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Experimental-ecological investigations on *Phaeocystis poucheti* (Haptophyceae): cultivation and waste water test

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KURZFASSUNG: **Experimentell-ökologische Untersuchungen an *Phaeocystis poucheti* (Haptophyceae): Züchtung und Abwassertest.** Die Haptophyceae *Phaeocystis poucheti* wurde im Laboratorium kultiviert und der Einfluß von Licht, Temperatur und Nährmedium auf die Vermehrungsrate der Zellen untersucht. Die Alge tritt im freien Wasser sowohl in Form von Kolonien als auch in Form von beweglichen oder unbeweglichen Einzelzellen auf. Zugleich können sich die Einzelzellen an festen Oberflächen anheften und bilden in Kulturflaschen wandständige Beläge. Um die quantitative Zählung aller Erscheinungsformen zu ermöglichen, wurden neben der Kultur in Petrischalen Massenkulturen in 1- und 10-Liter-Flaschen und Turbidostat-Kulturen angelegt. Im Turbidostat-Verfahren wurden die Algen unter ständiger Zuführung frischer Nährlösung bei niedriger Zelldichte über längere Zeiträume hinweg kontinuierlich gezüchtet. Als Kulturmedium wurden Erdschreiberlösung, Schreiberlösung und reines Seewasser verwendet. Unter optimalen Bedingungen wurden 2 Zellteilungen pro Tag beobachtet. Die Menge und das Verhältnis der Kolonien zur Zahl der freien Einzelzellen ist vom Nährmedium abhängig. Bei Zuführung ständig frischer Nährlösung dominiert die Koloniephase. Wächst die Kultur jedoch ohne Zufuhr frischer Nährlösung bis zu maximaler Zelldichte heran, so kommt es unter eutrophen Verhältnissen zu einer Massenentwicklung von Einzelzellen, während die Koloniephase schnell zusammenbricht. Im nährstoffarmen Milieu unterbleibt die Massenentwicklung der Einzelzellphase. In den Turbidostat-Kulturen wurde die Vermehrungsrate der wandständig in den Kulturgefäßen festgehefteten Zellen bestimmt. Sie geben ständig Einzelzellen und junge Kolonien in das umgebende Kulturmedium ab. Die Kulturversuche dienten als Grundlage für Versuche über den Einfluß industrieller und häuslicher Abwässer. Das Kriterium der Beurteilung bildete die Beeinflussung der Vermehrungsrate. Die Ergebnisse der unter Einfluß häuslicher Abwässer gezüchteten Algen werden mit den Ergebnissen der in Schreiberlösung und in reinem Seewasser gezüchteten Kulturen verglichen und diskutiert.

INTRODUCTION

The alga *Phaeocystis poucheti* is present in the North Sea plankton from spring to autumn. It occasionally occurs in such large numbers that it dominates all other plankton forms; such temporary "blooms" can influence the migration of herring *Clupea harengus* (SAVAGE 1930). Investigations on growth, multiplication rates and nutrient conditions are, therefore, of great interest in productivity studies and fisheries research. *P. poucheti* has been cultivated on Helgoland under different laboratory conditions employing several methods.

Ecologically orientated culture experiments require methods which, as far as possible, duplicate *in situ* conditions. Only results obtained under ecologically adequate laboratory conditions can help to clarify the complex events observed in the natural environment. The first aim of the present study, therefore, was to develop appropriate cultivation methods.

In our experiments we investigated the influence of light, temperature and nutritional requirements, as well as influences which can arise from industrial and domestic waste waters. Cultivation methods, elaborated for the purpose of basic research, can be employed without many difficulties for toxicity tests.

The life history of *Phaeocystis poucheti* has been described in a valuable paper by KORNMANN (1955). Due to position of the flagellae of the motile swarmers, the alga has been placed in the family Haptophyceae. The swarm cells possess two motile flagellae and one rigid haptonema. KORNMANN distinguished several stages within one life cycle: (1) A palmelloid colony stage, consisting of non-flagellated cells which are arranged in a regular fashion on the inside of an elastic precipitation membrane; the colonies are either spherical or lobed. (2) Single cells, which are released from the colonies and which can again develop, via a motile stage, to new colonies. (3) Microzoospores with independent vegetative multiplication, which can also develop to new colonies under favourable conditions. (4) Macrozoospores, the significance of which in the life cycle is, as yet, unknown.

In our experiment, conducted with mass cultures, possibly all 4 stages occurred simultaneously. However, only the colony stage (1) and the single cell stage (2) could be distinguished. Single cells, originating from mechanical destruction of colonies, multiplied vegetatively in some cases without forming colonies. Cell densities and multiplication rates of both stages, and their interdependence under various culture conditions, have been determined.

In toxicity tests, the multiplication rates of both phases served as criterion. In this way it was possible to assess sublethal long-term effects. Ecologically, critical sublethal concentrations are of special interest, since they provide the best basis for estimating permissible concentrations of waste water in the sea.

MATERIAL AND METHODS

Single colonies of *Phaeocystis poucheti* were isolated from Helgoland plankton and, after washing in filtered sea water, served as inocula for the cultures. The cultures were unialgal but not axenic.

At first, the algae were cultivated in petri dishes containing 10 ml culture medium. The growth of the colonies was measured by direct observation under the microscope from the single cell stage up to maximum dimensions. All cultures were set up in constant temperature rooms at 5^0, 10^0, 15^0 and 18^0 C, respectively. The influence of light was investigated on light-staircases.

Later, the algae were cultivated in 1 and 10 l bottles as mass cultures. Laterally positioned daylight fluorescent lamps served as light source. The culture medium in the bottles was moved about by aeration. Cell densities in the cultures were determined in samples by means of an inverted microscope.

Two methods were used for mass cultures: (1) In the constant volume culture the increase in cell numbers corresponded, at first, to an exponential function. After maximum cell density was reached, population size decreased as a result of nutrient limitation. The experiments had, therefore, a limited duration. (2) In the turbidostat culture (FOGG 1965) cell density was maintained at a constant optimum level by daily replacement of part of the culture medium. The daily amount of additional cell numbers produced was removed and an equal volume of fresh medium added. This method allows the culture to remain in the exponential growth phase without exhausting the nutrients in the culture medium.

Filtered seawater served as basis of the culture medium (32 ‰ ± 1.5 ‰ S, Millipore filters of 0.02 μ porosity). In accordance with experimental conditions the following substances were added to the seawater: nutrients ($NaNO_3$, $Na_2HPO_4 \cdot 12\ H_2O$); soil extract; domestic sewage (collected from the outflow of the Helgoland sewage plant) and industrial waste water. The latter was obtained from a titanium dioxide factory in Nordenham-Blexen (near the mouth of the River Weser, Germany). The industrial waste water consisted primarily of H_2SO_4 and $FeSO_4$. Disposal of this waste, by means of ships, has taken place since spring 1969 in quantities as large as 1,200 tons per day in an area about 14 nm NW of Helgoland. The composition of this waste water, together with its chemical and physical reactions when discharged into seawater, has been published in an earlier investigation which was concerned with the effect of waste water on marine flagellates (KAYSER 1969).

For interpretation of waste water tests, the development of *Phaeocystis poucheti* in pure seawater and in "Schreiberlösung" served as control. Domestic sewage was tested in dilutions from 1 : 1,000 to 1 : 10 (parts waste water : sea water); industrial waste water was tested in dilutions of 1 : 100,000 to 1 : 1,000 (parts waste water: "Schreiberlösung").

RESULTS

Cultivation experiments on influences of light, temperature and nutrients

Our first cultivation experiments were conducted employing petri dishes with 10 ml "Erdschreiberlösung" (1 l sea water + 0.1 g $NaNO_3$ + 0.02 g $Na_2HPO_4 \cdot 12\ H_2O$ + 25 ‰ soil extract). Figure 1 shows growth rates of *Phaeocystis poucheti* from single cells with diameters of 4.5 to 8 μ to spherical colonies, under different conditions of temperature and light. At 15° C, and with permanent light of 2,400 lux from a daylight fluorescent lamp, a single cell develops in 7 days into a colony of 1,000 μ diameter. Development time increases to 28 days when the alga is cultured under the same conditions but using 260 lux illumination. At 10° C, growth is obviously retarded; a diameter of 1,000 μ is reached 3 days later than in the 15° C cultures. At 5° C, under light intensities of 300 lux the colonies attain in 28 days a diameter of 140 μ. At 650 lux, the colonies stagnate at a diameter of 40 μ, and at higher light intensities (1,400 to 3,000 lux) the colonies die after a period of short

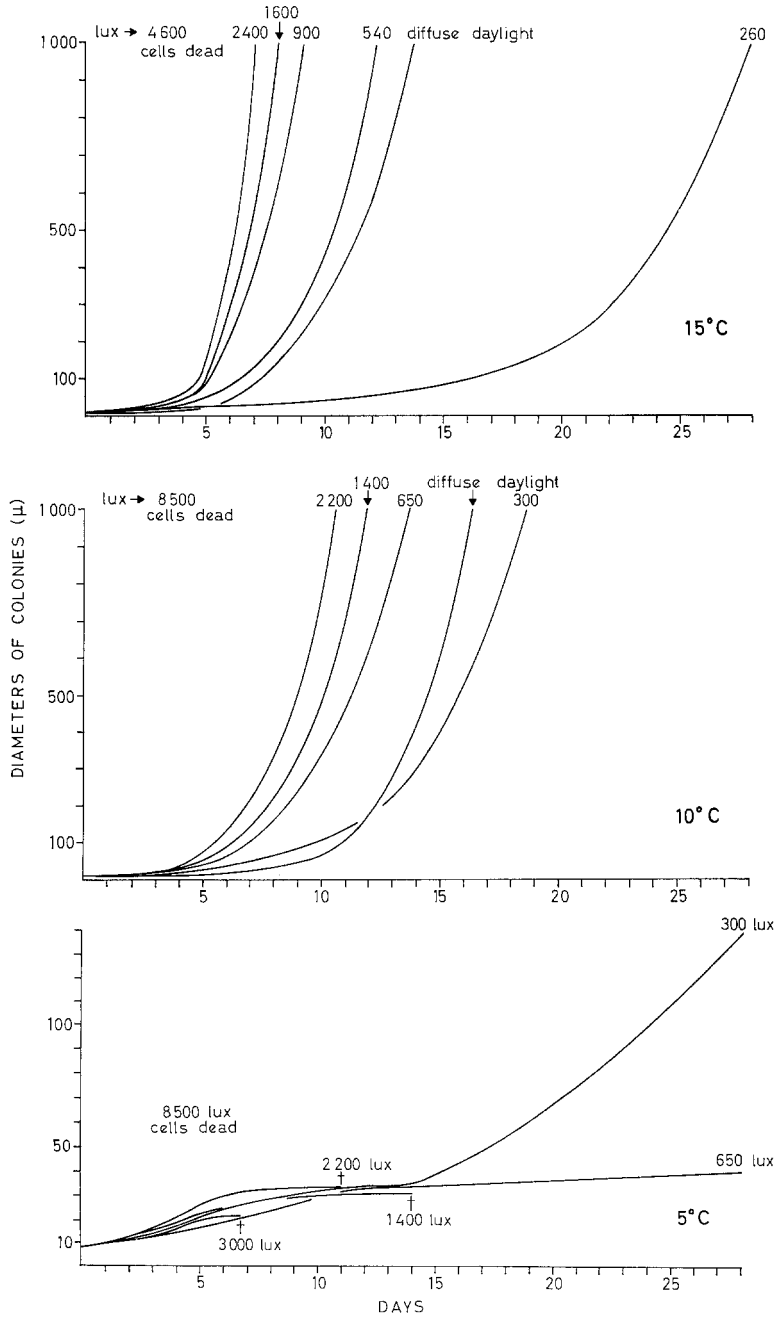

Fig. 1: *Phaeocystis poucheti*. Growth of spherical colonies from single cells with respect to their dependence on temperature and light (culture in 10 ml "Erdschreiberlösung" in petri dishes)

initial growth. 5° C seems to be the lower limiting temperature for colony development; such an assumption corresponds with the observation that, in March, the first detectable colonies in the spring plankton occur in water of this temperature. At

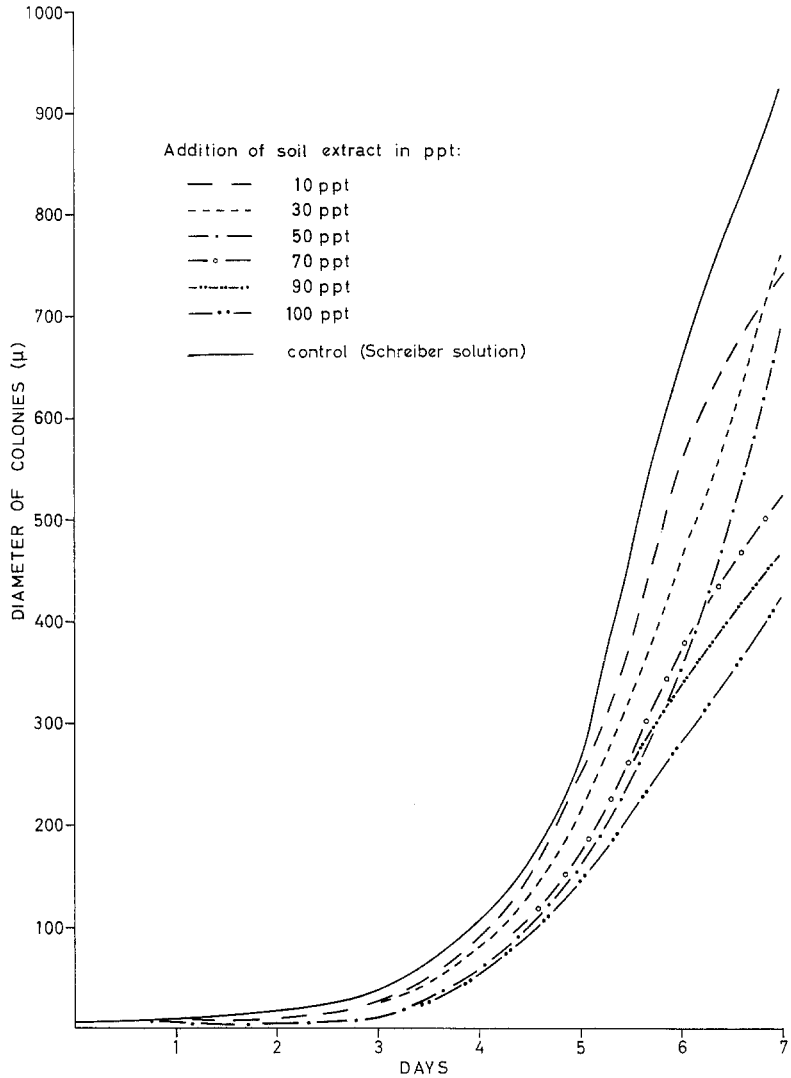

Fig. 2: *Phaeocystis poucheti.* Growth of spherical colonies from single cells in "Schreiberlösung" with respect to their dependence on the addition of soil extract (100–1 ml soil extract to 1,000 ml "Schreiberlösung") in petri dishes with 10 ml solution

15° C, colonies were cultivated until the maximum diameter was reached. In undisturbed petri dishes colonies assume the form of flat, irregular discs with a diameter of about 20 mm.

To investigate the influence of the soil extract a series of experiments was set up in petri dishes (10 ml solution) which contained "Schreiberlösung" (1 l sea water + 0.1 g $NaNO_3$ + 0.02 g $Na_2HPO_4 \cdot 12\ H_2O$) with addition of soil extract in descending concentrations (100 – 1 ml soil extract to 1,000 ml "Schreiberlösung"; control in

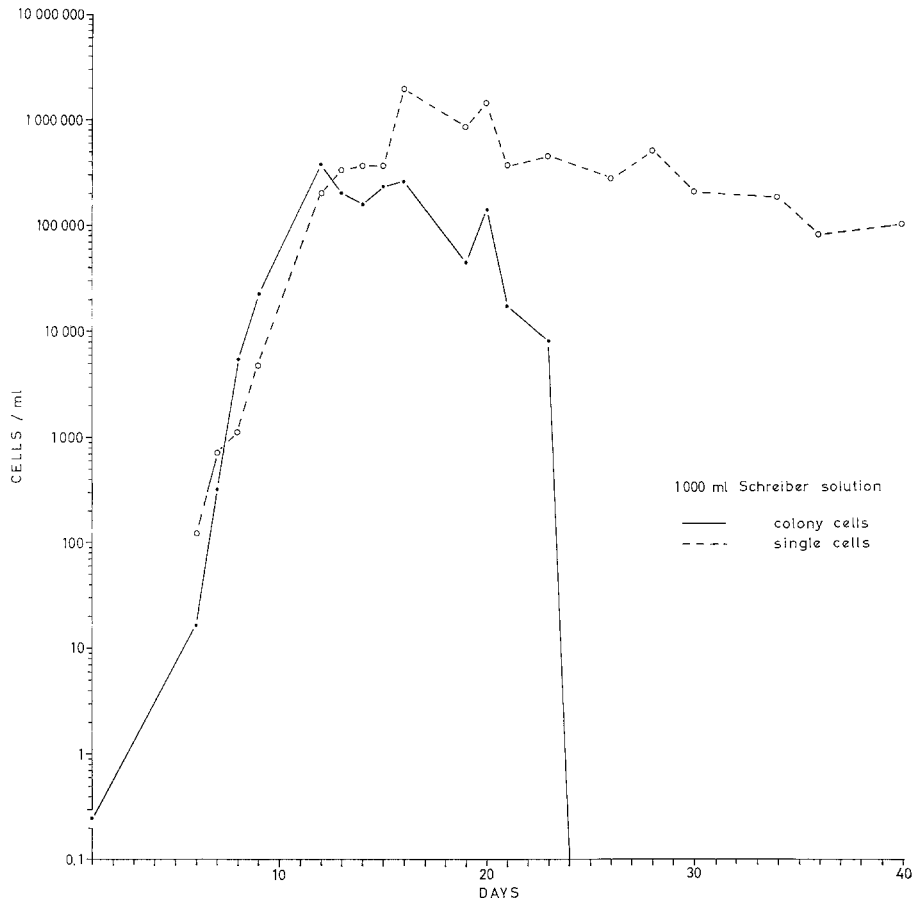

Fig. 3: *Phaeocystis poucheti.* Increase of cell densities, separated into colony cells and single cells, in a 1 l mass culture in "Schreiberlösung"

"Schreiberlösung"). Figure 2 demonstrates the surprising result that, throughout the 7 day experiment, colonies cultivated in pure "Schreiberlösung" show a higher multiplication rate than colonies cultivated in "Schreiberlösung" plus soil extract. In contrast, KORNMANN (1955) succeeding in cultivating *Phaeocystis poucheti* only in "Schreiberlösung" plus soil or mud extract ("Erdschreiberlösung"). In his experiments *P. poucheti* developed neither in sea water to which nitrate and phosphate had been added ("Schreiberlösung") nor in pure sea water.

Nevertheless, I attempted to cultivate *Phaeocystis poucheti* in pure sea water. In

10 ml sea water, colonies of 1 mm diameter developed in 8 days into irregular discs of about 7 mm diameter. Then, owing to lack of nutrients, the colonies died.

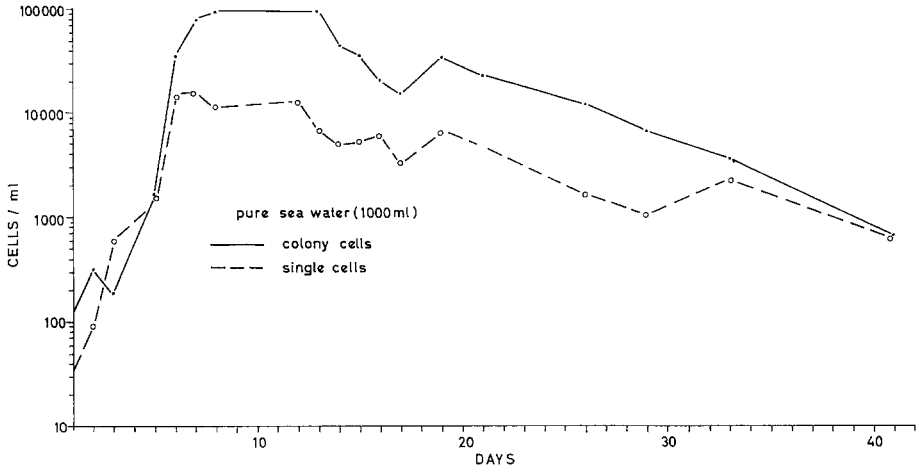

Fig. 4: *Phaeocystis poucheti.* Increase of cell densities, separated into colony cells and single cells, in a 1 l mass culture in pure sea water

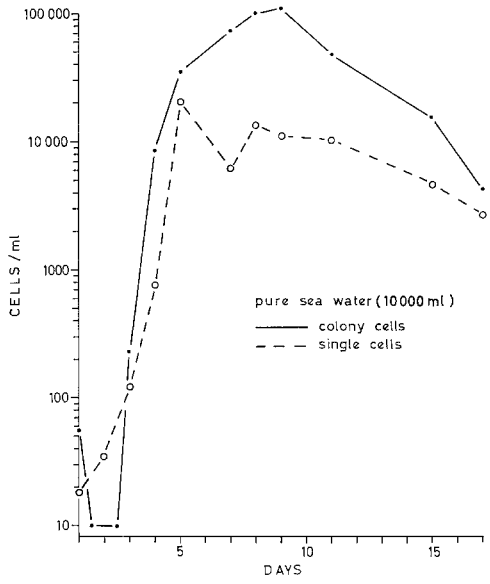

Fig. 5: *Phaeocystis poucheti.* Increase of cell densities, separated into colony cells and single cells, in a 10 l mass culture in pure sea water

In petri dishes, isolated single cells introduced into the culture medium always showed, at first, vigorous vegetative multiplication; only a small fraction of the cells developed into colonies. In the petri dish experiments the number of single cells could not be counted quantitatively.

We have, therefore, employed a different cultivation method: some small colonies or a few mechanically isolated single cells were introduced into a 1 l culture bottle and the rates of multiplication of both stages recorded with the help of an inverted

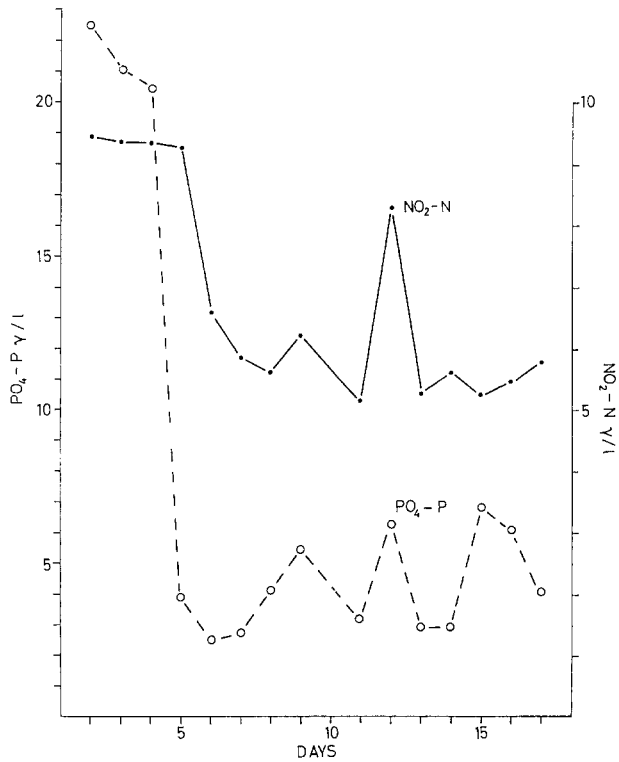

Fig. 6: Change of the PO_4-P and NO_2-N concentration in the culture medium (10 l pure sea water) during a mass culture of *Phaeocystis poucheti*

microscope (constant volume culture). Figure 3 shows the results obtained in "Schreiberlösung" (6,000 lux, 18° C, 14 h light/10 h darkness). At the beginning of the experiment, 2 spherical colonies of 132 μ diameter were introduced into the medium, corresponding to a cell density of 0.25 cells/ml. The number of cells, incorporated in colonies, reached a maximum of about 400,000 cells/ml after 12 days. Subsequently, the environmental conditions become less favourable for the colony-stage of the alga. The number of cells incorporated in colonies decreased and by the 24th day was 0. The number of free-swimming single cells was recorded from the 6th day. At first, the density of these cells remained below that of the colony cells;

however, after the 13th day the single cells predominated, and the colony cells decreased. The number of single cells reached a maximum of about 2,000,000 cells/ml on the 16th day. By the end of the experiment (after 40 days) single cells only could be detected in the culture medium.

During the first 12 days of the experiment the number and dimensions of the colonies increased steadily to a density of 238 colonies/ml with a maximum colony diameter of 1,350 μ (= 5.72 mm² surface area). During the next period of the experiment the large colonies were broken up by turbulence which resulted, on the 15th

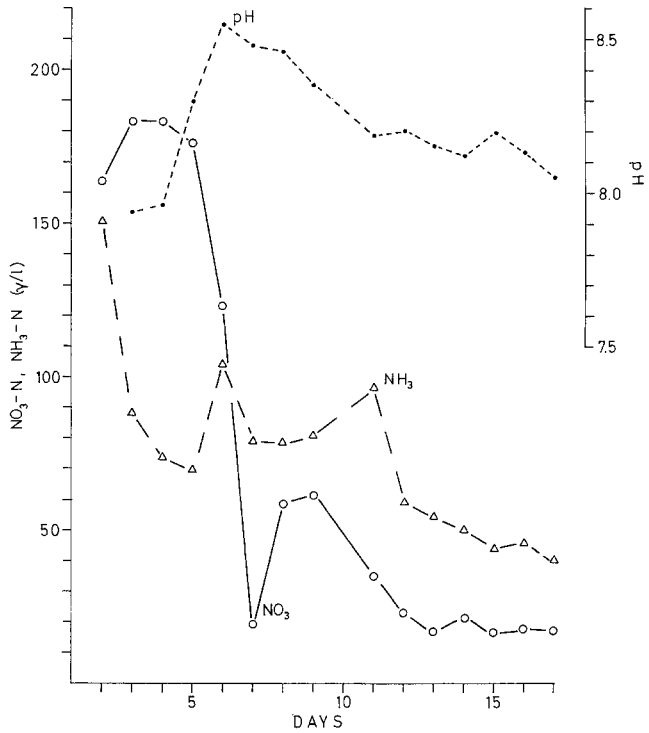

Fig. 7: Change of the NH_3-N and NO_3-N concentration and of the pH-value in the culture medium (10 l pure sea water) during a mass culture of *Phaeocystis poucheti*

day, in a mass development of small colonies. These colonies had a diameter of 50–300 μ and a density of about 1,100/ml. After the 20th day the number of colonies decreased.

In order to investigate the influence of the culture medium, a number of experiments were set up with pure sea water in 1 l and 10 l bottles. Figures 4 and 5 illustrate the results obtained. After 8 days the density of the colony stage in a 1 l culture (Fig. 4) reaches a maximum of about 100,000 cells/ml. This density remains until the 13th day. However, it decreases noticeably slower than in the experiments with "Schreiberlösung" (Fig. 3). After 41 days, the colony stage with a density of

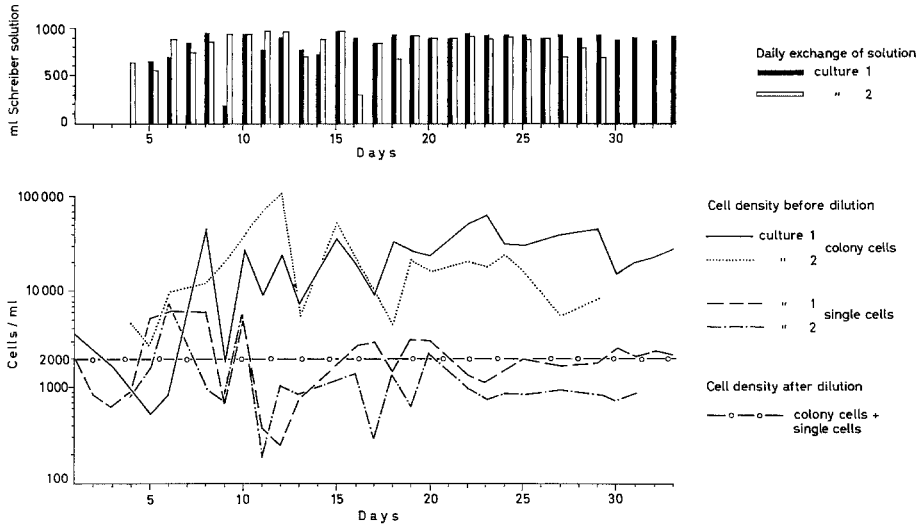

Fig. 8: Turbidostat culture of *Phaeocystis poucheti* in 1 l "Schreiberlösung". The amount of additionally produced cells was removed every day keeping the population density at a level of 2,000 cells/ml (colony cells + single cells). Above: the degree of daily medium dilution is shown in form of a column diagram for two experiments (culture 1 and culture 2). Below: the corresponding cell densities, determined before dilution, are shown for colony cells and single cells separately

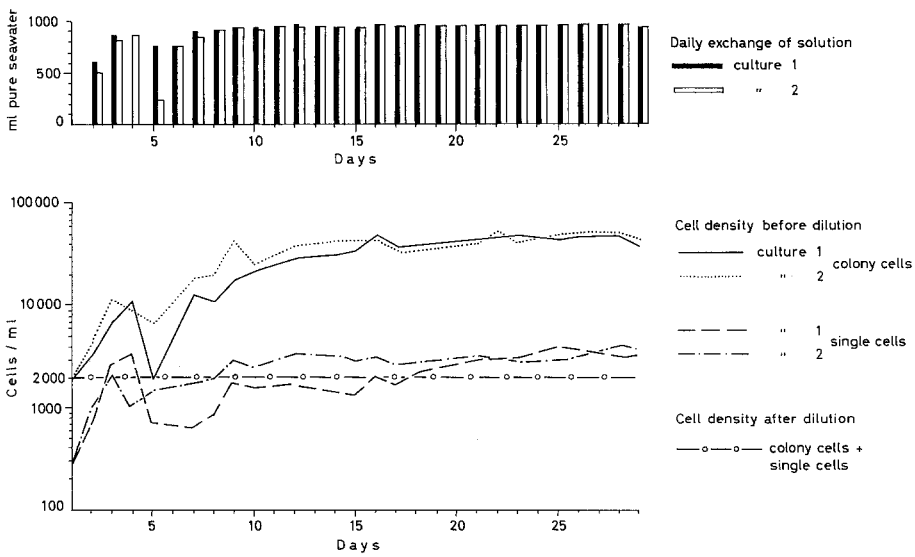

Fig. 9: Turbidostat culture of *Phaeocystis poucheti* in 1 l pure sea water. Experiments correspond to those illustrated in Figure 8

650 cells/ml could still be observed. The density of single cells is also different from that observed in the experiment with "Schreiberlösung"; the maximum density attained is only about 15,000 cells/ml. The number of single cells remains well below the number of colony cells even during the period of cell decrease. Corresponding results for a 10 l culture are shown in Figure 5.

The chemical data of the 10 l culture are shown in Figures 6 and 7. The nutrient content decreases with increasing cell density. After maximum cell density had been reached the nutrient content remains at a relatively constant level, which can be

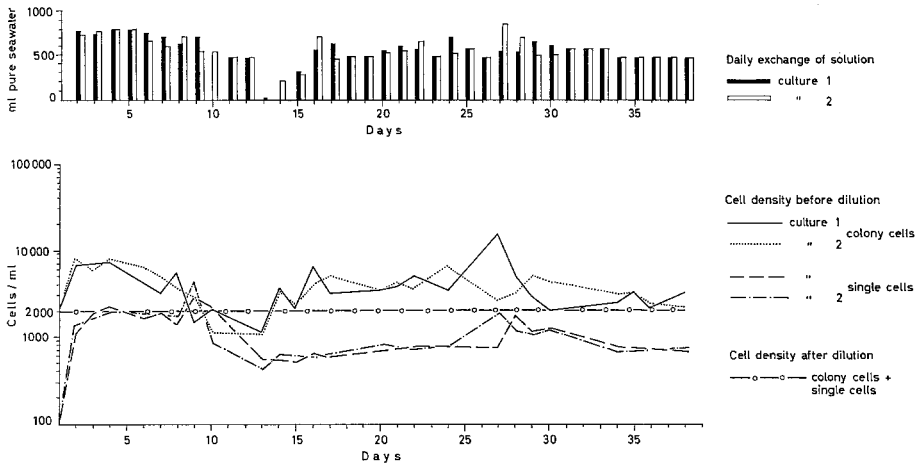

Fig. 10: Turbidostat culture of *Phaeocystis poucheti* in 1 l pure sea water by daily substitution of the culture bottle. Experiments correspond to those illustrated in Figures 8 and 9

assumed to limit the development of the algae. PO_4-P decreases from 22.5 to 4 γ/l; NO_2-N from 9.5 to 5.5 γ/l; NH_3-N from 150 to 45 γ/l; NO_3-N from 180 to 18 γ/l. The pH rose from 7.91 to 8.55 at the time of maximum cell density and decreased again to 8.05 at the end of the experiment.

In the turbidostat-cultures the daily increase in cell density was reduced by daily dilution to a level of 2,000 cells/ml (colony cells + single cells). Figure 8 shows the results of 2 experiments, conducted with "Schreiberlösung". The density of the colony cell stage (determined before dilution) reached a stable maximum level of about 20,000 cells/ml and the single cell stage attained a stable maximum of about 1,500 cells/ml. About 90 % of the culture medium was replaced every day. In Figure 8 the degree of daily dilution of the culture medium is illustrated in the form of a column diagram.

In a corresponding experiment, carried out with pure sea water as culture medium (Fig. 9), the cell density of the colony stage (before dilution) varied around a maximum of 40,000 cells/ml and the density of the single cell stage around a maximum of 3,000 cells/ml. It was necessary to renew about 95 % of the culture medium daily. Number and dimensions of colonies cultivated in pure sea water

attained a relatively constant ratio of small and large colonies. The majority of colonies had a diameter of 80 to 300 μ (0.02–0.27 mm² surface area). Sporadic colonies with 750 μ diameter were noticed. These observations correspond to information on the size of colonies obtained in the open sea.

In the two turbidostat experiments already referred to, it was observed that a thin layer of single cells was attached to the walls of the culture bottle. This layer remained in the bottle in spite of the partial daily exchange of the culture medium. As a result, these cells were not included in the daily count which dealt only with the free-swimming phase. The attached cells continuously released single cells and young colonies into the medium. In subsequent experiments, therefore, a new culture bottle was substituted daily and, in this way, the attached single cells were removed from the culture system. Thus, the multiplication factor caused by these attached cells was eliminated. The results are shown in Figure 10. The density of the colony cell stage remained (after a lag phase from the 7th to the 15th day) at a level of only 4,000 cells/ml. The density of the single cell stage reached 800 cells/ml only. About 50 to 60 % of the culture medium had to be exchanged daily.

Culture experiments under the influence of waste water

Industrial waste water

Addition of industrial waste water in the dilutions indicated, yielded the results shown in Figure 11. The industrial waste water had the following chemical com-

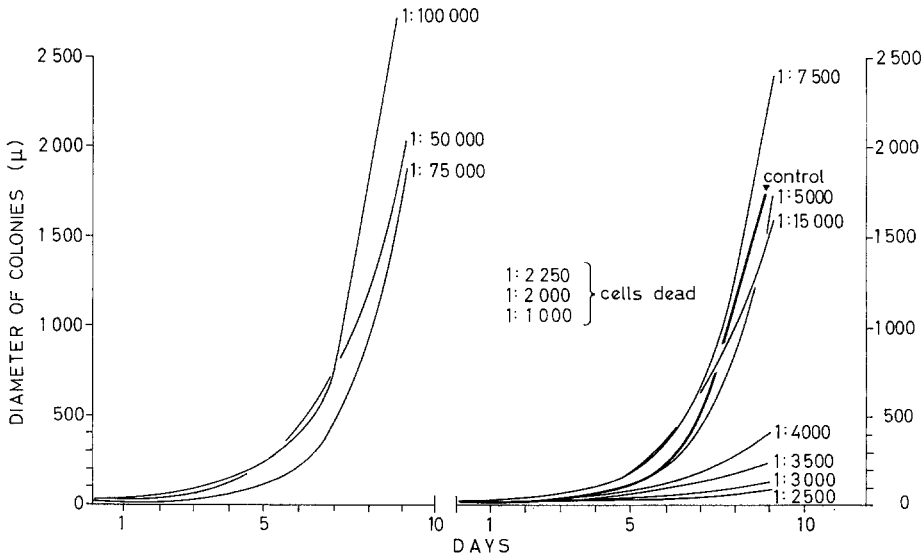

Fig. 11: *Phaeocystis poucheti.* Growth of colonies from single cells under the influence of industrial waste water (parts waste water: "Schreiberlösung" vary from 1 : 100,000 to 1 : 1,000). Petri dishes with 10 ml test medium

position: diluted sulphuric acid (14.4 % H_2SO_4, 74.7 % H_2O, 7.1 % $FeSO_4$, 1.6 % $TiOSO_4$ and small amounts of Mn, Mg, Cr, Vo, Al, Na and Ca) is combined with iron sulphate (50 % $FeSO_4$, 46.76 % H_2O, 0.25 % H_2SO_4 and small amounts of Ti, Mg and Mn) in a ratio of 10 l sulphuric acid to 3.3 kg iron sulphate.

In petri dish experiments, only the growth of colonies could be observed in 10 ml test medium; the single cells were surrounded by a flocculent precipitate of $Fe(OH)_3$. Single cells were, therefore, excluded from cell counts. In a concentration of 1 part waste water : 100,000 parts "Schreiberlösung" growth of colonies was slightly enhanced in comparison with the controls. This effect was presumably caused by the content of Fe-ions, added to the waste water. A distinctly negative effect became apparent at dilutions below 1 : 5,000. The lethal point was reached at a dilution of 1 : 2,250.

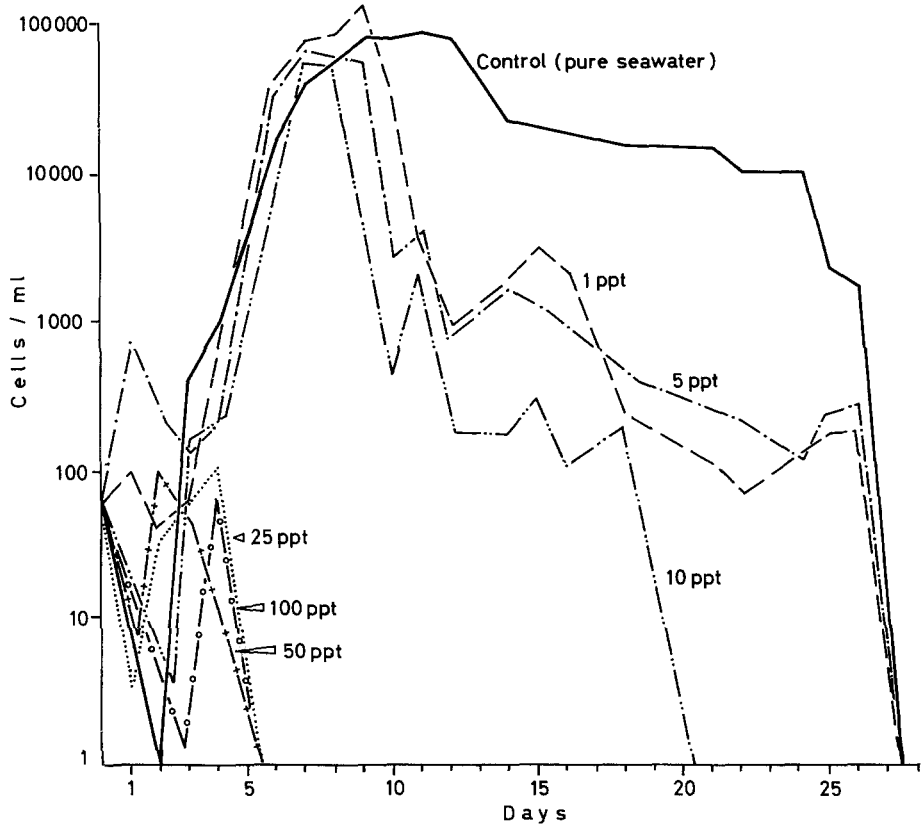

Fig. 12: *Phaeocystis poucheti*. Increase in cell density of colony cell stage under the influence of unfiltered domestic sewage water in concentrations of 1–100 parts sewage water to 1,000 parts sea water. Bottles with 500 ml test medium

Domestic sewage

The influence of domestic sewage was tested in bottles with 500 ml medium. The development of colony stage and single cell stage was investigated independently. The sewage water contained per litre: 3.46 mg PO_4^{---}, 311 γ NH_4^+ and 6.1 γ NO_2^-; NO_3^- was not detectable. The base of the culture medium was pure sea water.

Figure 12 shows growth curves of the colony stage. The control corresponds to results of experiments carried out in pure sea water (Figs. 4 and 5). On the 10th day, cell densities reached a level of about 90,000 cells/ml in colony stage and 16,000 cells/ml in single cell stage. After addition of 1 part domestic sewage to 1,000 parts sea water maximum density of the colony stage increased to 140,000 cells/ml, after addition of 5 and 10 parts sewage water to 1,000 parts sea water to about 70,000 and 60,000 cells/ml. In the control the density of the colony stage remained at its maximum until the 13th day, and then decreased very slowly, reaching a value of 10,000 cells/ml on the 25th day. However, in media contaminated with sewage water, growth rates began to decrease as early as the 10th day of the experiment. At the single cell stage, cultures in concentrations of 1–10 parts sewage water to 1,000 parts

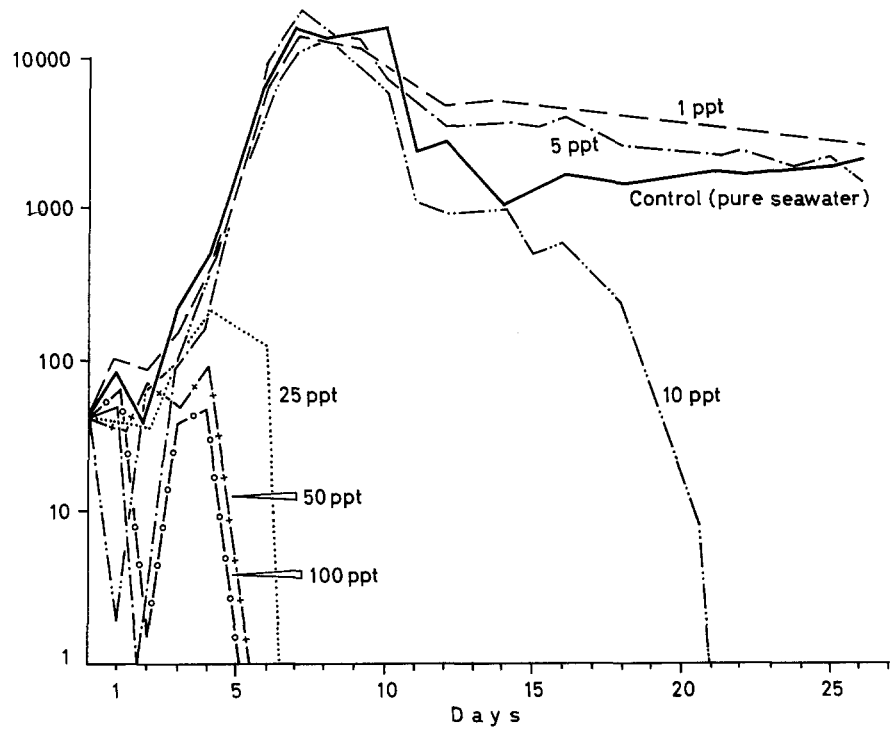

Fig. 13: *Phaeocystis poucheti.* Increase in cell density of single cell stage under the influence of unfiltered domestic sewage water in concentrations of 1–100 parts sewage water to 1,000 parts sea water. Bottles with 500 ml test medium

sea water developed in the same way as the control until the 11th day (Fig. 13). After this time cell densities in concentrations of 1 and 5 parts sewage water to 1,000 parts sea water remained somewhat above the control density, whereas in concentrations of 10 parts sewage water to 1,000 parts sea water the cell density decreased until the 22nd day. In concentrations of 25–100 parts sewage water to 1,000 parts sea water both stages revealed only low multiplication rates during the first 6 days.

DISCUSSION

The fascination, as well as the difficulty, in cultivating *Phaeocystis poucheti* lies in the fact that this alga occurs in two planktonic and one benthic form. In the planktonic stages the rate of multiplication reaches two cell divisions per day under optimum conditions. KORNMANN (1955) observed one cell division only per day in his colonies. In pure sea water, during the exponential phase of growth, *P. poucheti* multiplies at the same rate as in sea water enriched with nitrate and phosphate. Both components were present in the sea water in such high amounts that, temporarily, optimal conditions for development of *P. poucheti* already existed. Addition of soil extract has a slightly negative effect on multiplication rates. The ratio between the colony stage and the stage of planktonic single cells is clearly influenced by the culture medium. The colony stage invariably predominates during the exponential phase of multiplication. After the maximum cell density is reached in the nutrient enriched culture medium, however, the single cell stage is favoured, whilst the colony stage very quickly decreases. In contrast, in pure sea water the colony stage predominates all the time.

Under conditions of continuous supply of fresh culture medium, the colony stage dominates, both in pure sea water and in nutrient enriched medium. It is assumed that the colony stage requires a special nutrient supply. Under conditions of maximum cell concentrations, on the other hand, a negative effect caused by metabolic end products is suspected, which exerts a specific inhibitory influence on the colonies.

The extent to which the development of colonies is influenced by the amount of fresh culture medium added is indicated by the fact that single cells, taken from superannuated cultures will, to some extent, form new colonies when put into pure sea water.

The results can be related to the circumstances in situ: in the turbidostat a low density population is supplied with sufficient nutrients; hence the colony stage dominates. The constant volume culture (bottle experiment), with no daily dilution or renewal of nutrient supply, on the other hand, provides an example of "algal-bloom" in a given, limited water mass. After maximum population density is reached, the colony stage disappears and under eutrophicated environmental conditions a mass development of single cells takes place.

It is interesting to observe that single cells of *Phaeocystis poucheti* exhibit a tendency to attach themselves to solid surfaces. They constantly release new free single

cells and small colonies into the water. The significance of such multiplication is revealed in the turbidostat experiments. The percentage of culture medium renewed daily during experiments in which the attached cells are not removed is about 90–95 %; it is only 50–60 % when the attached forms are removed daily (exchange of bottles). In the first case, the culture is permanently enriched by multiplication of stages attached to the glass wall, resulting in very high growth rates of the total population (attached cells + free cells). In the second case, single cells were continuously withdrawn from the culture system. The growth rate of the total population in the experiment appears, therefore, unnaturally slow.

We may assume that in the open sea single cells are attached to solid surfaces on the sea floor or to motile particles and constantly supply the free water with single cells or young colonies.

Tests with industrial waste water, obtained from a German titanium dioxide factory, revealed that the rate of multiplication of *Phaeocystis poucheti* slightly increases in a concentration of 1 part waste water : 100,000 parts "Schreiberlösung", as compared with multiplication rates obtained in pure "Schreiberlösung". Increased reproduction is, presumably, caused by the presence of Fe-ions added with the waste water. The relatively high toxic lethal concentration of 1 : 2,250 shows, that *P. poucheti* is rather tolerant to this waste water. Investigations on other algae have shown that concentrations of 1 : 50,000 to 1 : 32,000 cause an evident negative influence on the multiplication rate (KAYSER 1969). Corresponding sensitivities were obtained by KINNE & ROSENTHAL (1967) and KINNE & SCHUMANN (1968) on juvenile fishes, fish larvae and fish eggs, exposed to industrial waste water obtained from the same factory.

Tests with domestic sewage have shown that, even in low concentrations, the initial positive effect on colony development gives way to severe damage. This fact corresponds to the observation that the colony stage ends more quickly in nutrient enriched medium ("Schreiberlösung") than in pure sea water. In a concentration of 25 parts sewage water to 1,000 parts sea water the toxic effect was evident. Since unfiltered sewage water was added, it can be assumed that the toxic effect was accentuated by mass development of bacteria and saprophilic protozoans which suppressed the test algae. Nevertheless, the results obtained are thought to be characteristic of the consequences of waste water discharge into the North Sea, because *in situ* waste water is also discharged unfiltered. Our experiments present an example of a combination of basic research and problems of applied science: the results of our waste water tests have been interpreted by employing methods and results developed in fundamental culture experiments.

SUMMARY

1. The planktonic North Sea alga *Phaeocystis poucheti* was cultivated in the laboratory. The influence of light, temperature and nutrients was investigated by employing several culture methods. The influence of industrial and domestic waste water was also studied.

2. The *P. poucheti* forms both colonies and free, motile or nonmotile, single cells. The single cells have the tendency to attach themselves to solid surfaces and, from there, to release continuously new free single cells and small colonies into the ambient water.
3. The multiplication rates of the two planktonic stages and of the sessile stage of *P. poucheti* have been determined and their quantitative inter-relations studied under various culture conditions.
4. *P. poucheti* was cultivated in "Erdschreiberlösung", "Schreiberlösung" and pure sea water. The cultures were set up (a) in petri dishes, (b) in 1 or 10 l bottles without renewal of nutrients as mass cultures, and (c) in turbidostats, limiting cell density to a constant optimal level by the daily removal of old, and the addition of new, culture medium.
5. Under conditions which ensure a permanent supply of fresh culture medium and a low cell density, the colony stage predominates over the single cell stage. However, if maximum cell density is reached in a nutrient enriched culture medium, the single cell stage is favored, whilst the colony stage very quickly decreases in number. In pure sea water the colony stage predominates all the time.
6. Cultivation in "Schreiberlösung" results in maximum cell densities of about 400,000 cells/ml in the colony stage, and of about 2,000,000 cells/ml in the single cell stage. Cultivation in pure sea water leads to maximum cell densities of about 100,000 cells/ml in the colony stage and of about 18,000 cells/ml in the single cell stage. Under optimum conditions the rate of multiplication in the planktonic stages reaches two cell divisions per day.
7. Industrial waste water (consisting primarily of H_2SO_4 and $FeSO_4$) from a titanium dioxide factory favors the growth of the colony stage of *P. poucheti* in a dilution of 1 part waste water : 100,000 parts "Schreiberlösung". A dilution of 1 : 4,000 significantly reduces the multiplication rate of colonies. A dilution of 1 : 2,250 is lethal.
8. After addition of unfiltered domestic sewage (from the sewage outflow on Helgoland) in concentrations of 1–5 parts sewage water to 1,000 parts sea water a vigorous development of the colony stage is followed very quickly by damage to the colonies. The single cell stage, however, shows (in these sewage water concentrations) slightly increased multiplication rates all the time. In concentrations up to 10 parts sewage water to 1,000 parts sea water the toxic effect predominates in both stages.

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