SCHEURING, L. and HÖHNL, G. 1956

Sphaerotilus natans seine Ökologie und Physiologie.

Schr. Ver. Zelstoff u. Pap. - Chem u. - Ing.

Bd. 26.

Translation of pp. 20 - 37.

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II. Morphology, Increase and Systematics of Sphaerotilus natans.

From previous work and our own research has been constructed the following description of the morphology, growth and systematics of Sphaerotilus natans.

Sphaerotilus natans forms, according to the state of nourishement and age 5-11μ long, and 2-3·5μ thick, cylindrical cells, which multiply by transverse division and rowed together like threads, are set in a 1-2μ broader slime-partings, with the latter one must discern between a delicate primary inner slime-film, which keeps the young cells together, and a somewhat firmer, gelatine-like outer layer, on which, especially in the case of older threads, finest inorganic particles from the water lodge themselves, especially chemical compounds of iron, mostly $Fe(OH)_3$.

The Plasma of the cells appears to be essentially homogenous, pierced by a few Granula. In the older cells there appear at times numerous ball-like structures, which are explained as being vacuoles used as storage places for reserve matter. As is generally the case with bacteria, the nucleus is missing in Sphaerotilus. However, it may be assumed, that the genetic stability, as was shown recently with other bacteria, is conditioned by nuclear materials, which are constructed of Ribonucleic-acids and of Desoxyribonucleic-acids.

The threads arrange themselves in many ways, sometimes parallel, sometimes woven plait-like, sometimes as if felted, and frequently show an unpure derivation. In this case a side branch is not formed by a side-proliferation of a cell, but by a cell at a sharpe angle adhering to another cell and sliding past it to make a further division. The aftergrowth, which can only take place without generic, can take place either through new proliferations from cells torn from the binding thread particles; also end-cells can detach themselves from the parting as unflagellated conidia or flagellated swarmers. The latter posses a tuft consisting of numerous flagellae. Swarmer development is seen especially with well fed, fast growing threads. The conidia which later

develop into "long-rods" are at this stage coccoid, they multiply immediately after adhering to a solid surface by transverse division. The swarmers attach themselves with the unflagellated end of the cell by means of a slimy adhesive hold fast disc, they discard flagellae and grow similarily by transverse partings into a new thread.

The adhesion of the thread to a support allows proliferation of the root cells into long lengths of filamentous chains.

The threads unite themselves according to surroundings and nourishment conditions into fine cotton wool-like flocs, or into more compact-tail-like tufts, also into fur like cushions. These varied forms of growth, to which many different names have been given by separate authors - Numan e.g. discerned between a "felt" and "tuft" types, - this will be dealt more closely with in one of the next chapters.

It is not entirely necessary for the growth of Spaerotilus threads to be attached to a firm support. In strongly polluted fast flowing streams and rivers rich in nourishment it forms a fungal-type of growth which having been torn from their supports can, while drifting, grow on; and the same fact we also confirmed in our experiments with differing nourishment solutions.

The colour of small, newly growing Sphaerotilus tufts is white to light-grey, on the other hand older and larger ones are more yellow-brown to brown-grey in colour. The large tufts and fur-like cushions have light colours only on the tips and on the surface, otherwise they are yellow-brown, brown to grey-brown in colour.

In part the discolouring of the older colonies is brought about by the formerly mentioned adhesion and precipitation in the slime partings, but partly also as a result of all kinds of matter floating in the water which sediment on to the filaments, this consists of an inorganic clay like cloud and microscopic algae, these are caught and held in the slimy network of threads if the Sphaerotilus tufts. These "deposits" are more marked in slow moving water than when there is a strong current which cleans the

foreign particles away from the tufts again. The colour and the suspended matter content of the Sphaerotilus colonies reflects from the richness and the composition of the suspended particulate matter present in the water.

Eventually one can also find quite frequently "artificially" coloured Sphaerotilus tufts. A whole range of dyes, which are also of use in the production of paper, colour the cells of Sphaerotilus for a long time, and we could establish the existence of yellow, rose, carmine red, brownish and violet dye material in various streams and could produce these as we liked.

The pleomorphism of the appearance-and growth forms of Sphaerotilus natans is obviously, as has already been stated, the result of the many different names given by authors to the thread-bacteria. After we determined five species that they were morphologically and physiologically identical in their behaviour, we believed ourselves to be of the opinion of Pringsheim. He discerned, as has already been mentioned, for the genus Sphaerotilus natans (Kutzing 1833) two species the one being Sphaerotilus natans and the other Sphaerotilus discophorus.

For the pollution of rivers only the first "collection-species", which appears abundantly in masses, is of significance; the five forms which are discerned :

1) Sphaerotilus natans = forma eutrophica
2) Sphaerotilus natans = forma ochracea (syn.u.a. Leptothrix ochracea Kützing).
3) Sphaerotilus natans = forma dichotoma (syn.u.a. Cladothrix dichotoma Cohn)
4) Sphaerotilus natans = forma sideropus.
5) Sphaerotilus natans = forma fusca.

However, since the systematics is only of secondary interest in our work a more detailed discussion on these species will have to be foregone.

III. Culture Methods and Experimental Apparatus.

On grounds of extensive research on all possible literature on Sphaerotilus natans a programme of research was planned in May 1952 and experimental apparatus was designed. The result of the first preliminary attempts raised up more questions and demanded a constant adaption and transformation of methods in analytical and manipulative procedures until this was adequate in responding to our demands.

The majority of earlier authors from Büsgen (1894) to Stokes (1954) had in the main cultivated Sphaerotilus according to the conventional methods, only few like Naumann and co-workers (1932-34), Streeter (1930), Blöchliger and Husmann (1939), Sierp, Wuhrmann (1949/50), Lackey and Wattie (1940) had tried to study cultures in apparatus which imitated the environmental conditions of running water.

Since Sphaerotilus grows best in organically polluted waters flowing not too quickly or in static waters moved by wind, the predominant opinion of most bacteriologists and polluted-water-biologists was that a certain amount of water current and a relatively high oxygen content was necessary; for growth and there was every evidence that it would not grow on the usual bacteriological culture medium in agar plates and liquid medium under static conditions.

Naumann and Mitarbeiter tried to rear Sphaerotilus in pure culture in round aquariums, whose water contents were kept in motion by a stirring device, Sierp (according to unpublished information) tried to culture it in high glass columns filled with clay balls or Raschig-ringen, which were aerated and then slowly circulated by water. Streeter and Blöchliger and Husmann used inclined, 80 meter-long wooden water drains, which were a pattern for Wuhrmanns twisting narrow concrete-channels (Gerinnen) Lackey and Wattie used 6-8 liter glass containers to imitate the conditions under which Sphaerotilus "flakes" grow in the aerated slime denaturing tanks.

For our questions we could hardly make use of these examples because

we were not concerned with whether or not Sphaerotilus grew well or badly; we wanted to know how its change-of-matter could be quantitatively followed and determined. Rather more apparatus had to be devised, which made it possible with different nourishment-media under different conditions, to observe the change-of-matter, and rate of growth of a known amount of Sphaerotilus-threads impure and pure cultures which served as an inoculum and had also to be measured quantitively at a given time.

We solved this problem according to four methods:

1) In circulation-apparatus in which the same culture-liquid was kept constantly in motion.
2) In "through-flowing-apparatus, through which constantly new culture-liquid was circulated.
3) In a specially kept static and shaken-cultures (Kolben), and
4) In apparatus for the determination of the gas-matter-change, (Gasstoffwechsel).

In the circulation apparatus work was done throughout with impure-cultures, mostly Sphaerotilus "flakes" from effluents in the other apparatus work was done with pure cultures.

a) Obtaining pure cultures

Five specimens of different origin were available to us, which in spite of their differing hydrological requirements at the places found, and different forms of growth belonged to the typical form of Sphaerotilus natans:

Specimen C had been for quite some time in the culture collection of the Botany School, Cambridge (Note of thanks)

Specimen M was isolated from the Rhine at Karlsruhe down-river from a drain of the cellulose factory at Maxau. Through a reaction of the water of pH 6•3 - 6•4 Sphaerotilus natans typ.-forma eutrophica showed there, in March 1953, on well "washed" river stones, a mass-growth of white-grey to brown-grey tufts of 2-3 cm length.

Specimen E was obtained in Jan. 1953 from the strongly organically polluted Danube below the cellulose factory at Ehingen. The pH value of the Danube water varied between 7·6 and 8·0 with average river current and the bacterial-masses showed a typical profuse growth, of Sphaerotilus natans typ.-forma, eutrophica.

Specimen S was also isolated from the effluent of a cellulose and paper factory, der Paar, at Schrobenhausen-Mühlried, in March 1953. The reaction of the slowly flowing water was pH 6,9 to 7·1. Small and very small "flakes" colonised the shore plants and stones. Microscopic examination showed an "unpure", collection of branched threads so that this form was determined as Sphaerotilus natans forma dichotoma.

Specimen P originates from a moor-ditch (pool) between Prien and Stock on the Chiemsee (March 1953). The almost static water had a pH value of 6·0 - 6·3, was coloured yellow-brown, and on its surface there swam a thin, opaque pellicle of decomposed iron oxide. Weak yellowish, encrusted particles, which gave a distinct Prussian-blue reaction, formed brownish slime-accumulations on the pellicle. In between there were found colourless threads, which under the microscope showed themselves as being a number of single cells in rows. This particular form corresponds to the type of Sphaerotilus natans forma ochracea. From the raw material at times was taken the cleanest possible "flakes" having a little detritus adhering to them. These were put into fine mesh wire bags which were thoroughly scoured by tap-water for 1/2 - 1 hour. Small flakes were extracted from the bacteria-stock by means of tweezers and floated in distilled water. Under the microscope their purity was controlled and if necessary washed again by means of capillary Pipette. Then the separate threads were transferred on to petri dishes containing 1·2% Agar and 0·05% Liebigs meat extract.

Gelatine was found to be unsuitable because of the rapid

"liquidation" by saprophytic bacteria attached on the threads. The incubation temperature was 22-25°C, and after two to three days the thread bacteria had moved out so far from the contaminating attached bacterial colonies that they could with microscopic control by transferred on to sterile petri dish trays in the normal way. By transferring further suboultures pure cultures were eventually obtained. Since the colonies were distributed thickly on the agar and partly grown into it, sometimes small cubes were extracted from the agar by means of an injection needle and brought onto a new petri dish or a liquid nourishment solution. The pure cultures thus obtained were stored in agar slopes with 1-2% agar and 0·2% Liebigs meat extract.

b) Description of the Apparatus.

1) Circulation-apparatus.

In step-wise series or staircase notched set-up an ellipse-shaped groove made of sheet iron of 3·5 m length and 0·2 meters width having a 31 x 20 cm. cross-section at rt. angles, was set up on a socle under a shelter hut. The basin got a slight covering of concrete, and the covering which consisted of glass plates resting on little bits of wood was put aside and the basin was filled with 400 liters of culture-liquid, which was kept in constant circulation by means of a paddle-wheel and was also enriched with oxygen (Fig. 1).

By means of changing the gear on the motor, the paddle wheel could be turned at various speeds quickly, so that the liquid could be circulated at a speed of 5-25 cm/sec. The normal working tempo was 18 cm per/sec so that the contents of the basin were whirled round once in every 46 secs. In the basin wire-mesh of V4A steel were suspended for the adhesion of the Sphaerotilus "flakes. But Sphaerotilus "bunches" also adhered to the sides and the bottom of the basin.

According to the same reciepe a circulation apparatus made from a large wash-basin which was divided by an elliptic wall was set up in Ehingen; the

capacity was 100 liters. At first it was built into a case with glass-surfaces but was later covered with a cellophane sheet to prevent dust from floating in.

In both of the apparatus the culture-liquid was protected from larger temperature changes by a cooling pipe through which tap water flowed.

At the conclusion of the experiments and after the harvest of the growth of the Sphaerotilus all the liquid was drained off through a stopper at the bottom and discarded; the sieves or steel meshes were brushed with a soft painting brush as well as the sides of the basin. Both apparatus imitated the biotope of a slowly flowing stream.

For experiments with pure cultures a completely closed sterilisable circulation-apparatus was developed by Jenke und Nagel. It only consisted of glass and V4A-steel and had no moving parts attached. (Fig. 2).

From elevated storage-containers which were stopped with sterile cotton-wool (1) the nourishment solution flows through a constant-level via an aerating chamber (2) with oxygen connection (3) and a sintered aeratum diffuser (4), to the rearing and culture vessel (5) with an "injected" germinating filter (6) which is scoured from below by the culture-liquid. The latter then falls through a pipe (7) into the 40 liter collection and pressure chamber (8), from where the culture liquid is, by means of sterile air-pressure (9) lifted to the storage containers (1) in 4-5 minutes, or according to the requirements. For the "de-aeration" and pressure equalisation in the culture-chamber is a "de-aeration pipe" (10) and a pressure equalisation chamber (11). For the sampling of the culture liquid the collection chamber possesses a tap (12) and for the purpose of sterilising of the apparatus a "steam-connection" (13). A thermometer and a vacuum pump for the "injecting" of the germinating filter (6) complete the apparatus.

2. Constant-flow apparatus.

The development of these apparatus there was in the previously mentioned article over the results, up till now, on the "Research on the

Biology and the change-of-matter of Sphaerotilus natans" many illustrations.

The final form of the constant flow apparatus as given again in Fig 3 consists of the following parts:

The vital part of this apparatus is a closed inspection vessel (1) serving as culture container with a generating filter (2) for the adhesion of the Sphaerotilus growth, a pipe with drain (3) and a pressure equalising tube (4) with a sterile cotton-wool stopper. As in the circulation-apparatus the germinating filter is scoured by the nourishment liquid from the bottom. For the "injection" of the latter, a Sphaerotilus suspension is introduced through a tube (not shown in the diagram) into the culture-vessel, and is sucked onto the germinating filter by means of a vacuum pump.

A mixing and "aerating" vessel (5) is put in front of the culture-container from which former the culture liquid flows via tube (6). The mixture vessel consists of a wide glass pipe which takes in sterilised tap water through a tube with tap fixed on to the top of the mixing vessel (7). The latter is also "aerated" by a glass tube melted on at the apex and reaching to the bottom (8) with a sinterglass diffuser (9). Next to this is the tube conducting (10) the nourishment liquid (Ablauge- alkali) is connected. The nutrient medium similarly comes from an elevated storage container (11), mixes in the aerating and mixture vessel with tap-water and is enriched with oxygen. Superfluous air can escape through a tube (12) stopped up with a sterile plug of cotton wool.

After many attempts to use dried and sterilised plant umbles certain cloth and metal sieves, glass and metal-spirals, sintered glass and clay-candles, as "injection" and adhesion surfaces for the Sphaerotilus-growth, it eventually was shown that one-sided shut germinating filter made of unglazed porcelain which were made to special order by the Stattlichen Porcellan Manufactur Selb), were the ideal "fungi" carriers. These allowed by a pore-size of 1µ a uniform distribution of the fine, suspended "injected" substance over the whole surface and also allowed the growing Sphaerotilus-threads to get good support.

The bacterial suspension used for "injecting" was grown in 200 ml of a 0·2% meat extract solution in 1000 ml Erlenmeyer flasks at 25°C. These were closed by sterile cotton-wool through which again two glass tubes were placed which also had cotton-wool stoppers, one of these reached only al little way in to the flask the other right to the bottom. The danger of foreign infection by the transferrence into the experimental-apparatus was extremely great. In order to prevent this therefore, the experimental-apparatus was sterilised three times on consecutive days before an "injection", in a specially constructed steam-pot. Then, with sterile technique the longer of the two glass tubes of the connections of the pre-culture-container was connected with the culture vessel after the latter had been filled with water from the tap; on the other hand the shorter was connected to the sterilized tap-water. By means of a vacuum pump the "injection" - suspension was sucked into the culture-containing and onto the germinating filter. The scouring of the pre-culture-vessel lasted for 10 - 15 minutes, that is until the last of the meat extract solution which had been sucked in with the "Injection"-culture, had been washed away.

For the germination of the tap water, used for dilutions, it was shown after numerous experiments with the most varied methods and the most varied germination-filters necessary for the high rate of flow - 3-4 apparatus were often in operation at the same time - that the special-filter "Grade - Z" of the Seitz-Works GmbH, was best suited. The experiments which lasted day and night had to have essentially complete freedom from germination; this was guaranteed with the change of filter once weekly.

After the termination of the experiments the germinating filters could be taken out with the adhering Sphaerotilus-growth these were dried, weighed and the formed growth was then used for chemical analyses.

3. Static and shaken-cultures.

In order to solve such fundamental questions as to the nourishment, and change-of-matter-physiology of Sphaerotilus natans, pure culture experiments

on static and shaken cultures in simple round-bottomed flasks were made. So the Erlenmeyer flasks served as rearing containers for a period normally exceeding ten days. The 200 ml spheres were each prepared with 50 ml nourishment solution. For the rearing of larger amounts of bacteria for "injection" and constant flow apparatus and for the manometric examinations, which are still to be described, over the respiration of Sphaerotilus with different nourishment-substrata, 1000 ml Erlenmeyer flasks were used (1500 ml culture-spheres according to Fernbach); these were treated each with 200 ml (300 ml) of solution. After two sterilisations, each for 25 minutes at 120°C under pressure, or after three sterilisations of 30 minutes at 100°C and control of the reaction of the nourishment-medium, the "injecting" of the flasks was done.

In order to have uniform material to a certain extent for the research series, it was intended to make the "injections" with three day old cultures.

When working with heat labile subtrates for which the above mentioned sterilisation method was not usable, the treatment had to be such, that the heat-sensitive material was filtered through a "Seitzfilter", and with sterile pipettes was transferred after sterilisation into the culture-flask.

The breeding normally took place in a climatized place by a temperature of 27°C or in a stepwise thermostatically controlled water-bath at 5-40°C when the optimum temperatures at which Sphaerotilus is still capable of living and growing, could be ascertained.

Difficulties arose in the production of homogenous bacterial suspensions. In liquid nourishment-media Sphaerotilus develops usually comparatively large swimming, "tufty" colonies. Once these have come to being, no homogenous suspension can be gained even by strong shaking. After many experiments it was shown to be advantageous, to shake the culture-flask within the first 12-24 hours after the "injection", and so obtain a small tuft growth, which was suitable for "injections" as also the Warburg-apparatus which will not be described.

4. Apparatus and methods for determining the "Gasstoffwechsel" (gas-matter-change).

The "Gasstoffwechsel" was determined according to the Warburg-method The apparatus (Fig. 4) consists of a ca. 300 l. rectangular water basin as thermostat with "heating-spiral", contact thermometer and stirring machinery. Through the latter as well as the large quantity of water itself a very constant temperature is maintained. Along the two long sides 6-7 skittle-like, small respiration-troughs are completely submerged, which by means of a low-geared motor are constantly kept in shaking-motion, (120-180/min), and which are connected to the simple manometers which are filled with Brodie-solution coloured blue with methylene (Fig. 5).

The small, glass respiration-troughs each have one or two side arms for the nourishment-solution as also a "middle" insertion for the CO_2 - Absorbent (0·5 ml 4n NaOH). For the enlargment of the absorbing surface a folded filter-paper strip is used.

The volume of the vessels which had been calibrated with mercury lay between 18·2 and 19·9 ml. The vessel constants were 1·0 - 1·5 so that one millimeter pressure difference ment 1·0 - 1·5 mm^3 Oxygen. Usually the respiration troughs received 2 ml bacterial suspension and 1 ml of the pH^6 substrates. Any temperature changes were corrected by a Thermo-barometer in the gas-room there was normally air only with the more lengthy experiments was oxygen or carbon dioxide (5% CO_2 and 95% O_2) introduced.

The determination of the respiration quotient (RQ) was usually as a rule undertaken with the help of the Dixon-Vessels. For that purpose 3 ml aqueous suspensions of bacterial suspension with the corresponding, neutral reaction substrate were mixed and pipetted into the vessels. The determination of the carbon acid production and the oxygen intake followed one after the other. During the time of the temperature equilibrium (15 minutes) oxygen was introduced into the gas room, in order to completely remove any traced of carbonic acid. Immediately after locking the manometer, Trichloressig-acid (0·5 ml of a 60% TES-solution) which was contained in a seitenbirne was added into the resp-

iration-vessel to the bacterial suspension, and so by killing off the latter hinder any further carbon-acid production and also to expell all the remaining carbon-acid from the trough vessel. Next, by way of turning a tap a connection was made with the absorbing alkali to the side-arm tube. The absorbing alkali was situated in a cavity of the vessel. The preliminary pressure increase in combination with the said pressure decrease after the absorbing through the Natron-alkali, results with consideration of the necessary vessel-constants, the residual carbon-acid, (page 31, 12 lines up), at the end of the experimentating the bacteria matter was killed off in the same way by the TES-Solution, the residual carbon-acid is expelled at the same time and then the total carbon-acid is absorbed through the Natron-solution, which is seen in a sudden pressure decrease on the manometer. The total taking off gives with due regard for the vessel constants for carbon-acid, the total amount of carbon-acid in mm3 which was present in the vessel, from which then the carbon-acid determined for the Anfangsretention must be separated, in order to maintain the carbon-acid which was produced from the substrate. The oxygen volume present at this can be seen in the difference of pressure between the chosen zerovalue, and the lowest manometer level resulting through the Natron-alkali absorption under multiplication with the relative vessel constant for oxygen.

The time of incubation for the cultures for the manometric respiration measurements was as a rule three days at 27°C. The bacteria-suspension which was obtained after centrifuging in order to learn more of the effect of the substrate was denatured for 90 minutes in the airstream. The aeration was carried out by using a "water-spray-pump. After that the bacteria were again centrifuged and suspended in the necessary water or Buffer solution in a measuring cylinder. After the experiments were concluded the suspension was taken quantitatively from the respiration-troughs, centrifuged, washed at 105° Centigrade and weighed.

IV The relation of the Sphaerotilus growth to physical-chemical conditions.

a) dependence on temperature.

Fishermen and fish-experts, water users and those who pollute water to a great or lesser extent, powerstation and mill owners and not least pollution prevention authorities all made again and again the observation that the appearance, especially the mass-appearance of Sphaerotilus shows seasonal changes and is strongest in the spring and autumn months. In some cases Sphaerotilus disappears altogether in the summer months which are warmer and only makes itself uncomfortably noticeable in the winter, or colder times of the year. In many works and also in numerous legal advice for jurisdiction this fact has been discussed, and it is as has just been said, impossible to re-tell even a small portion of these publications; for this reason we will refrain from trying to enumerate them altogether.

The conclusion, which was taken mainly from the fact of its strongest appearance in the colder months of the year, was that Sphaerotilus was a typically cold-loving organism and it was assumed that it grew at low temperatures optimally, without being able to refer to any specific work or author of such an observation in more recent years this view was changed and the greater appearance of the "tufts" (or fungal growth) in the colder months was tried to be explained by the assumption, that the higher temperature bacterial groups which take part in the breaking-up of organic substances in polluted waters, are prevented to a certain extent by the low temperatures from their activity and so cease to be in food competition with Sphaerotilus so that this through its plentiful food supply can grow exuberantly.

From more temperate climates observations are in the end admissible that Sphaerotilus can grow in a relatively broad temperature-belt, as is also proved by the results with experiments with pure cultures by Linde (1913) Zikes (1915) and Stokes (1954). According to these authors, Sphaerotilus can grow between 10-40°C. 5-29°C and 15-40°C. The lower temperature limits for the growth of Sphaerotilus in culture-experiments varies considerably.

Linde (1913) believed on ground of observations in nature that Sphaerotilus grows at 0°C even if this could not be proved in his cultures under a temperature of 4°C. Up till now no experiments have been able to confirm this belief also not for similar beliefs by Kolkwitz (1914/22) and Liebmann (1951) who

claimed that Sphaerotilus can still grow abundantly under 4°C; also not for the statement of Naumann (1933) - that he had still observed a growth between 2°C - 0°C. Within the temperature belt tolerated by Sphaerotilus, its growth according to all experiments up till now has also been recorded very differently. Thus Linde believed to have found optimal development between 30 and 35°C and Stokes at 30°C, Zikes at around 25°C while Cataldi (1937/39) and Lackey and Wattie (1940) gave 37°C as an estimate, but without having determined the "boundary" temps. All authors have, however, determined the strength of the tuft-growth only according to visual demonstration, and thereby they have been subjects to understandable mistakes. They have neither followed the growth gravimetrically, nor did they give any information as to the length of their cultures nor as to the pH number of the culture mediums.

In our experiments each single one of the culture-vessels, Erlenmyer flasks with 50 ml of the optimal convertable nourishment-mediums- 0·2% solution of Liebigs meat-extract- in series with the same pH values, graded in pH 4,5,6,7,8,9,10 - this was exposed to temperatures between 0-50°C.

It was very difficult to maintain a constant low temperature with only an uninsulated thermostatic water-bath so that in the range between 5 and 15°C an allowance of plus or minus 1° must be made. Still more difficult was the setting of temperatures under 5°C in a DKW refrigerator. Here the temperature of 0°C could only have been reached temporarily and must have lain usually between 2-3°C.

In order to make absolutely certain with the results, 162 experiments in three grades with each 54 "single" cultures were "in operation" and were controlled daily. When the experiments terminated, the increased bacteria-mass was centrifuged by means of a highly geared centrifuge at 7 x 10^8 G (8000-10 000 RPM), and washed out with distilled water for a number of times, dried at 105°C for 24 hours and after being placed into the desiccator for two hours, they were weighed. In the dried substance the total-nitrogen was determined analytically for reasons explained later.

The nitrogen-determination was carried out with a Micro-Kjeldhl-Apparatus. The dried and weighed material first reduced to ashes at 80°C for 12 hours with

2 ml concentrated sulphuric acid, and transferred into "Aufschlusskolbchen" (?). In order to promote the reducing to ashes a few drops of Perhydrol added proved to be advantageous. If the Nitrogen content of the bacteria-mass lay between 0•3 and 1•0 mg, then the over-distilled ammonia was introduced into n/50 hydrochloric acid and the superfluous acid by using a mixed indicator Brothymolblue-Methyl-red and titrated back again. 1 ml of used up acid corresponds to 0•28 mg Nitrogen. The average diviation amounted to plus or minus 5% after control-analyses.

With a small Nitrogen-content of the Sphaerotilus matter a n/200 sulphuric acid served as an indicator in which the residual acid was determined iodometrically. 1 ml of used up sodium thiosulphate corresponds to 0•07 mg Nitrogen.

In order to compare two cultures - species - which have been in culture for different length of time, the "laboratory-species" C and the "wild-species" E were compared. They as a matter of fact did not show the same results completely. The first showed very good growth in the temperature belt between 10 and 40°C, the last showed better growth between 5 and 30°C. Under the given lower temperature the rate of growth diminished quickly, yes, even came abruptly to an end (Fig. 6).

Through the above given speculative some it can be assumed as certain, that Sphaerotilus as it is represented by the "wild species" E reaches its lowest growth boundary between 5 and 4°C so that we cannot confirm the publications and assumptions of Linde, Kolkwitz, Naumann and Liebmann.

See Fig. 6 (Dependence on the temperature of the growth, given in mg of the dry-weight.)

In Fig. 6 the "harvest" weights at different temperatures of the species C and E are represented graphically. They are average values of the dry-weights of the bacteria-masses, which have increased in "single" cultures at the same temperatures and different pH numbers. As can be seen, the rate of growth for both species is different. Whereas species E showed at 5°C almost as large bacteria amount as in the optimal increase which was attained at 10°C, species C showed at the last temperature the first significant amount increase, which

reached its optimum at 15°C. In both cases the "harvest" weight sank with the increase in temperature, but at such a rate that species E at 30°C and species C at 40°C showed almost the same values. There remains only to be seen if there are species of Sphaerotilus in colder regions which are more adapted to the climate, i.e. that there are species which are more cold resisting. This can only be proved by culture-experiments.

In any case the values given here for the upper temperature zone largely apply to this literature.

The determination of the temperature at which Sphaerotilus grows optimally is hindered by the fact that at the same temperature the development continues evenly for several days.

von unten nach oben von der Kulturflüssigkeit umspült wird. Diese fällt durch ein Wasserstandsrohr (7) in das 40 Liter fassende Sammel- und Druckgefäß (8), von dem aus sie mittels steriler Druckluft (9) in 4–5 Minuten, je nach Bedürfnis, zu den Vorratsgefäßen (1) gehoben wird. Für die Entlüftung und den Druckausgleich im Kulturgefäß sorgen ein Entlüftungsrohr (10) und ein Druckausgleichsgefäß (11). Zur Probenahme von Kulturflüssigkeit besitzt das Sammelgefäß einen Hahn (12) und zwecks Sterilisierung der Apparatur einen Dampfanschluß (13). Ein Thermometer und eine Vakuumpumpe zur Beimpfung der Röhrenfilterkerze vervollständigen den Apparat.

2. Durchflußapparaturen

Die Entwicklung dieser Apparate wurde in dem eingangs erwähnten Bericht über die bisherigen Ergebnisse der »Untersuchungen über die Biologie und den Stoffwechsel von *Sphaerotilus natans*« eingehend an Hand von Abbildungen geschildert.

Die in Abb. 3 wiedergegebene definitive Form der Durchflußapparatur besteht aus folgenden Teilen:

Das Herzstück der Apparatur ist ein geschlossenes Schauglas (1) als Kulturgefäß mit der Röhrenfilterkerze (2) als Ansatzstelle für den *Sphaerotilus*-Bewuchs, einem Wasserstandsrohr mit Ablauf (3) und dem Druckausgleichs-

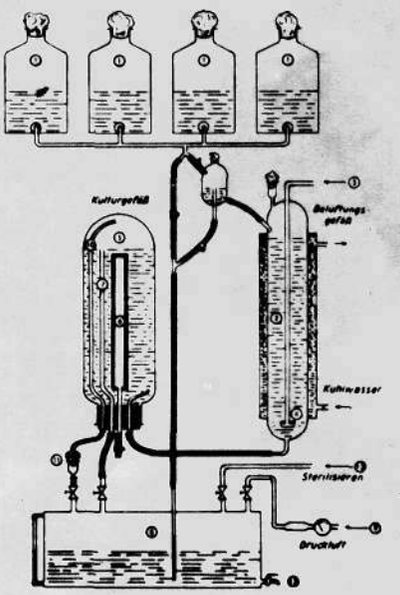


Abb. 2. Geschlossene Zirkulationsapparatur (Erklärung im Text)

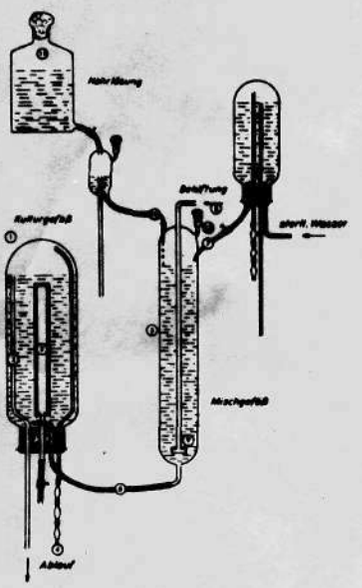


Abb. 3. Durchlaufapparatur (Erklärung im Text)

konzentrierter Schwefelsäure in Wägegläschen bei 80° C 12 Stunden vorverascht und in die Aufschlußkölbchen überführt. Zur schnelleren Veraschung erwies sich ein Zusatz einiger Tropfen Perhydrol als günstig. Lag der Stickstoffgehalt der Bakterienmasse zwischen 0,3 und 1,0 mg, so wurde das überdestillierte Ammoniak in n/50 Salzsäure geleitet und die nicht verbrauchte Säure unter Verwendung eines Mischindikators – Bromthymolblau-Methylrot – zurücktitriert. 1 ml verbrauchter Säure entspricht 0,28 mg Stickstoff. Der durchschnittliche Fehler betrug nach Kontrollanalysen ± 5%.

Bei geringerem Stickstoff-Gehalt des *Sphaerotilus*-Materials diente n/200 Schwefelsäure als Vorlage, in der die Restsäure jodometrisch bestimmt wurde. 1 ml verbrauchter Natriumthiosulfat entspricht 0,07 mg Stickstoff.

Um zwei verschieden lang in Kultur befindliche Stämme zu vergleichen, dienten zu diesen Versuchen der »Laborstamm« C und der »Wildstamm« E. Sie lieferten tatsächlich nicht völlig gleiche Ergebnisse. Ersterer zeigte gutes Wachstum in dem Temperaturbereich von 10–40° C, letzterer in dem von 5–30° C. Unter dem jeweiligen niedrigsten Temperaturgrad kam das Wachstum rasch, ja abrupt zum Stillstand (Abb. 6).

Bei der oben angegebenen Fehlergrenze kann als sicher angenommen werden, daß *Sphaerotilus*, wie er durch den »Wildstamm« E repräsentiert wird, seine untere Wachstumsgrenze *zwischen* 5 und 4° C erreicht, und wir können die Angaben oder Vermutungen von Linde, Kolkwitz, Naumann und Liebmann nicht bestätigen.

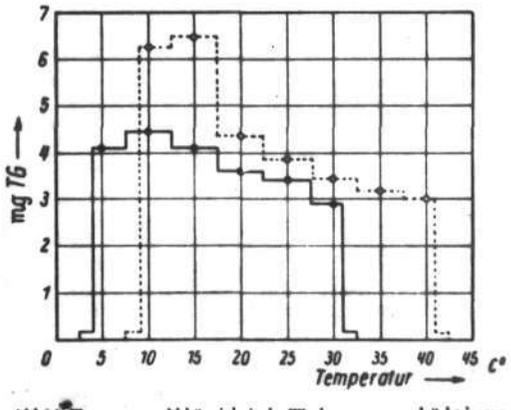
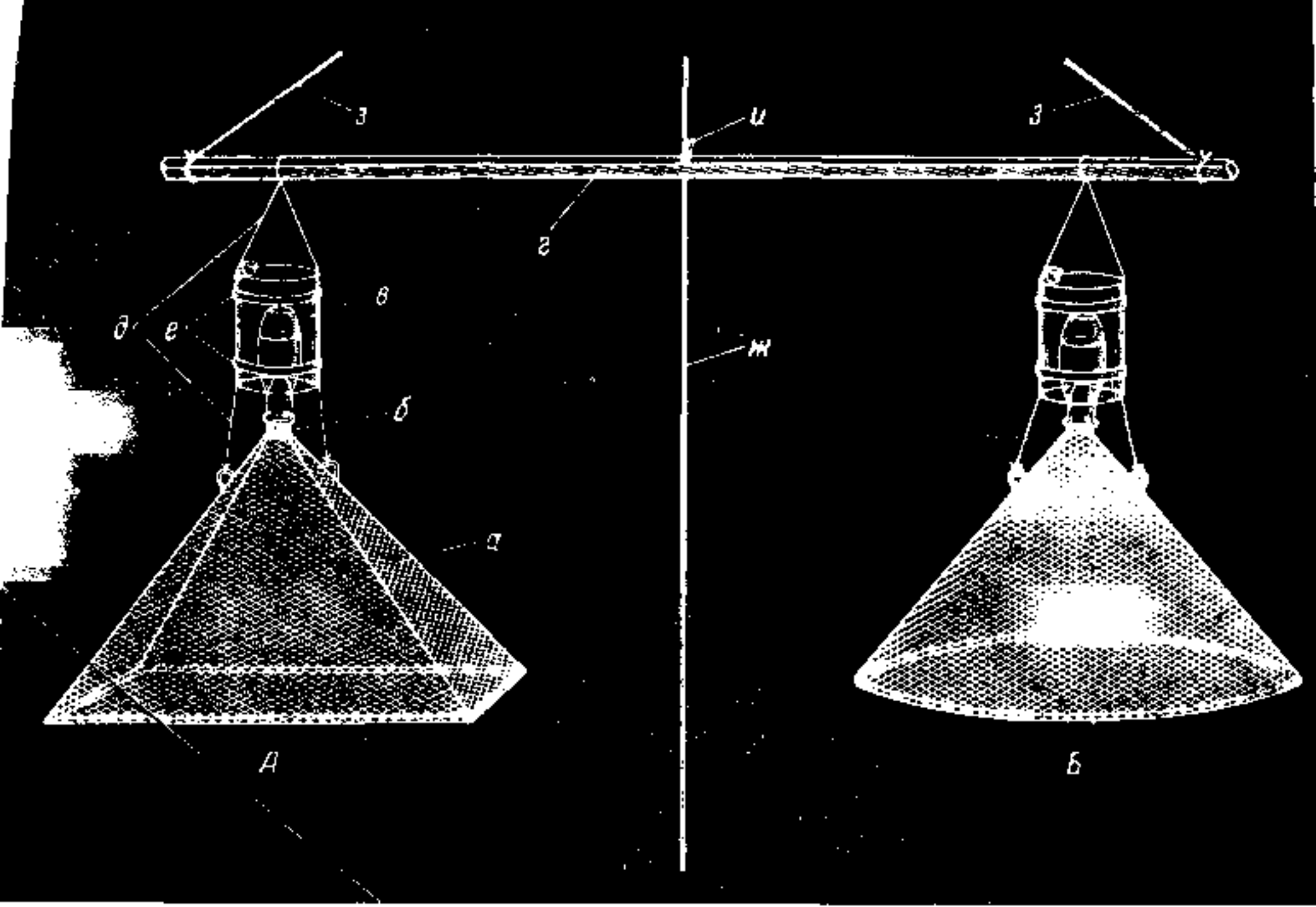


Abb. 6. Temperatur-Abhängigkeit des Wachstums, ausgedrückt in mg des Trockengewichtes; ····· Stamm C, —— Stamm E

In Abbildung 6 sind die für die Stämme C und E bei verschiedenen Temperaturen erhaltenen Erntegewichte graphisch dargestellt. Sie sind Mittelwerte aus den Trockengewichten der Bakterienmengen, die in Einzelkulturen bei jeweils gleichen Temperaturen und verschiedenen pH-Zahlen zugewachsen waren. Wie deutlich zu ersehen, verläuft der Zuwachs für die beiden Stämme verschieden. Während Stamm E schon bei 5° C eine fast ebenso große Bak-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Notice

Please note that these translations were produced to assist the scientific staff of the FBA (Freshwater Biological Association) in their research. These translations were done by scientific staff with relevant language skills and not by professional translators.