both between filaments and within the same filament. Areas with a striated appearance, which were most opaque in phase-contrast microscopy also stained most intensely. This staining was associated with the presence of the protein (or protein-sheath complex) as shown by the following observations.

A suspension of the alga was washed and resuspended in buffer at pH 3.4 containing 0.1% BSA. To this was added a volume of 1/3000 nigrosin (G. T. Gurr 'Nigrosin W.S.') in buffer at pH 3.4. The amount of nigrosin taken up by the protein-sheath complex was estimated by comparing the optical density of the nigrosin solution at 750 nm with the optical density of the supernate of the centrifuged nigrosin/alga mixture. Control experiments were conducted under identical conditions, except that BSA was omitted. These experiments showed that consistently more nigrosin was absorbed by BSA-treated filaments at pH 3.4 than by untreated filaments at pH 3.4, and very much more than BSA-treated filaments at pH 7.2. From 50–70% of this absorbed nigrosin could be removed by 1 or 2 washes with buffer at pH 7.2, leaving the filaments quite unstained under the microscope. (The greater the absolute amount of nigrosin taken up, the greater was the percentage recoverable in buffer at pH 7.2.) Washes at low pH released less than 10% of the absorbed nigrosin from BSA-treated filaments. A similar experiment with a capsulate *Bacillus* sp. gave similar results. Typical experimental findings are shown in the Table.

Stainability of *Nostoc* and *Bacillus* capsules with nigrosin

Organism	BSA	pH	% available nigrosin absorbed	% of absorbed nigrosin released at pH 7.2
Nostoc FP23	+	3.4	58%	69%
	—	3.4	29%	36%
	+	7.2	7%	–
Bacillus sp.	+	3.4	80%	77%
	—	3.4	27%	52%
	+	7.2	<10%	–

The sheath of FP23 is likely to be chiefly polysaccharide[5]. Its reaction with proteins at pH values near their isoelectric points is very similar to that displayed by bacterial capsules[4]. From a morphological point of view also, it would seem that the 'sheath' of FP23 is more comparable with bacterial capsules than the sheaths found in Chlamydobacteria. Whether this can be said for all blue-green algal sheaths is not clear, but it is unlikely to apply, for example, to genera like *Tolypothrix*.

The affinity of nigrosin for protein absorbed by polysaccharide gels was quite unexpected. Possibly the experiments reported here may afford a means of estimating such protein. Nigrosin is widely used in bacteriology as a negative stain, and it is occasionally used in histology as a positive stain for nervous tissue, but it does not seem to have any recorded affinity for particular cell components[6,7].

Zusammenfassung. Die Polysaccharid-Schale, resp. Kapsel der Schleimalge *Nostoc* sp. (Myxophyceae) kann nach Serumalbuminbehandlung mit Nigrosin bei niedrigen pH-Werten gefärbt werden.

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PRO EXPERIMENTIS

A Simple Method for the Estimation of Yeast-Growth in Hydrocarbon-Substrates by Determination of Turbidity

When microorganisms are cultivated in substrates containing hydrocarbons, the estimation of growth is in general more difficult than it is in the case of a sugar substrate. The reason for this disadvantage lies in the fact that those culture media always consist of an aqueous and of a hydrocarbon phase. As long as the culture is agitated mechanically the hydrocarbon phase is emulsified and a more or less homogeneous suspension can be maintained. This emulsion is supported and stabilized to a certain degree by the presence of fatty acids and proteins, which are produced during the growth of the yeast population. Whenever agitation ceases, the emulsion is decomposed and the largest part of the hydrocarbon phase, containing the bulk of the microorganisms, accumulates as a surface layer. Under these circumstances, sufficient mixing of the culture medium is necessary during the sampling procedure. Furthermore, representative sampling is rendered more difficult by the strong adhesion of the hydrocarbon phase to the glassware, and inhomogeneity is again the result.

GATELLIER et al.[1] reported several methods suitable for the estimation of growth. In their experiments the only source of nitrogen supplied to growing yeasts in the medium were ammonium salts. The amount of NH_4OH supplied, which was necessary for maintenance of a constant pH, was recorded continuously. By plotting the logarithm of volumes of ammonia solution consumed as a function of time, a straight line was obtained representing exponential growth. For the determination of cell concentration a method using filtration on a millipore membrane was described. The cells were washed by isopropyl alcohol and weighed after drying at 100 °C. Consumption of oxygen and production of carbon dioxide were also measured. It has been shown by the authors that all these methods gave the exact same value for the generation

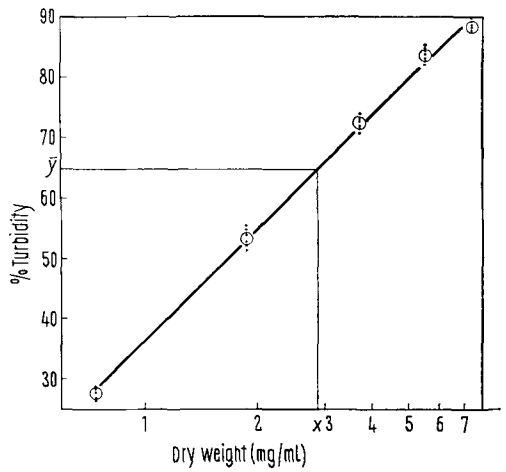
[1] C. GATELLIER, G. GLIKMANS and D. BALLERINI, Kinetics of Alkane Oxidation and Assimilation by Yeasts. Presented at the 154th Congress of the Am. chem. Soc., Chicago Meeting, Sept. 1967.

time of growing yeast. Another method was used by TAKAHASHI et al.[2]. They determined cell concentration by packed volume of cells which were collected centrifugally (10 min at 3500 *g*) at the bottom of a measuring tube. There are several preliminary conditions for the application of one of the methods mentioned above. When the cell concentration is determined by packed volume of cells or by the filtration method a rather large volume of sample is necessary.

It is well known that cell yield can be determined more conveniently and this in very small samples by measuring the optical density. For this purpose the hydrocarbon phase must be separated from the culture broth. TANAKA and FUKUI[3] used this method for the determination of cell yield. They prepared the sample for measurement by successive washings with n-hexane and water. However, only few data were given for the relationship between optical density and dry cell yield. Another preparative procedure of cell suspension, implying washing with Aerosol OT and petroleum ether was described by ARIMA et al.[4].

Initially we used in our experiments the following method. 1 ml of sample was pipetted into 25 ml of a solvent mixture consisting of 80% isopropyl alcohol and 20% hexane. Despite immediate measurement of the optical density after addition of the solvent, the cells flocculated immediately and irreversibly through dehydration by the solvent. Furthermore, mineral salts precipitated in the solvent and caused an undesired error. The reproducibility of the results obtained with this method was not within the limits of $\pm 10\%$.

For a turbidimetric assay we used the following simple method advantageously: In taking samples the culture must be agitated vigorously to attain a homogeneous suspension. With a bulb operator the sample is sucked directly from the stirred suspension into the pipette up to the desired mark. One must be careful not to exceed the upper mark of the pipette, since this would cause a considerable sampling error, due to the adherence of the oil phase to the glass surface. The sample is transferred into a centrifuge tube; the hydrocarbon phase adhering to the pipette wall is rinsed into the glass by repeated washings with small portions of hot water. A few drops of concentrated detergent solution such as Teepol are now added. The stoppered tube is then mixed manually for about 2 min and then centrifuged for 15 min at 1600 *g*. The hydrocarbon phase free from yeast cells accumulates as a turbid surface layer. The cells sink quantitatively to the bottom of the glass as a compact sediment. The liquid phases can be sucked up by means of a capillary attached to a vacuum pump. For the measurement of turbidity a desired amount of saline is pipetted into the centrifuge tube and small glass-beads are added. A homogeneous and stable cell suspension is obtained by shaking for 1 min. The method was tested on a *Candida tropicalis* strain cultivated in a medium containing liquid paraffins, and the results are shown in the Figure. As can be seen from the diagram, the amount of yeast dry matter can be determined over a relatively wide range using the same sized turbidometer cell. If the content of yeast cell dry matter exceeds 7 g/l the sample must be diluted. The desired dilution is prepared directly in the centrifuge tubes with physiological saline solution.



Turbidity was determined in a Lange-turbidometer using rectangular cells of 1 mm pathlength. The medium size of yeast cells was 6.7 × 4.0 µ. Regression line for the relationship between % turbidity and content of dry weight of cells; regression equation: $Y = a + bX$; constant $a = -25.7 \pm 1.4$; regression coefficient $b = 62.0 \pm 0.7$; correlation coefficient $r = 0.99$.

It will be realized that the correlation between the turbidity and the yeast dry matter depends on the size and shape of the yeast cells and to a certain degree on the growth conditions. If other strains or substrates are used a new correlation characteristic for those specific conditions must be set up. Despite the fact that yeast dry matter has been determined here with this method, the determination of turbidity alone will in many cases satisfy the demands. Then the benefits of this easy, quick and reliable method become especially apparent.

Zusammenfassung. Es wird eine einfache und schnelle Methode zur Bestimmung der Zellkonzentration in kohlenwasserstoffhaltigen Kulturflüssigkeiten beschrieben. Zur Abtrennung der Kohlenwasserstoffe wird die Probe nach Zusatz von Teepol zentrifugiert, danach die Zellkonzentration durch Trübungsmessung bestimmt.

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A New Air Sampler

In the past, studies of air spora have been made by the exposure of sticky surfaces for various periods. The catches represent a total during the period of exposure and it is not possible to relate them closely to meteorological or other data. GREGORY[1,2], and GREGORY and STEDMAN[3], have shown that the efficiency of these methods is very low and dependent so greatly on the wind speed, that it is almost impossible to give a reliable interpretation of the counts made. This has led to the development by HIRST[4] of a suction-trap which suffers