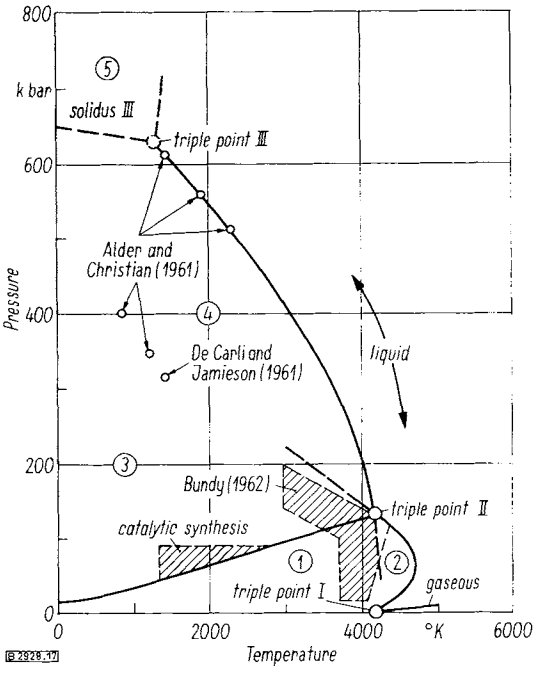

Fig. 17. The phase diagram of carbon (after *Bundy* [43]).
1) Graphite + metastable diamond; 2) graphite; 3) metastable graphite + diamond; 4) diamond; 5) metallic phase.

explosion shock waves using an iron catalyst (Du Pont), the technique being developed from *Moissan*'s experiments, and even a catalyst-free transformation seems feasible, although this could only be done at much higher pressures still, *i.e.* in the region of "metallic carbon" (see Fig. 17). The conditions so far attainable for diamond synthesis are still inadequate to produce large diamonds of gem quality. However, it is already possible to control specific properties of diamonds within certain limits, *e.g.* to vary the color by admixtures to the catalysts and to influence the habit of the diamond crystals. Nitrogen imparts semiconductor properties to diamond and gives it a yellow to brown coloration, boron is reported to make it blue. Figure 18 shows a schematic arrangement for

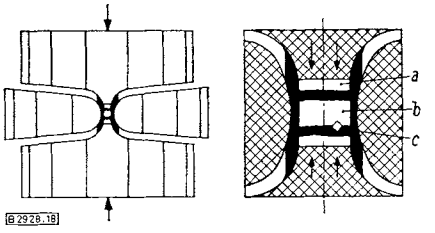

Fig. 18. Schematic arrangement for diamond synthesis.
Left: sectioned cemented carbide anvil and die; right: detail of the reaction space: a) metal; b) graphite; c) diamond crystal.

diamond synthesis. The carbon tube heating element, which is insulated with pyrophyllite, is introduced between cemented carbide anvils into annular shrunk cemented carbide dies. Cobalt-bonded tungsten carbide is used for the anvils and dies. This material continues to form the basis of the cemented carbide industry and synthetic diamonds would be inconceivable without it.

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Modern Industrial Microbiological Fermentations and Their Effects on Technical Developments[**]

By H.-J. Rehm[*]

Owing to the development of many new processes and techniques, the production of primary and secondary metabolites with the aid of microorganisms has not only expanded greatly, but has also been simplified in many details, and has therefore become more widely feasible on the industrial scale. The present article surveys the principal fermentation processes and the main types of equipment, which shows a trend toward continuous processes and increasing automation. Some recent developments observed e.g. in the cultivation of animal cells or the production of electrical energy, and particularly in the production of proteins by microorganisms, are also described.

1. Isolation of Primary Metabolites

Man has been trying for thousands of years to develop apparatus for the controlled cultivation of microorganisms by processes and special techniques best suited to the needs of the microorganisms. Following the discovery of large numbers of microorganisms and the elucidation of many of their biochemical properties, cultivation methods that have proved adequate almost to the present day were developed in the late 1800's and early 1900's. The cultures were carried out in open or closed tanks, usually without protection against extraneous organisms. As far as

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possible, the growth conditions were kept such that only the desired organisms could develop. The object of the process is essentially either the production of the cells themselves, as in the growth of yeast, or the production of their primary metabolites, such as ethanol, butanol, acetone, and glycerol, as well as of many simple organic acids. The first phase of industrial microbiology may be regarded, for simplicity, as the preparation of primary metabolites. This phase dates from the first attempts to obtain alcoholic drinks or organic acids up to around the middle of this century.

2. Isolation of Secondary Metabolites and Derived Processes

Penicillin was the first important secondary metabolite to be required in very large quantities. Its production led to the development of revolutionary new processes, which can be described collectively as sterile aerated submerged fermentation, and which allowed considerable simplification as compared with the extremely laborious sterile surface methods that were already known. The microorganisms are grown in aerated and stirred culture solution under constant conditions in large fermenters with effective capacities of 50000 to 250000 l, and there is no possibility of infection by extraneous organisms (for details, cf. [1–3]).

Many difficulties had to be overcome in the development of these processes:

1) The fermenter and the nutrient solution were sterilized by introducing superheated steam into the pipes and the fermenter, heating the culture solution as it flowed, and introducing the concentrated culture solution into the fermenter, the contents then being made up to the required volume with condensing steam.

2) The entire fermenter was kept sterile throughout the fermentation (which took several days) by ensuring that its internal pressure was slightly above atmospheric.

3) Sterilizable measuring equipment had to be developed.

4) For sterile foam reduction, automatic defoaming equipment was designed in which a sterile defoaming liquid was added to the substrate. The agents used are mainly fats, oils, higher fatty alcohols, and silicone oils.

5) The introduction of sterile air initially presented special difficulties. The air was first passed through wash towers containing acids, alkalis, or disinfectants, but was later forced through sterilizable glass wool-carbon filters.

The solution of these problems and the adaptation of microorganisms for submerged culture provided a fermentation method for the production of many other secondary metabolites as well as penicillin with the aid of microorganisms. The variety of products, which include most antibiotics, many enzymes, various alkaloids, polysaccharides, nucleotides, gibberellins, and various vitamins, frequently necessitated the design of new fermenters, and the result was an extremely diversified development.

The development of further microbiological processes naturally did not stop at the submerged process. Great interest was also stimulated in the production of laboratory shakers suitable for the cultivation of microorganisms under practically submerged conditions. Besides a number of variants based on the conventional principle, *e.g.* with constant temperature regulation, with special shaking speeds, *etc.*, completely new designs have also appeared. Because of the unfavorable center of gravity in conventional shakers, stabilization with several platforms (tablars) is often difficult. For this reason, *e.g.* the point of application of the drive in a new design by Infors AG (Basel/Switzerland) is situated exactly at the center of gravity of the moving upper part as well as of the counterweight.

Further work was also carried out on the surface process, which was still needed for the preparation of certain products. One example is the fermentation cup process (a method for surface culture) described by *Schröder* and *Brandl* (cf. [1]). These authors developed a sterile aerated surface process in which the

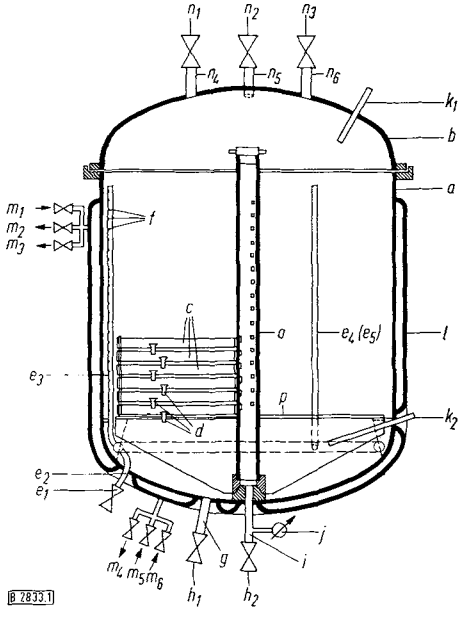


Fig. 1. Longitudinal section of a fermentation cup container (after [1] p. 158).

a, Container; b, lid; c, exchangeable fermentation cups; d, overflow devices; e_1 to e_5, aerators; f, air outlet holes; g, discharge pipe; h_1, h_2, valves; i, deaeration pipes; j, manometer; k_1k_2, thermometer glands; l, double wall; m_1 to m_6, valves for heating steam (m_1,m_4), cooling water (m_2,m_5), and recirculation water (m_3,m_6); n_1 to n_6, nutrient feed; o, perforated central bracing tube; p, perforated base.

[1] *R. Brunner* and *G. Machek*: Die Antibiotika. Vol. I, Die grossen Antibiotika. Verlag Hans Carl, Nürnberg 1962.

[2] *R. Müller* and *K. Kieslich*, Chemie-Ing.-Techn. *38*, 813 (1966).

[3] *H. J. Rehm*: Industrielle Mikrobiologie. Springer Verlag, Berlin-Heidelberg-New York 1967, and further literature cited therein.

many laborious operations are largely combined and automated, *e.g.* automatic introduction of the culture medium, automatic sterilization of the many surface-growth dishes (making good use of experience gained in the submerged process), simple inoculation of the complete solution, possibility of sterile aeration, and simple temperature control (cf. Fig. 1).

3. Kinetics of Batch Submerged Fermentations

The submerged processes with their constant conditions for the growth of microorganisms made it possible for the first time to obtain really accurate information on the kinetics of the fermentation process. Since about 1946, and particularly since 1954, many articles dealing with this problem have been published.

3.1. Patterns of Development of the Microorganisms in the Fermenter

Whereas very long incubation times must be expected in normal microorganism cultures, the exponential phase of the growth of the microorganisms can be considerably prolonged and the incubation time shortened in the fermenter (cf. Fig. 2). Microbiological fermentations are influenced and characterized in particular by substrate consumption, occurrence of the end product, and development of the biomass. Movement of the substrate, aeration, accumulation and disappearance of intermediate products, and other factors such as temperature and pH changes, which can be relatively easily kept constant, are very important.

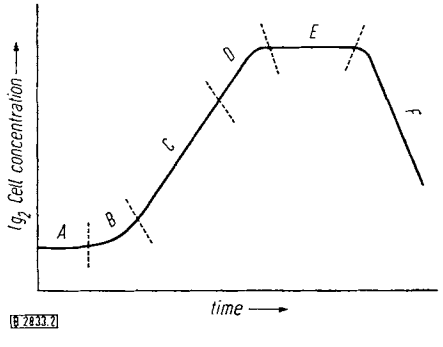


Fig. 2. Growth curve of bacteria (from [3]).
Various phases of development: A, incubation phase; B, acceleration phase; C, exponential growth phase; D, transition phase; E, stationary phase; F, lethal phase.

3.2. Patterns of Formation of the Fermentation Product

The fermentations are classified according to various rules. One classification that seems reasonable is that proposed by *E. L. Gaden* and *F. H. Deindorfer*, who distinguish three types (cf. [4]):

Type I: The main fermentation product is formed as a result of the primary energy metabolism. It is often formed on direct oxidation of the primary carbohydrate, *e.g.* in the alcoholic fermentation or the formation of lactic acid from glucose. The mass cultivation of many microorganism cells must also be regarded as fermentations of this type (Fig. 3a).

Type II: The main fermentation product is formed only indirectly from the energy metabolism. Examples are the formation of itaconic acid and of citric acid; the amino acid fermentations may also belong to this group. The reaction steps are complex, and inhibited or anomalous metabolism occurs. Figure 3b shows that the growth and the sugar consumption have two maxima. The specific yield curve

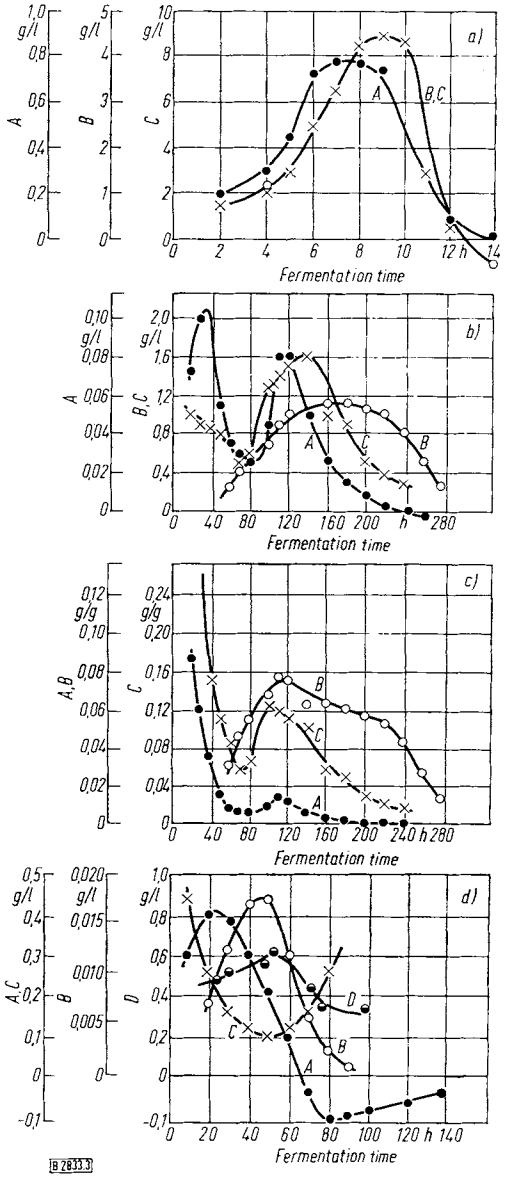


Fig. 3. Classification of fermentations according to *E. L. Gaden* and *F. H. Deindorfer* (cf. [4]).
a) Type I: Alcoholic fermentation. Volume growth rates; A, growth; B, alcohol synthesis; C, sugar consumption.
b) Type II: Citric acid formation. Volume growth rates; A, growth; B, citric acid formation; C, sugar consumption.
c) Type II: Citric acid formation. Specific growth rates; A, B, and C as in (b).
d) Type III: Penicillin formation. Volume growth rates; A, growth; B, penicillin formation; C, sugar consumption; D, oxygen absorption.

[4] *N. Blakebrough*: Biochemical and Biological Engineering Science, Vol. 1, Academic Press, London-New York 1967.

(Fig. 3c) shows that growth and sugar consumption are very closely related in the initial phase, whereas growth and product formation are closely related in the secondary phase.

Type III: This type of fermentation is found in all biosyntheses in which the main fermentation product is formed not from the energy metabolism, but relatively independently of it in the cell, *e.g.* in the formation of penicillin and streptomycin (Fig. 3d).

Thus, in Type I complex compounds are broken down into simpler compounds (catabolic processes), and in Type III complex molecules are formed from simple ones (biosyntheses, anabolic processes), while Type II lies between these.

4. Aeration of Microbiological Cultures

Aeration is one of the fundamental problems of aerobic microbiological fermentations. The oxygen requirement C of the cells varies widely, according to the nature of the microorganisms and the biochemical process taking place in the cells. The oxygen requirement per gram of newly formed dry cell substance [5] is:

$$C = A/Y - B$$

(A = theoretical quantity of oxygen necessary for the combustion of 1 g of substrate to CO_2, H_2O, and NH_3; B = theoretical quantity of oxygen necessary for the combustion of 1 g of dry cell substance to CO_2, H_2O, and NH_3; and Y = yield of dry cell substance per g of substrate consumed). The hourly consumption of oxygen per g of dry cell substance present Q_{O_2} is the product of the absolute oxygen requirement C and the growth rate k. For example, the hourly oxygen consumption for the formation of gluconic acid with *Pseudomonas ovalis* is 6.2 ml of O_2/100 ml h [6]. In the submerged formation of acetic acid by *Acetobacter suboxydans*, the oxygen consumption is 55 ml of O_2/100 ml h.

The rate of supply of oxygen is determined by the rate of transfer through the liquid film at the air bubble/nutrient solution interface. This transfer, and hence the entire oxygen supply, can be represented in the form of the general oxygen transfer equation (cf. [7,8]). The rate of oxygen transfer is thus the product of three factors:

1) The oxygen concentration gradient between the liquid film and the mass of the liquid (this concentration gradient is the driving force of the oxygen transfer).

2) The transfer area, which corresponds to the total surface area of all the gas bubbles present in the liquid.

3) A transfer coefficient, which is not a constant, but depends essentially on the thickness of the interfacial film and on the state of the interface.

The transfer rate is a maximum when the concentration of the oxygen dissolved in the liquid mass is zero, *i.e.* when the concentration gradient is greatest. However, the oxygen concentration must not fall to zero in cell cultures, but must always be greater than the critical oxygen concentration, at which the cell respiration starts to become affected. If the concentration of the dissolved oxygen is kept slightly above the critical value for the cells being grown, the rate of oxygen supply in cell cultures is optimal, and the capacity of an aeration system is fully utilized [9].

The development of oxygen-metering equipment has been greatly promoted by the fermentations. Many devices for the continuous determination of dissolved oxygen in fermentation cultures have been described in recent years. These make use of three principles:

1) paramagnetic measurements; 2) measurements based on the polarographic principle; and 3) measurements based on the galvanic principle.

Of the many aeration systems developed, the devices used for microbiological fermentations can be placed in the following basic groups (cf. *e.g.* [8]):

1) Bubble column fermenters. The air is finely distributed into individual bubbles at the base of the tank, and these ascend in the fermenter. The air is distributed in some cases by static (Fig. 4a) and in others by rotating (Fig. 4b) and oscillating (Fig. 4c) devices.

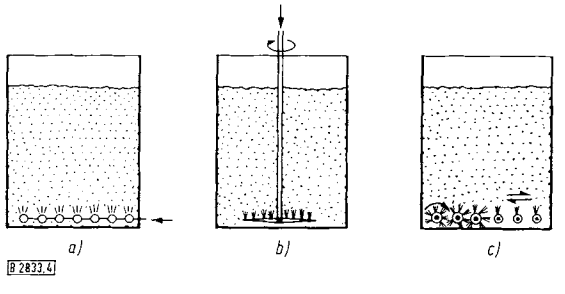


Fig. 4. Bubble column fermenters (from [8]).
a, aeration by jet pipes or sintered glass; b, rotational aeration; c, aeration by rotating or oscillating units.

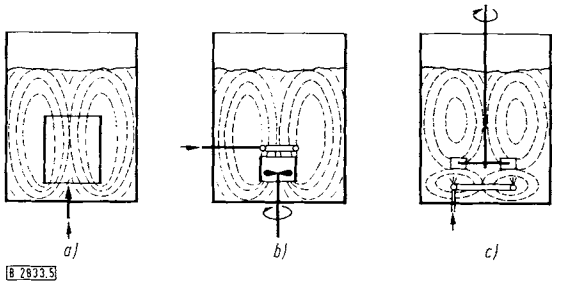


Fig. 5. Stirrer and air-jet fermenters (from [8]).
a, air distributor with guide cylinder; b, circulation of liquid under the aerator; c, circulation of liquid over the aerator (universal fermenter).

[5] *M. J. Johnson*, Chem. and Ind. *1964*, Sept. 5.

[6] *Sh. Aiba, M. Hara*, and *J. I. Someya*, J. gen. appl. Microbiol. (Tokyo) *9*, 163 (1963).

[7] *F. C. Webb*: Biochemical Engineering. D. van Norstrad, London 1964.

[8] *W. K. Bronn* in: Arbeitsmethoden und aktuelle Ergebnisse der technischen Mikrobiologie. Fischer Verlag, Stuttgart 1967, p. 45.

[9] *D. Hegner* and *H. Glossmann*, Z. Naturforsch. *20b*, 234 (1965).

2) Stirrer and air-jet fermenters. These have static fixed air distributors past which the liquid is pumped by built-in guide cylinders (principle of air lift pump) or stirrers (cf. Fig. 5).

3) Fermenter with rotating aerators. The air is distributed through hollow stirrers rotating at relatively high speed. Circular movement of the liquid is usually prevented by baffles on the walls of the container (cf. Fig. 6).

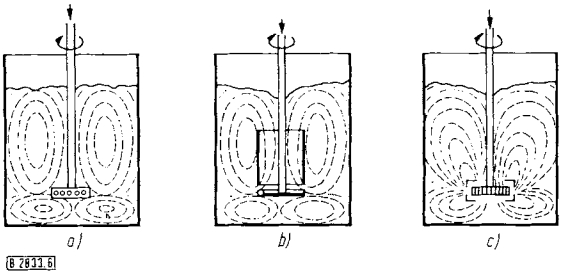


Fig. 6. Fermenters with rotating aerators.
a, hollow stirrer rotating freely; b, rotating aerator with guide cylinder; c, air sucked in by static inserts on the rotating aerator.

4) Shaking, rotating drum, vortex, and vibromix aeration. These types depend on movement of the culture liquid and absorption of oxygen from the air over the liquid (cf. Fig. 7).

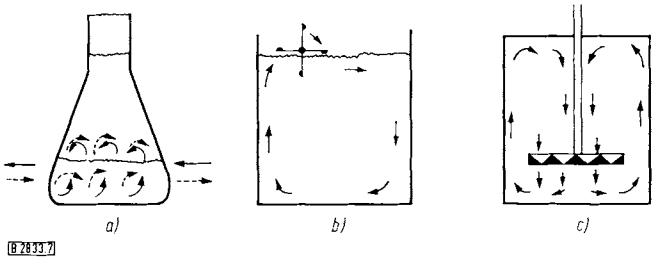


Fig. 7. Shaking, rotating drum, and vibro aeration (from [8]).
a, shaking flask; b, paddle aerator; c, vibromix aerator.

5) Surface aeration. In this case air is allowed to pass over the surface of the stationary culture solution (*e.g.* in the fermentation cup method) or is led past the solution by special devices (self-heating *e.g.* in the production of vinegar) (cf. Fig. 8).

It was only when a means of maintaining an adequate supply of oxygen to the microorganism cells in solutions had been found that it became possible to develop processes in which intensive biological oxidations could be carried out. Some examples are the sorbitol/sorbose oxidation with *Acetobacter* (including new biosyntheses for the production of vitamin C), the oxidation of ethanol to acetic acid, and many other oxidations with *Acetobacter* species, as well as steroid oxidations, particularly 11α-hydroxylations by microorganisms of various genera (*e.g.* for the production of cortisone/hydrocortisone or estrogen preparations), and finally oxidations of cardiac glycosides, antibiotics, and other substances.

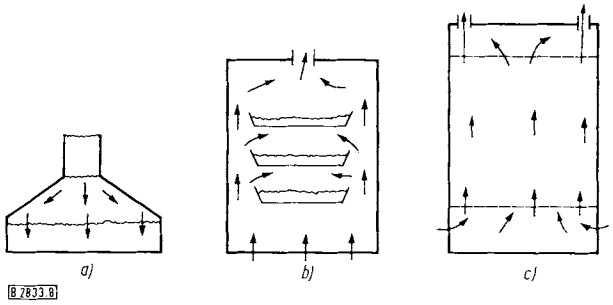


Fig. 8. Aeration of stationary surfaces.
a, stationary surface culture (Fernbach flask, penicillin flask); b, stationary surface culture (fermentation cups, enzyme production); c, filled tower.

5. Oxidations with High Oxygen Consumptions (Hydrocarbon Oxidations)

In the oxidation of steroids and of ethanol the microorganisms have a high oxygen consumption, but this can still just be satisfied by the aerators mentioned so far. Whereas only 33 moles of O_2 are consumed in the formation of 1 kg of cell material from 2.3 kg of glucose, about 75 moles of O_2 are necessary for the production of 1 kg of cell material from 1.1 kg of *n*-hexadecane [10]. This higher consumption is partly explained by the biochemistry of the oxidation of hydrocarbons. In this process a methyl group of a molecule that contains no oxygen is oxidized, first at one end and then possibly also at the other, to a carboxyl group, and ultimately, by continuous β or β and ω oxidation, to CO_2 and H_2O. If the degrading microorganisms are exposed to a reduced O_2 partial pressure, C_3, C_5, and C_7 fatty acids are formed in the substrate as intermediate products of incomplete degradation. For aromatic compounds we must first expect preoxidation, and then (as far as is known at present) three different types of ring cleavage, which can then lead by further oxidation to β-ketoadipic acid and finally also to CO_2 and H_2O.

The oxygen transfer in the four-phase system oil/water/microorganisms/air is a problem of primary importance. The more intensive the mixing of the air introduced with the substrate, the better is its utilization by the microorganisms. In many plants the air is finely emulsified in the substrate to provide the greatest possible surface and to allow optimum oxygen absorption for the oxidation of the hydrocarbons. Several types of fermenters have been developed for this purpose. In the multistage reactor of Chemap AG (Männedorf/Switzerland), for example, two-sided emulsifier wheels are fixed one above another on a central rotating shaft, and are surrounded by a multistage rotating tube with relatively fine outlet apertures (see Fig. 9). Midway between the wheels are large suction holes. At a speed of 2500 to 3000 rpm, the particle sizes of the insoluble phase are between 1 and 10 μm. The air supply can be increased to 5 v.v.m. [volumes of air/(volume of fermentation liquid × min)] in fermentations, so that oxidation

[10] *F. Wagner*, Lecture to Colloquium held at Botan. Inst. der Universität München 1969, unpublished.

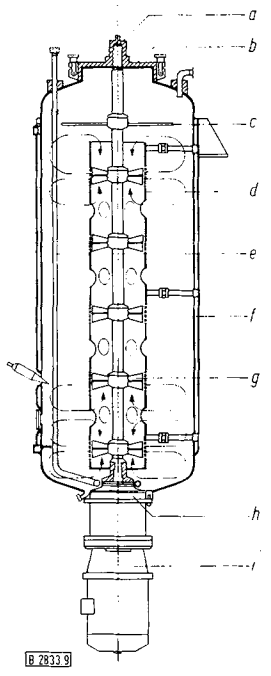


Fig. 9. Multistage reactor (Chemap AG).
a, top bearing; b, cover; c, defoamer; d, reaction tube with emulsifier and suction holes; e, gas feed tube; f, double jacket; g, multistage propeller shaft with double-sided wheels; h, lower bearing; i, motor.

rates of 350 mmole/l h can be obtained. Other less complicated types allow aeration rates of 1 to 2 v.v.m. and oxidation rates of 150 to 200 mmole/l h.

The removal of the large quantities of foam formed during the extremely intensive aeration and motion is particularly important in such processes. When the usual defoaming agents are used, however, there is a danger of oxidation by the microorganisms (*e.g.* in the case of fats and oils), or else the substances must be separated later, and are also very expensive (silicone oils). A design that has given good results is a mechanical foam separator (cf. Fig. 10) in which inverted conical plates are fitted to a rotating hollow shaft. The defoaming action is increased by radial

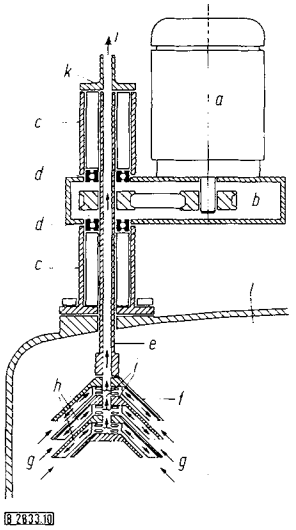


Fig. 10. Mechanical foam separator (Chemap AG).
a, motor; b, belt drive; c, slide ring seal, pressure equalizer; d, bearing; e, hollow shaft; f, rotating plate; g, foam inlet; h, liquid outlet; i, gas outlet; k, bearing housing with cooler; l, contents of tank.

baffles in the plates. The gas/liquid mixture passes upward in the fermenter into the rotating plate. Because of the speed of rotation, the liquid is thrown back into the fermenter vessel, while the lighter gas is forced through the hollow shaft and can escape.

Further problems are presented by the nature of the carbon source and the separation of the microorganisms from the oily substrate. Various industrial processes suitable for the oxidation of hydrocarbons can be derived from such biological fermentations with high oxidation rates, as is shown by the following examples:

1) One-step oxidation of pure paraffins. In these processes the paraffins have already been separated from other petroleum fractions. Such a process is currently being developed with yeast (species of *Candida* and *Torula*) by BP in Scotland.

2) One-step oxidation of paraffins without previous separation from the petroleum. The microorganisms in this case have the tasks of producing protein, *i.e.* cell material, on the one hand and freeing the petroleum from paraffins on the other. In a second stage the petroleum is processed further after separation of the microorganisms. BP are at present building a plant of this type in Southern France with a capacity of 16000 t/year, which is planned to start operation in 1970.

3) Multistage and complete microbial oxidation of petroleum. The long-chain paraffins are oxidized in the first stage, the cycloparaffins in the second, and the aromatic compounds in the third. Processes of this type are not very far advanced at present, and practical difficulties are being encountered because of the long times required for the oxidation of the aromatic compounds. One-step processes are also possible.

4) Production of metabolites (particularly secondary) from petroleum fractions. These processes are mostly one-step with respect to the carbon source. Paraffins that have already been separated from the petroleum are being used at present. The large-scale microbiological production of glutamic acid and 5'-nucleotides has already been in progress in Japan for several years. The industrial microbiological production of salicylic acid from naphthalene in the USA shows that there are also good prospects for the use of aromatic hydrocarbons as carbon sources for microorganisms.

These processes mark an entirely new development of microbiological fermentations. Many new secondary metabolites of microorganisms will soon be produced from hydrocarbons, and suitable processes for the various microorganisms and groups of substances will be devised.

6. Promotion of Certain Phases of Microorganism Growth

Once the various phases of microorganism growth in industrial fermentation plants had been established, the next problem was to prolong the phase in which

the desired metabolite is formed as much as possible or to intensify activity in this phase in order to optimize the yield of this product and to minimize the often expensive secondary cultivation of cell material. Semicontinuous and continuous culture processes were developed for this purpose.

6.1. Semicontinuous Culture

The submerged culture mentioned earlier is carried out in batches, *i.e.* a tank is filled with substrate and inoculated. After the fermentation, the entire contents of the tank are emptied out and the operation is started anew. If part of the fermented solution is held back for inoculation of the fresh nutrient solution, the first step has been taken toward continuous fermentation, since part of the microorganisms grows continuously, provided that the fermentation of the "inoculation liquid" retained has not been interrupted.

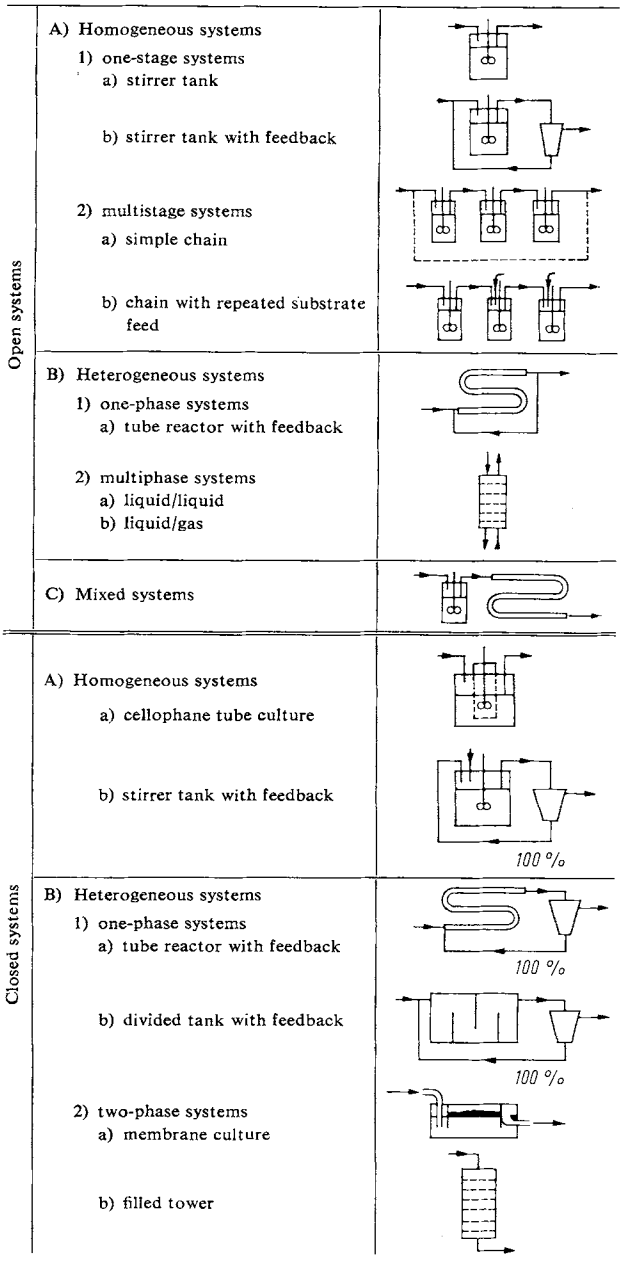
A "semicontinuous process" of this type has proved effective *e.g.* for the production of vinegar, and has led to a complete change in the microbiological methods commonly used for this purpose [11]. In this process, which was developed by Messrs. Heinrich Frings (Bonn/Germany) only about 60% of the contents of the tank are discharged after the fermentation (*i.e.* the oxidation of ethanol to acetic acid) is complete, and the remaining 40% are left in the tank with the microorganisms (*Acetobacter suboxydans*). The tank is then refilled with fresh nutrient solution. Since the microorganisms are extremely sensitive to ethanol and acetic acid if insufficient oxygen is present, aeration is necessary during the discharging and refilling operations. An oxygen utilization of 70 to 80% is achieved by intensive mixing of air with the liquid. Using this method, vinegar can be produced with an acetic acid content of 12%. The alcohol content in the acetator is determined with an Alcograph, and when it reaches a value of 0.1 to 0.3% an automatic discharge mechanism is started up. This causes part of the contents of the tank to be drawn off, and fresh nutrient is added to the remainder.

6.2. Continuous Culture

If the fresh nutrient solution is added continuously instead of in batches, fermented nutrient solution must be drawn off at the same rate.

In one-stage continuous fermentation only a stirring fermenter is used (cf. Table 1). In this fermenter the growth of the microorganisms begins as in a batch plant. At a suitable moment, *e.g.* almost at the end of the exponential growth phase, fresh nutrient solution is allowed to flow into the tank, and an equal quantity of fermentation product is simultaneously allowed

Table 1. Continuous fermentation systems (from [3]).



to flow out. These methods are suitable for processes in which the object is to obtain either large quantities of microorganism cells or substances that are formed in the exponential growth phase.

In multi-stage continuous fermentation, a set of tanks are connected in series with one another. A fermentation is started up in the first tank, and the introduction of fresh substrate is started at a suitable instant. The overflow is not collected for isolation of the product, but is allowed to continue its fermentation in the second tank, until this is also full. A third tank is then filled with the overflow, and so on. In a series of this type one stage can be specially promoted, or suitable precursors may be added in certain stages.

There are also other continuous systems, among which filled towers (Table 1) are important in the fast production of vinegar, and will undoubtedly be important in the future for the fermentation of gaseous

[11] *H. Ebner* in: Arbeitsmethoden und aktuelle Ergebnisse der technischen Mikrobiologie. Fischer Verlag, Stuttgart 1967, p. 65.

products. In this system the microorganisms are grown on a solid support inside the tower. The nutrient solution trickles down over the microorganisms, and a gas (*e.g.* oxygen or gaseous hydrocarbons) is brought into contact with the microorganisms in countercurrent from the bottom. Two gases may also be used, *e.g.* a heavy gas from the top and a light gas from the bottom; in most cases a liquid must also be allowed to trickle downward as the nutrient solution.

Cellophane cultures or cultures in dialysis vessels are still difficult to carry out continuously for industrial purposes. The microorganisms are grown in the dialysis vessel (Cellophane) which is immersed in a tank containing concentrated nutrient solution. The nutrient solution diffuses through the Cellophane membrane to the microorganism culture under the influence of the concentration gradient, while the metabolites diffuse out.

A new thin layer fermentation plant has been developed by *Gorbach* [12]. The microorganisms are grown on rollers in a pipe system, which dips into a nutrient solution on rotating (cf. Fig. 11). The thin layers of microorganisms can be intensively aerated in this process. Removal of the metabolism products and the introduction of nutrients are also very easy. The plant, which operates continuously, can be considerably increased in size by the use of a large number of tubes, and can also be automated.

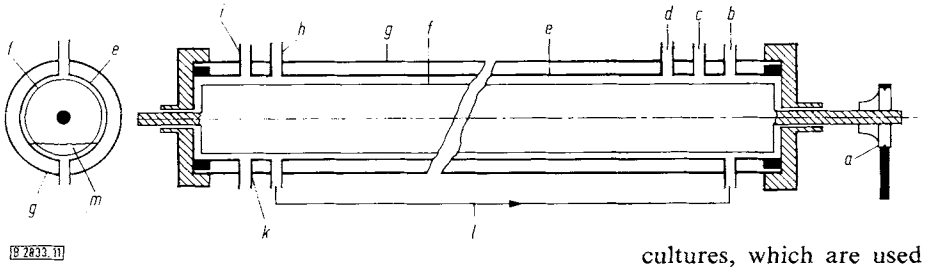


Fig. 11. Thin layer fermentation reactor (after [12]).
a, drive; b, nutrient solution inlet; c, air inlet; d, introduction of additional nutrient solution and pH control; e, fermentation tube; f, drum; g, heating and cooling jacket; h, air outlet; i, foam outlet; k, outlet for transformed nutrient solution and microorganisms; l, microorganism return; m, immersion of drum in nutrient solution.

The trend toward continuous fermentation has resulted in a number of industrial developments. Since the periods of uninterrupted operation must be as long as possible, the provisions for sterility in the fermentation plants must be better than in plants for batch operation. The nutrient feed and the growth of the microorganisms must also be carefully matched. This is ensured by external or internal control systems. With external control systems the rate of growth of the microorganisms is kept somewhat below the maximum by limiting the nutrient concentration ("chemostat"). With the internal control system, which is particularly suitable for high growth rates, the density of the population is measured and used as a basis for the control of the feed of nutrient solution ("turbidostat"). In view of the many parameters involved in the continuous system, it seemed feasible to use analog computers to control and optimize the fermentation. Experiments with this in view have already yielded promising results.

Continuous processes have long been established in practice for the cultivation of cell material (yeasts, algae, *etc.*). However, they have so far been used only in a few sectors for the production of primary and secondary metabolites, though *e.g.* dextran, antibiotics, fermentation products (including beer), oxidation products, amino acids, vitamins, and organic acids can be produced continuously. Nevertheless, once our knowledge in this field has been extended, continuous fermentation will probably be preferred in the future.

7. Cultivation of Very Sensitive Cells

Since microbiological methods are used in the cultivation of cells and tissues, this is generally regarded as belonging to the realm of microbiology. Despite the difficulty of maintaining the sterility of the equipment for the production of suitable substrates, it is already possible to grow a whole range of animal cells and very sensitive microorganisms, *e.g.* tetanus bacilli (for antitoxin production), by submerged culture in fermenters. The animal cells are used for the cultivation of viruses or for the production of cell cultures, which are used in turn for the testing of cancer-inhibiting substances.

Vibration systems are used instead of stirring systems for the cultures, since the conventional propellor

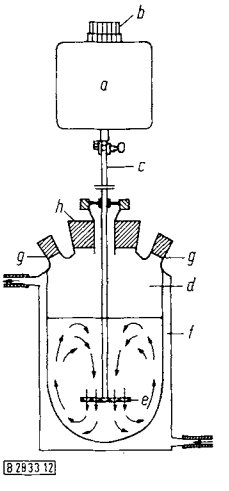


Fig. 12. Vibromix fermenter (Chemap AG).
a, motor; b, speed regulator; c, stirrer shaft; d, fermentation vessel; e, vibration plate; f, water jacket for cooling and heating; g, inoculation points (+ air inlet); h, opening for nutrient solution (↑ air inlet).

[12] *G. Gorbach*, Fette, Seife, Anstrichmittel *71*, 98 (1969).

stirrers would cause excessive damage to the cells. The action of the vibromixer shown in Figure 12 is based on the Bernoulli effect. Instead of a stirrer, the shaft inside the vessel is fitted with a plate having holes that taper in the downward direction. The plate is made to oscillate up and down, and so produces pressure differences in the holes, with the result that the liquid flows from the wide end to the narrow end of the holes. This ensures adequate movement of the cells and minimizes cell damage of the type caused by stirrers.

Since the metabolism products of growing cells are mostly the cause of cessation of growth of the cell cultures, *G. B. Gori*[13] developed a process for dialysis of the toxic metabolism products (see Fig. 13). A laboratory apparatus with an effective volume of 300 ml gives generation times of 40 to 50 h with high cell densities of up to 2.0 to 5.0 million cells/ml.

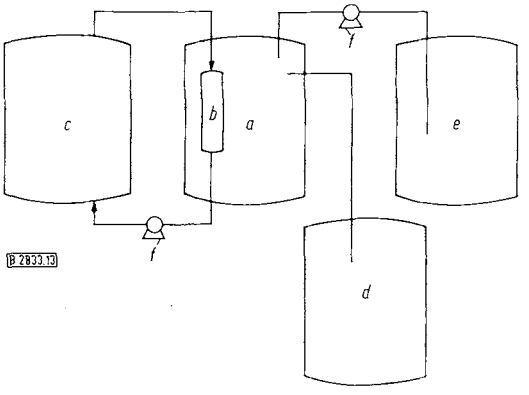


Fig. 13. Apparatus for continuous growth of animal cells in the submerged process with dialyzer (from [3]).

a, fermenter; b, dialysis membrane; c, reservoir for dialysis solution; d, reservoir for collected cells; e, reservoir for eluent; f, pumps.

The need for animal cells will undoubtedly increase greatly if it is desired to use apathogenically grown viruses which can only be cultured on animal cells, *e.g.* in aerosols for the mass immunization of whole population groups, as they are being used at present for the immunization of farm animals.

8. Continuous Culture of Hydrogen-Oxidizing Bacteria

Another group of microorganisms, known as hydrogen-oxidizing bacteria (species of *Hydrogenomonas*), can be used for the production of microorganism proteins. In a synthetic nutrient solution containing nitrogen, with CO_2 as the only carbon source and molecular hydrogen as the hydrogen donor, these bacteria produce organic substances, including proteins, as cell material under aerobic conditions. The oxidation proceeds with the aid of a hydrogenase in accordance with the following scheme:

$$\begin{array}{llll} 2\,H_2 & + CO_2 & \rightarrow & (CH_2O) + H_2O \\ 4\,H_2 + 2\,O_2 & & \rightarrow & 4\,H_2O \\ \hline H_2 + 2\,O_2 + CO_2 & & \rightarrow & (CH_2O) + 5\,H_2O \end{array}$$

Thus the hydrogen-oxidizing reaction is carried out very slowly by the microorganisms. The resulting energy is used for the reduction of CO_2.

The introduction of the hydrogen-oxygen mixture into the bacterial culture in this fermentation is currently presenting a technical problem in large-scale plants. *Schlegel et al.*[14] have developed a continuously operating laboratory apparatus in which water is converted electrolytically into $H_2 + O_2$ in the nutrient solution itself, which contains no NaCl (cf. Fig. 14). Since some of the hydrogen is used for the reduction of CO_2, the corresponding part of the oxygen formed by electrolysis is available for other purposes. If CO_2 from the air were used in the plant, it could be developed as an air-regeneration system. Approximately 100 l of such a culture would be enough to regenerate the respiration air for one person. However, no suitable apparatus designs have been produced as yet.

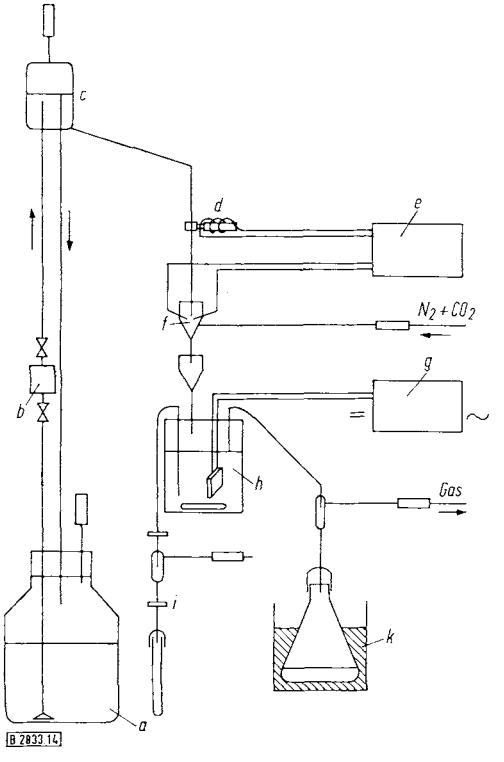


Fig. 14. Plant for continuous cultivation of hydrogen-oxidizing bacteria (after [4], taken from [3]).

a, storage vessel; b, pump; c, leveling vessel; d, magnetic valve; e, timer; f, contact; g, constant current source; h, H_2O electrolysis cell as fermenter; i, sampler; k, collecting vessel in cooling bath.

9. Production of Electrical Power by Microorganisms

Bacteria can change the redox potential of the nutrient substrate or its hydrogen-ion concentration, and can thus form a biological "half-cell" under certain

[13] *G. B. Gori*, Appl. Microbiol. *13*, 909 (1965).

[14] *H. G. Schlegel, F. Schuster*, and *Ch. König* in: Arbeitsmethoden und aktuelle Ergebnisse der technischen Mikrobiologie. Fischer Verlag, Stuttgart 1967, p. 73.

circumstances. A sterile medium can form another half-cell, which may be connected to the microorganism half-cell *e.g. via* a KCl/agar bridge to form an electric cell. The electric energy is produced by the metabolic activity of the microorganisms. Organic substances are oxidized, and the electrons ultimately flow to the oxygen.

Since the electron transport is not continuous but proceeds in steps, conditions must be provided such that a build-up of electrons is avoided, *i.e.* such that an electron flux occurs. A redox cell system with glucose as the substrate (combustion energy) and sea

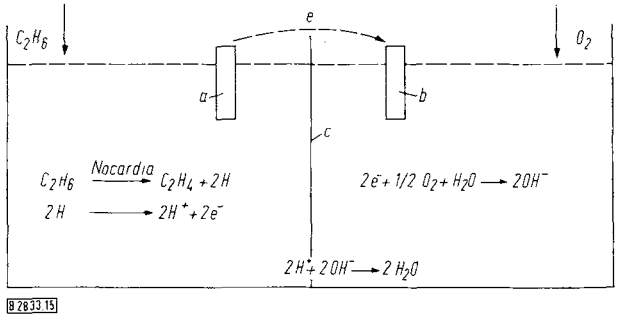


Fig. 15. Scheme of a cell for the biological production of energy (from [3]).

a, anode; b, cathode; c, semipermeable membrane.

water or other salt solutions as electrolytes may be regarded as a very simple form of half-cell (cf. Fig. 15). Gaseous hydrogen may be used instead of glucose as the fuel. Systems of this type have been tried with various microorganisms (*e.g. Escherichia coli, Desulfovibrio desulfuricans*, algae, and yeasts).

Enzymes may be used instead of microorganisms. Thus a urease system by which NH_3 is formed from pure urea or from urine has proved suitable for this purpose. The anode consists *e.g.* of nickel, and the cathode is a glass cell with a fitted carbon stopper at the end, on which cathodic material such as aluminum is sprayed to produce an electrically active surface. An output of 20 W at 28 V has been obtained with a battery of 64 such cells (cf. *e.g.* Fig. 16). About 17 g of urea and 13.6 g of O_2/h were consumed over a working time of two weeks. Microbiological energy cells are still in the development stage at present, but they could be very important some day if naturally occurring reducing substances such as methane or ethane, or even effluent, could be used in this way for the direct production of electricity. This would present a totally new field of industrial microbiology.

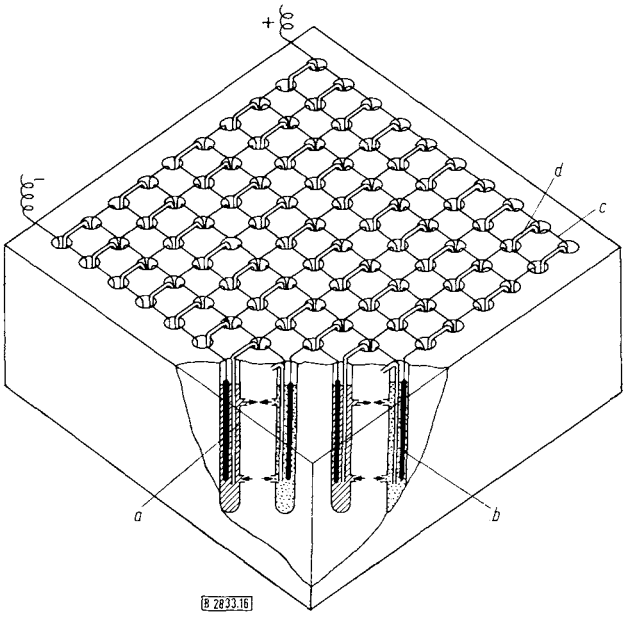


Fig. 16. System of biochemical-biological energy cells (from [3]).

a, tubes with reducing contents; b, tubes with oxidizing contents; e, wire; d, KCl/agar bridge.

10. Conclusions

In view of the quantity and diversity of the microbiological processes that are already being carried out, as well as those that seem promising or are still prospects for the "remote" future, it has only been possible to give a few examples in this review. Thus, no mention has been made of the mass growth of algae (which presents special technical problems), the submerged cultivation of mushrooms, attempts to obtain sulfur by industrial microbiological processes from water containing sulfate, sulfite, or hydrogen sulfide, and many other industrial microbiological processes. Many interesting metabolism products are being produced at present from and with the aid of microorganisms. However, there are also innumerable biochemical-microbiological reactions that could be utilized in practice, but which cannot be exploited at present. There are many reasons for this. One important reason is that most engineers who have the experience and know-how to develop an industrial microbiological process lack the necessary biochemical knowledge, while those who possess the biochemical knowledge do not know whether the development of these reactions might be of interest or which technical lines should be pursued. A future aim should therefore be to close the gaps by suitable training, to rise above classical fermentation technology, and to build up a modern science of biochemical-microbiological engineering.

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