UV-Irradiation and Measuring of the Optical Density of Microorganisms in a Microcalorimeter

Microcalorimetric experiments on microorganisms only allow one to measure continuously the heat flux during biochemical reactions of the culture, whereas by reason of technical difficulties – unfavourable installation of the reaction vessel in the internal thermostate block – other biophysical parameters can only be determined before or after the reaction but not during the experiment (BELAICH[1], BOIVINET[2], LAMPRECHT[3]). The recently developed flow microcalorimeter of the LKB type renders tests and measurements during the experiment (ERIKSSON[4], BRETTEL et al.[5]), but one must put up with a time delay of a few minutes because of the separation of calorimeter and culture vessel, so that fast processes, which for instance occur immediately after irradiation, cannot be detected. In this paper 2 optical arrangements are described by which further information of a culture in a calorimeter is obtained.

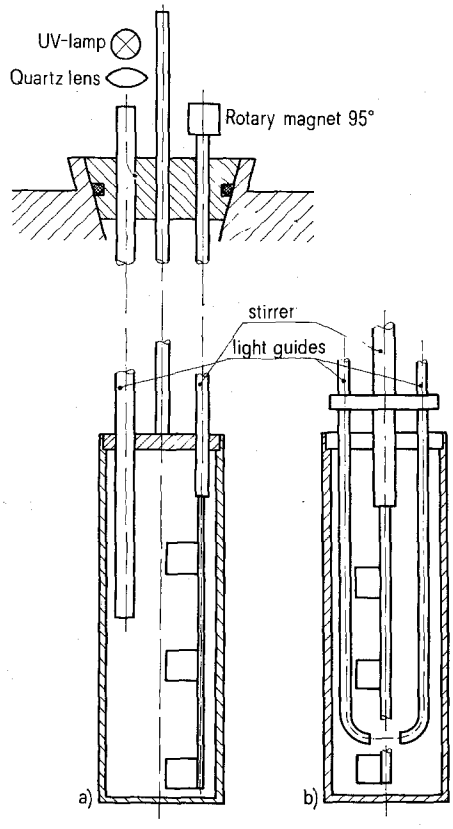

Fig. 1. Arrangement of the fibre optics in the calorimetric vessel, for irradiation (a) and measurement of optical density (b).

Microcalorimeter. A differential-microcalorimeter E. CALVET is used (type MS 70, Setaram/Lyon) (CALVET et al[6]). The reaction vessels have a height of 128 mm, diameter of 35 mm and a volume of 100 ml, they are positioned 675 mm deep in the calorimetric block. To avoid settlement of the cells of the organism and to obtain a homogenity of air and medium, the culture is mixed by a stirrer which is operated by a rotary magnet and a multivibrator (LAMPRECHT et al.[7]).

Optical density device. Two fixed light guides (⌀ 1 mm, sterilizable at 115°C) bent to a distance of 5 mm (Figure 1b) lap into the suspension of the reaction vessel, whereas the flexible ends lead beyond the calorimeter. One of the ends is illuminated by an electric bulb (4 V, 5 W) through a green glass-filter, the light passing through the glass fibres and the suspension between the guides in the vessel. The transmitted light is measured by a photomultiplier tube (type 931 A, RCA), the output of which is amplified and recorded as optical density. The noise level, especially the switching pulse of the multivibrator, is minimized by an electrostatic shielding, and the heat emission produced by stray light can be diminished to a value smaller than $\pm$ 1 μW if a similar device is used in the second vessel for compensation.

The optical density of a growing cell suspension chiefly represents the dry weight, that is the cell mass per ml. If one uses a homogen strain with an uniform cell volume one may obtain the number of cells per ml from the optical density. Figure 2 shows the growing curves of the yeast *Saccharomyces* at 2 different temperatures. The heat flow (d Δ H/dt), the total heat production (Δ H) and the optical density (OD) are obtained as functions of time. Comparing the two sigmoid curves (OD and d Δ H/dt) one finds no coincidence which means that there must be enacted some other heat-producing processes in addition to the mass production. The effect preferably takes place at the transition from the logarithmic into the stationary

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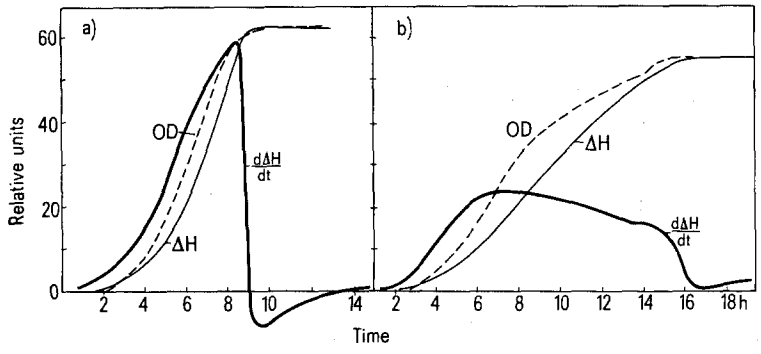

Fig. 2. Growth curves of yeast cells. OD, optical density; dΔH/dt, enthalpy flux; ΔH, total heat production.

phase. The irregularities of the heat flux curve in Figure 2b obviously originate from the variable increase of cell mass. An interpretation of the results, together with a mathematical description, will be given in a later paper.

Irradiation device. UV-irradiation in the calorimeter is carried out by a low pressure mercury lamp (HNS 12, Osram/Berlin, maximal emission at 2537 Å) combined with a light guide of quartz glass (⌀ 10 mm, type QLG, Schott/Mainz). If the irradiation lamp is exactly focussed upon the surface of the fibres the intensity is 2.3 erg/mm^2. s measured by a photon flux detector (SCHAARSCHMIDT[8]). The radiant energy is partially converted into heat with a maximum value of 3 mcal after 10 min of irradiation. This additional heat may be diminished by compensation with a similar device placed in the opposite vessel, which requires a difficult and tedious adjustment of the lamp and the lenses. A heating resistor of 100 Ω proofed as an exact compensation unit if the values of current and voltage are arranged to a similar heat output. The irradiation device was used to record growth curves of radiation sensitive mutants of *Saccharomyces* during short intervals of UV-irradiation as well as heat flux measurements of resting cells in buffer. The changes of enthalpy in these thermograms are discussed in detail together with questions of repair processes elsewhere (SCHAARSCHMIDT[9]).

At the moment experiments with irradiation of visible light are being prepared to test the photoreactivation of radiation mutants of microorganisms and the photosynthesis of algae.

Zusammenfassung. Mit einer Lichtleiteranordnung können optische Messungen in einem Mikrokalorimeter E. CALVET durchgeführt werden. Eine weitere Anordnung ermöglicht Bestrahlungsversuche im Kalorimeter mit UV- und sichtbarem Licht.

B. SCHAARSCHMIDT and I. LAMPRECHT

Zentralinstitut für Biochemie und Biophysik der Freien Universität Berlin, Habelschwerdter Allee 30, D–1 Berlin 33 (Germany), 13 September 1972.

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A New Method for Measuring Guanidine in Uremia

Due to lack of methods for the measurement of guanidine (G) in body fluids, no extensive studies are available on its serum and tissue concentrations and its renal excretion in normal and uremic subjects. In the present paper a procedure is described for measuring it in body fluids, and figures are reported concerning its serum levels and urinary excretion in normal persons and renal patients, its serum and muscle concentrations in normal and anuric dogs and its contents in certain widely used foods.

Materials and Methods. Normal serum (50 ml) and urine or uremic serum (25 ml) were diluted ten times with distilled water and passed through a column (6 × 20 mm) of Dowex W 50 resin (100–200 mesh) in the H+ form. The column was then washed with 100 ml of distilled water followed by 100 ml of 2*N* NH_4OH to elute creatine, creatinine (CR) and arginine. After a second water washing (100 ml) G and methylguanidine (MG) were eluted with 50 ml of 2*N* HCl. This was dried at 75 °C in a ventilated oven and the dry residue, dissolved in 50 ml distilled water, was passed through a column of Amberlite IRA 400 resin in the OH–form (1.5 × 28 cm), to remove Cl–. The eluate was dried again and the residue, quantitatively collected with 3 aliquots of 95% ethanol (2–3 ml each), pooled and reduced to exactly 1.0 ml. This solution was employed (in amounts from 0.2 to 0.5 ml) for chromatography on paper Watmann No. 1 with the system: ethanol: NH_3: water (90:5:5). After about 12 h descending migration at room temperature, G was satisfactorily separated from MG: Rf 0.39 and 0.47, respectively. The paper strips (55 × 5 cm) were dried in an air current and then sprayed with a mixture (2/3) of reagent No. 1 (diacetyl in water, 0.06%) and No. 2 (freshly prepared α–naphtol 1.0 g dissolved in 100 ml water containing g

Serum and muscle concentrations of creatinine, methylguanidine and guanidine in 2 normal dogs and in 7 dogs on the 3rd day of anuria following the ligature of the ureters

	Creatinine		Methylguanidine		Guanidine	
	Serum (mg/100 ml)	Muscle (mg/100 g)	Serum (mg/100 ml)	Muscle (mg/100 g)	Serum (μg/100 ml)	Muscle (μg/100 g)
Normal						
dog 1	0.60	10.0	0.008	0.06	0.8	3.2
dog 2	0.50	12.2	0.010	0.08	1.0	3.5
Anuric						
dog 1	11.8	19.1	0.12	0.29	10.0	14.0
dog 2	9.8	16.0	0.09	0.30	10.0	17.0
dog 3	19.0	21.0	0.19	0.34	5.5	20.6
dog 4	12.2	24.0	0.23	0.54	6.2	14.8
dog 5	14.0	22.0	0.08	0.20	9.1	28.3
dog 6	9.2	21.6	0.07	0.20	7.5	27.2
dog 7	6.6	11.4	0.05	0.17	10.6	30.3