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The influence of the dissolved oxygen concentration on the physiology and ecology of *Sphaerotilus natans* Kütz

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Summary. Using a hermetically closed experimental system the influence of continuously decreasing oxygen concentration on the metabolism of *Sphaerotilus natans* was investigated by concurrently measuring the temperature, pH, oxygen, sucrose, organic acids, diluted organic carbon (DOC) and dry weight.

Oxygen and sucrose were eliminated linearly until the detection limit of $<0{,}1$ mg O_2/l for dissolved oxygen concentration was reached. Under anaerobic conditions neither sucrose uptake nor organic acid formation occured and autolytic processes were evident. The results confirm that *Sphaerotilus natans* is an aerobic organism with no capacity for fermentative metabolism.

Introduction

Sphaerotilus natans, one of the most important indicator organisms for polluted water, assumes a substantial ecological significance by causing a nuisance by its frequent mass development in both polluted natural waters and in sewage treatment plants. The ecological factors that primarily influence the development of the organisms such as substrate availability (Heukelekian and Ingols 1940; Ruchoft and Kachmar 1941; Harrison and Heukelekian 1958; Mulder 1964; Pipes 1967; Adamse 1966/1968; Phaup 1968; Curtis 1969; Mechsner and Wuhrmann 1974; Roberts 1977) and light (Favre 1975; Mechsner, unpublished results) have been comprehensively investigated. However, the question of the oxygen demand of *Sphaerotilus natans* has received little attention. Preferential surface growth of the bacterium in laboratory culture demonstrates its aerobic character but indications of anaerobic growth by *Sphaerotilus natans* (Heukelekian and Ingols 1940) have been reported but never independently confirmed. Many authors emphasis the ability of *Sphaerotilus natans* to grow at low dissolved oxygen concentrations (0,1–2 ppmO_2) and to successfully compete with saprophytes under conditions of low dissolved oxygen concentration in the natural surface waters and in sewage treatment plants (Ruchoft and Kachmar 1941, Stokes 1954, Gaufin and Tarzwell 1956, Phaup 1968, Curtis 1969, Barnard 1978).

This communication deals with experiments designed to answer the questions, whether respiration and/or substrate uptake by *Sphaerotilus natans* are affected by decreasing dissolved oxygen concentrations and whether the oxidative metabolism of *Sphaerotilus natans* is replaced by fermentative processes under conditions where oxygen depletion occurs. Using a hermetically sealed experimental system it was possible to establish a continuously decreasing oxygen partial pressure as a result of the respiration of the suspended organism and to investigate the above mentioned problemes by quantitatively measuring relevant parameters.

Methods

Organism: Sphaerotilus natans Kütz

Isolation and culture. Naturally occuring *Sphaerotilus natans* flocs from model flowing water channels were rinsed with sterile buffer solution and ground by vigorous shaking with sterile quartz sand. The suspensions were increasingly diluted and plated on the following medium: peptone 0,1 g, lactic acid 0,5 g, gelatine 150 g per liter of m/150 phosphate buffer (ph 8,0). Incubation at 22° C followed. The outer filaments of the colonies were cut off and reinoculated. This procedure were repeated until the isolated colonies were no longer contaminated with other organisms. Freshly isolated *Sphaerotilus natans* strains are cultured and maintained at 22° C in 200 ml-Erlenmeyer-flasks containing 50 ml of the following substrate solution: peptone 1 g, sucrose[1] 1 g, trace metal solution 1 ml per liter of m/150 phosphate buffer (pH 8,0).

Experimental substrate. 25 mg $MgSO_4$, 15 mg $CaCl_2$, 30 mg NH_4Cl[2], 100 mg sucrose[2], 1 ml tracemetal solution.

Inoculum. To prepare the inoculum for the experiment, *Sphaerotilus natans* was precultured for 24 h at 25° C in a flask with a magnetic stirrer (750 rpm), harvested by centrifugation and washed in sterilized beakers and resuspended in the reaction vessel so as to give a concentration of 50–100 mg/l on a dry weight basis.

Reaction vessel. (Fig. 1) 2 l double wall fermenter "Schmizo" (M. Schmid, CH-4800 Zofingen) with flanged lid, O-ring gasket and fixing ring. The screw capped Sovirel SVL fittings in the lid were closed either by diaphragms (Fig. 1.1) or by a rubber bladder (Fig. 1.2a) connected to

1 To inhibit degeneration of the organisms either carbohydrates or lactic acid are required.

2 1,000 mg sucrose and 150 mg NH_4Cl for preculture.

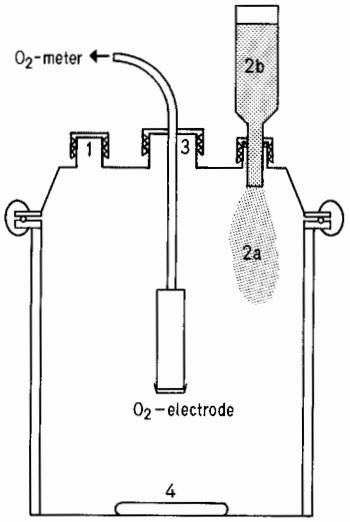

Fig. 1. Reaction vessel (explantation see text)

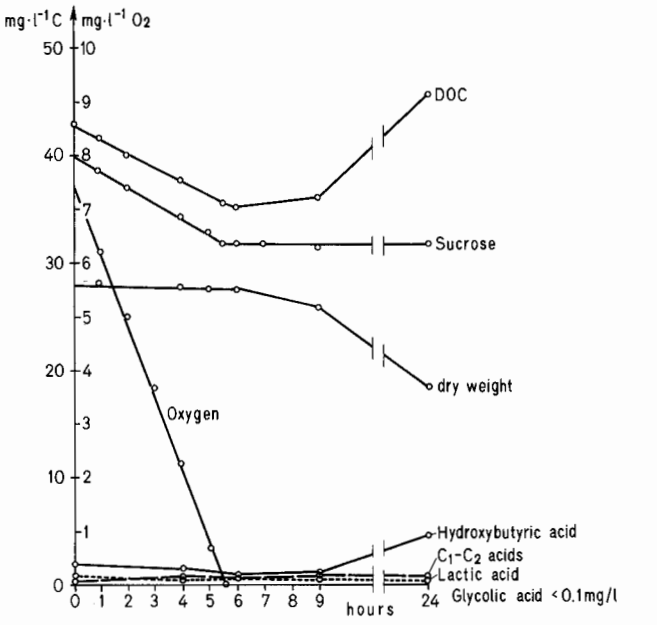

Fig. 2. Metabolic activity of *Sphaerotilus natans* during a decrease in oxygen concentration. All analytical data – incl. dry weight – reduced to organic carbon. Elimination rates, oxygen: 26,6 mg O_2 · g dry weight^{-1} · h^{-1}; sucrose: 59,7 mg sucr. · g dry weight^{-1} · h^{-1}

a column (Fig. 1.2b). Both the bladder and the column were filled with oxygen free water to balance volume differences caused by sampling. The oxygen electrode (YSI 5739) connected with the oxygen-meter (Yellow Spring Inst., Mod. 54, U.S.A.) was introduced through a gastight gasket in the central fitting (Fig. 1.3). The contents were magneticly stirred. The medium and the vessel were sterilized by autoclaving at 120° C for 20 min and the oxygen electrode was treated with 96% ethanol, rinsed with sterile water and pasteurized for 5 min at 75° C in a water bath. The temperature for the experiment was maintained at 24.5 ± 0.5° C by putting the vessel in a water bath. The buffer capacity of the medium was sufficient to keep the pH at 6.9.

Analysis. The dry weight was determined by membrane filtration of the bacterial suspensions and subsequent drying of the residue for 1 h at 103° C. The carbon content of the dry matter was measured using a "F+M" CHN Analyzer Mod. 185. It varied between 48% and 51% and was assumed to be 50% for calculating the substrate balances.

The sucrose was measured enzymatically, using the Bergmeyer method, adapted by Ruchti and Kunkler (1966). Organic acids were analysed by capillary gaschromatography after esterification (Gloor and Leidner 1976).

Experimental. The reaction vessel was 90% filled with substrate and aerated until oxygen saturation was reached. After addition of the inoculum (10 ml) the vessel was completely filled with oxygen-saturated substrate and the Sovirel caps carefully closed preventing any gas bubbles. By connecting the dissolved-oxygen meter with a recorder, the oxygen uptake was followed continuously. Samples for pH, dry weight and substrate concentration were taken with a syringe every 30 min, immediately membrane filtered (Nuclepore PC membranes, pore size 0,4 µm) and stored for analysis at −25° C.

Results. The course of the experiment is shown in Fig. 2. The carbon balances (Table 1) established at the start, direct after exhaustion of the oxygen and at the end of the experiment after 24 h indicate that, very largely, the substrate components, products and intermediates were accounted for. The respiration rate, −26 mg O_2/g dry weight · h, remained constant until the detection limit of 0,05 mg O_2/l was reached and corresponded with values which had been obtained earlier with manometric measurements. Parallel with the respiration, the uptake of the sucrose followed

Table 1. Balance of medium components during the several stages of the experiment. All data reduced to mg org. C/l, DOC: diluted org. C

Sampling$_{time}$	DOC	Identified Components	Dry weight
Start		39.7 Sucrose	
		1.8 Hydroxybutyric acid	
		0.7 Lactic acid	
		0.1 Glycolic acid	
		0.3 C_1–C_2 acids	
	42.9	42.6	28.0
6 h (oxygen exhausted)		32.1 Sucrose	
		0.8 Hydroxybutyric acid	
		1.0 Lactic acid	
		0.1 Glycolic acid	
		0.7 C_1–C_2 acids	
	35.05	34.7	27.5
24 h		32.4 Sucrose	
		4.9 Hydroxybutyric acid	
		0.5 Lactic acid	
		0.8 C_1–C_2 acids	
		38.6	18.5
		+ 9.5 Autolysis (dry weight)	
		− 3.1 Increase Hydroxybutyric acid	
	45.7	45.0	

an unlimited course as long as oxygen could be detected. No intermediates, indicative for fermentative metabolism, could be detected. Throughout the experiment, organic acids, with exception of β-hydroxybutyric acid, could only be identified in insignificant traces. β-Hydroxybutyric acid can be assumed to be an autolytic product of *Sphaerotilus natans* produced from its main storage material, poly-β-hydroxybutyric acid, as its appearance correlated with the decay of biomass as indicated by a reduction in the dry weight.

The increased cell lysis as soon as oxygen is exhausted completes the interpretation of the results, proving *Sphaerotilus natans* to be a strict aerobic organism unable to perform any fermentative metabolism in order to grow anaerobicly. However, the low critical oxygen concentration for *Sphaerotilus natans,* i.e., less than 0,1 mg O_2/l, enable the organism to grow under microaerobic conditions. This capacity is of great ecological relevance and has to be interpreted in the light of the concepts discussed by Dias et al. (1968), who suggested the reason for the positive effect of low oxygen concentration on *Sphaerotilus natans* development in natural mixed systems was a competitive advantage with respect to other heterotrophes resulting from a higher affinity for oxygen.

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