yeast concentration fell to 1/3 of its original value. Each steady state analysis comprises a few evaluations, e.g. RNA, DNA, pyruvic acid content and respiration rates data. It has been demonstrated that under aerobic conditions and until 4 % sucrose in steady state the cytochromes ($a+a_3$, b, c) may be noticed. Under anaerobic conditions only cytochrome b_1 was found unless sugar concentration reached 1 %. In conclusion it should be pointed out that the protein content in yeast biomass is higher when cultivating under anaerobic conditions (even under aerobic conditions but in high substrate concentration media, i.e. "aerobic fermentation"). However, the protein content under such conditions obtained can be seriously compared with values for fodder yeasts. All experiments were carried out in small laboratory fermenters.

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YIELD ENHANCEMENT OF FLAVOMYCIN® - FERMENTATIONS

Sukatsch, D.A., Dietrich, J. and Fritsch,G.
Hoechst AG, Frankfurt/M. - BRD

Flavomycin® R & D-work was carried out in a pilot plant with conventionally equipped 4.0 m^3 bioreactors using standard conditions concerning biological and physico-technical parameters.

The aim was to improve the yield of Flavomycin® and to get new data with respect to geometrically stirrer display, number and type of stirrers, stirrer distance on the well and of liquid level for production of this antibioticum in 40,0 m^3-vessels.

The increase of Flavomycin® - output should be achieved by optimization of mass transfer in order to make use of the full potency of the microorganism.

A quantitative measure of the improved mass transfer was considered to be the volumetric oxygen transfer coefficient $K_L \cdot a$. This measure was applied to three comparable 4.0 m^3 bioreactors, which were technically modified.

The $K_L \cdot a$-value represents the most critical step of the reaction i.e. the oxygen-transferrate. It was determined after each technical change in equipment and operation conditions. Then fermentations of Flavomycin® under standard production conditions, but with these new technical optimized variations were carried out.

As a result an increase in yield was observed with larger diameter of the impeller combined with increasing power input per unit volume $P/_V$ and the max. $K_L \cdot a$ obtainable i.e. the $K_L \cdot a$ and the antibiotic yield can be considered as a function of $P/_V$.

In order to get economically tolerable power requirements for the production level the max. $K_L \cdot a$-values of the pilot-plant reactors could there not be applied.

This postulated further biological and physiological improvements to balance the technical conditions.

The last step of optimization was achieved simply by addition of different tensides. Fermentations with additions of tensides displayed shorter and faster growth- and production-rates compared with the control runs.

The surface active action of these compounds caused as well a reduction of resistance across the cell membrane for substrates and product in both directions as a decrease in medium viscosity. Another advantage was that most of the immissible substrates of the medium became emulsified, which gave a better distribution of all phases in the vessel including an increase of the mass transfer capacity.

These methods (changed stirrer geometry, better mixing times, $K_L \cdot a$ and OTR-values and tenside addition) were transferred from high yield pilot plant fermentors to the production units (40.0 m^3).

Using more or less constant energy for agitation, and required OTR, the microorganism showed in these bioreactors comparable and reproducable yields in antibiotic production with less problems in substrate-metabolism and viscosity of the medium.

AERATION WITHOUT AIR

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Oxygen is normally supplied to a suspension of aerobic microorganisms in the gaseous form. Only oxygen dissolved in water is taken up by the cells. The solubility of oxygen is very small compared with the solubility of conventional energy and carbon sources. A suspension of aerobic microorganisms is, therefore, dependent on a continuous addition of oxygen to the suspension medium. Usually, oxygen is supplied by air. The efficiency of aeration is a function of those parameters which are contained in Fick's Law of Diffusion. The phase boundaries can be increased by several means, the oxygen partial pressure may be increased as well; however, the rate of transfer of oxygen from the gas into the liquid phase depends on the diffusion constant D; therefore the oxygen transfer rate can be manipulated only within narrow limits. The question has been raised whether there is an oxygen concentrate which can be supplied to the nutrient medium in liquid form; the availability of such an oxygen concentrate would drastically simplify the means of aeration. In addition, oxygen could be easily supplied to those cells which do not tolerate the high shearing forces due to strong aeration and agitation.

Such an oxygen concentrate is perhydrol. About 100 litres of oxygen are produced if 1 l perhydrol containing 30 percent hydrogen peroxide is decomposed by catalatic cleavage. 1 l perhydrol is, therefore, equivalent to 500 litres of air. As hydrogen peroxide has to be considered as a potential oxygen source for aeration purposes, experiments were made to examine the conditions under which a suspension of growing microorganisms can be supplied with oxygen through continuous addition of diluted perhydrol solutions.

Hydrogen peroxide is toxic to living cells. The concentration of H_2O_2, which is just tolerated by microorganisms, depends on the catalase content of the cells. The catalatic activity of those cells studied so far is not sufficient to produce the oxygen necessary for respiration from H_2O_2. Catalase has, therefore, to be added to the nutrient medium. If bovine liver catalase (20 mg/ml; 50 000 units/mg protein) was added at a concentration of 0.1 or 1.0 µl/ml nutrient medium even 1 M H_2O_2 was tolerated by bacterial cells. In most cases, 1 µl catalase/ml was added. Commercial perhydrol did not contain growth inhibiting substances in addition to H_2O_2; the hydroquinone added as a stabilizer was without effect on the microorganisms studied. So far, the growth of the following microorganisms has been studied: Acinetobacter calcoaceticus, Alcaligenes eutrophus H 16, Candida oleophila, Paracoccus denitrificans, Pseudomonas putida, Saccharomyces cerevisiae.

Growth experiments were carried out in 250 ml Erlenmeyer flasks each containing 30 ml cell suspension of an initial optical density (436 nm) of about 1.0. The flasks were shaken in a thermoconstant water bath at 30° C. The gas phase was either air or oxygen-free nitrogen. Diluted solutions of hydrogen peroxide were added at a rate of 2.5 ml/h by means of Vario-Perpex pumps (LKB, Sweden). The continuous dilution of the suspension by the perhydrol solution was considered for calculation of optical densities.

A few examples of growth curves are presented in Figure 1. In each instance growth of the cells in a complete nutrient medium was followed under air. Under nitrogen atmosphere no growth occurred. If under nitrogen, however, hydrogen peroxide solution was added continuously, the cells grew at a rate identical or similar to the rate of the aerobic

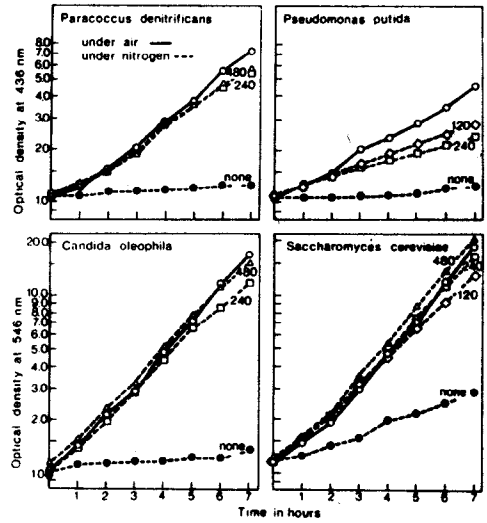

Figure 1. Growth of cells of Paracoccus denitrificans (on fructose), Pseudomonas putida (on fructose), Candida oleophila (on glucose) and Saccharomyces cerevisiae (on glucose) with oxygen supply by continuous addition of hydrogen peroxide solutions. Hydrogen peroxide (amount added per hour indicated in µmoles) was continuously added to 30 ml cell suspension of an initial optical density of about 1.0 and containing 1-10 µl catalase/ml; vessels filled with O_2-free nitrogen and shaken at 30° C. Control under air (o).

control. The rate of H_2O_2 supply had to meet the oxygen consumption rate of the culture. When the supply rates were lower than the demand rates, the growth rates decreased. When the supply rate exceeded the demand rates, growth was impaired, too. In submerged culture, growth occurred as well. 300 ml suspension of A. eutrophus H 16 were stirred in a 300 ml Erlenmeyer flask at 150 rpm, and the gas space was flushed with O_2-free nitrogen. A 175 mM H_2O_2 solution was continuously added, the flow rate was increased from 2.5 to 11.0 ml per hour. The cells grew exponentially at a doubling time of 150 min.

The experiments indicate that oxygen supply through hydrogen peroxide is possible. In the presence of catalase in the nutrient medium H_2O_2 is decomposed fast enough to keep the steady state concentration of H_2O_2 below the threshold of toxicity. A reaction of H_2O_2 and superoxide radicals to form hydroxyl radicals had at least to be considered.

So far, various growth responses have been observed with different microorganisms. The reasons for the differences are obvious: They concern an overdosage of oxygen and the rise of carbon dioxide concentrations. At partial pressures higher than in air, oxygen is toxic to most microorganisms. Aeration by air is a self-regulatory system; the oxygen absorption rate is proportional to the oxygen deficit of the medium, and the oxygen concentration cannot exceed that of air saturation. Oxygen supply by H_2O_2 is not based on a self-regulatory system. The method requires information on the p_{O_2} optimum for cell growth and continuous control of the oxygen concentration by means of an O_2-electrode.

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STERILE FILTRATION OF FERMENTATION AIR USING MEMBRANE FILTERS

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A fermentation process must be performed under aseptic conditions in a practically closed system, as any alteration of the controlled microbiological condition of the system by extraneous organisms can jeopardize the entire charge.

One of the basic problems for an economical production is the sterilization of the supply air for the fermenter. This problem becomes more and more important as charges and air volumes required increase.

Therefore, a sterile filtration system for fermentation air has to be safe and reliable. An optimum system is expected to continuously and over a long period of time supply sterile air for many fermentation process at low operating costs. Furthermore, it should comply with the following requirements:

- Reliability of sterile filtration even with high air velocities
- Low pressure loss for the flow rate required
- In-line sterilization
- Testability of the effeciency of the entire system
- High flow rates with relatively small dimensions
- Reliable and efficient even with air containing condensate
- Low maintenance costs

Due to their typical depth filter characteristics, traditional filter types of glass wool or fiber glass cannot meet these requirements. With high flow rates they do not offer an adequate safety, and their efficiency considerably decreases when the air contains condensate. Moreover, these filter systems require a lot of space which, especially with high capacity fermenters, constitutes a considerable disadvantage.

Recently, membrane filters of Fluoropore (PTFE) material with pore sizes of 0,5 µm - or 0,2 µm for critical applications - are increasingly and successfully used for the sterile filtration of fermentation air. These filters meet all requirements that can be applied to an optimum sterile air filter. Due to their uniform pore structure, they guarantee a safe sterile filtration independent of varying operating conditions such as pressure and flow rate. The very high percentage of pores - approx. 80 % - provides high air flow rates per area unit at low differential pressures. This results in relatively small filter sizes as related to a given throughput capacity for air and also, due to the low pressure loss across the filtration system, considerably reduces the costs of the air compressor.

The PTFE membrane filter is hydrophobic and therefore cannot be penetrated by water. Consequently, air containing condensate will neither affect the safety of the filtration nor can it, due to the capillary effect of the pore structure, block the filter and make it impermeable for air.

The filters are available as discs as well as as cartridges. They are placed into adequate stainless steel housings of various sizes and can repeatedly be steam sterilized in-line at a temperature of 125°C. Prior to and after every fermentation process the entire filtration system can be integrity tested by means of the bubble point test.

For higher flow rates, Aerotube membrane filter cartridges are installed in single or multiple cartridge housings. In this way, up to 20 filter cartridges can be operated in parallel in a single housing. In order to increase the life time of the filter cartridges, these are provided with an integrated prefiltration layer of micro glass fiber material. Experience shows that by this method a life time of approx. 6 - 8 months per filter cartridge can be achieved.

The following are flow rates for some typical cartridge systems, related to a pore size of 0,5 um and a pressure differential of 0,2 bar. With permissible higher pressure differentials the flow rates will go up proportionately.

1-cartridge housing:	up to 3 m^3/min or 180 m^3/h
3-cartridge housing:	up to 9 m^3/min or 540 m^3/h
7-cartridge housing:	up to 20 m^3/min or 1200 m^3/h
20-cartridge housing:	up to 50 m^3/min or 3000 m^3/h

For Aerotube cartridge filter systems special computer programs have been developed which, based on the initial data for each process, can determine the technically and economically optimum filter for fermentation air. With this optimizing process, the economical computations also include the electricity costs for the compressor as well as the amortization of the system and the current costs for expendables.

APPLICATION A NEW FILTER MATERIALS FOR AIR STERILIZATION IN ANTIBIOTIC PRODUCTION

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In air cleaning and sterilization systems for antibiotic production, as well as in other branches of the industry of microbiological synthesis the multistage filtration method is often used. Filter materials for fine air cleaning or sterilization may be divided into some basic groups: super fine filter materials in the form of mats, paper, cardboards, hard granular partitions / ceramic, metal ceramic , polymer/, membrane filters.
Successful using of a new filter material for air sterilization may be guaranteed by optimal solving of the following problems such as filter media, filter elements and filter housing . It is necessary to use also the abreviative non-destractive industrial method of the efficiency control of filter in situ.
The materials chosen by us belong to two different classes, such as paper-like filtering meterials and polymer granular partitions. Paper filter materials were prepared of superfine mineral fibers with the median size of the diameter, equal to one micron with addition of cellulose and glue substances.
The results of the experiments showed, that the filtering properties of the paper depend on the sample square meter and composition of additional substances. The properties of some types of the mineral filter papers are presented in Table I.

The properties of some types of the mineral filter papers

Table I

Material	Mass of $1 m^2$	Permeability for air	Resistance to air flow I cm/sec	Penetration coefficient of oil aerosol
	g/m^2	$cm^3/cm^2 min$	mm.W G	%
I	2	3	4	5
BFB-60	60	40	6,09±0.29	I.4630±0.I757
BFB-I20	I20	23	9.08±0,42	0,009I±0.0009
BFB-I80	I80	I5	II.52±0.85	0,00094±0.000I8
CFB-I	300	50	4,83±0,I7	0,0020±0.0003
CFB-2	700	35	I2.48±0.34	0,00046±0.00004
CFB-3	I000	25	I6.7I±0.43	0.00024±0.0000I

Note: I)air permeability of materials I-3 was determined by pressure drop 8 mm W.G., of materials 3-6 by pressure drop 30 mm W.G.

2)in graphs 5-6 average values with trusted intervels for probability 0,95 are presented.

Hard granular partitions were prepared of polytetrafluoroethylene. Polytetrafluoroethylene as a filter media have a number of advantages. It is heat resistant at a temperature of 250°C, hydrophobic and has no effect on vital activity of microorganisms. As a result of the experimental study of a large number of samples polytetrafluoroethylene used for air sterilization must have the diameter of pore about I0-20 micrometers and thickness of 5-I0 mm.
The basic characteristics of the filter elements made of new filter materials are presented in Table 2.

The basic characteristics of new filter elements made of new filter materials

Table 2

Filter elements	Filtration area	Productivity	Initial resistance	Efficiency of filtration for	
				oil aerosol	methylene blue aerosol
	m	m³/h	atm	%	%
I	2	3	4	5	6
I	0.I8	250	0.I	99.999	above 99.996
2	0.06	250	0.I	99.900	99,990

Note: in graph 1 for filter element 1 the goffered mineral paper materials were used, for filter element 2 were used polytetrafluoroethylene materials.

The filter materials developed were successfuly employed in antibiotic production. During operation of the filters their safety and effeciency were controlled by means of the methylene blue test. The results of the trials under industrial conditions showed that for sterilization of air filters it is necessary to use purified steam.
In case of filters with productivity of above 250 m³/h the required numbers of the filter elements mentioned above in Table 2 were assembled in one installation.