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EXCRETION OF S- AND O-METHYL ESTERS AND OTHER VOLATILE COMPOUNDS BY *OCHROMONAS DANICA*

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Key Word Index—*Ochromonas danica*; Chrysophyceae; metabolism; volatile excretion products; O-esters; S-esters; microaerobiosis.

Abstract—The formation of volatile excretion products was studied in axenic cultures of *Ochromonas danica*. Under microaerobic conditions in the light, an accumulation of O- and S-methyl esters, alcohols, dimethyldisulphide and dimethyltrisulphide was observed in the medium. Most of the compounds are structurally derived from the amino acids methionine, valine, leucine and isoleucine. The excreted volatile compounds, particularly the methyl thioesters, were rapidly metabolized in the dark.

INTRODUCTION

Among the freshwater algal groups which form blooms in lakes and reservoirs, Chrysophyceae are unique in their ability to produce most unpleasant odours [1]. These observations can be explained by the excretion of numerous volatile compounds with very low odour thresholds. The spectrum of these excretion products including alcohols, aldehydes, ketones, esters, organic acids and nor-carotenoids has been studied for *Synura petersenii* [2, 3], a natural bloom of *S. uvella* [4] and *Poterioochromonas malhamensis* [5]. Here, we report the pattern of odour compounds excreted by *Ochromonas danica* into the medium and the growth conditions which lead to maximum accumulation of these substances.

RESULTS

O. danica requires a large number of different organic compounds for optimum growth [6, 7]. In addition, light is necessary to attain rapid rates of cell multiplication. We obtained no growth in the dark despite the use of a complex medium enriched with numerous organic compounds. The anaerobic conditions which soon appeared in the dark may be responsible for the lack of growth. Rapid growth was observed in illuminated 1 l. fermenters although oxygen concentrations in the medium were extremely low as determined with an oxygen electrode. Oxygen may have been evolved by the photosynthetic process but the rate of uptake must then have surpassed that of evolution.

To minimize the loss of volatile compounds, these were determined in illuminated fermenter cultures without external gas exchange. In Table 1, the compounds identified by GC and GC/MS are listed and their approximate amounts in the culture suspension are given. With the exception of dimethyltetrasulphide and 1,2,4-trithiolane whose identification is based on known data from the literature [8, 9], authentic reference substances were available to check the R_ts and mass fragmentation patterns. The time-course of formation of volatile compounds present in sufficient amounts to be analysed was determined in an illuminated, anaerobic 1 l. fermenter culture. After a lag phase, a marked increase in the concentration of the methyl thioesters and 3-methylbutan-1-ol was observed from the second to the fourth day after inoculation (Fig. 1). The concentrations of dimethyldisulphide and dimethyltrisulphide increased steadily. The synthesis of these substances was restricted to illuminated cultures. Cultures precultivated in the light and then transferred on the fifth day to the dark showed a decrease in the concentrations of chlorophyll *a* and all volatile compounds present in the medium (Fig. 2). The most rapid metabolism was observed with the methyl thioesters which showed an immediate drop in concentration after transfer of the culture to the dark.

DISCUSSION

O. danica synthesizes an unusually broad spectrum of different O- and S-esters. Unlike other species of the Chrysophyceae studied before, only methyl esters have been found. The methyl residues of these compounds may originate from methionine added as a source of reduced sulphur to the growth medium. Degradation of methionine that leads to the formation of methyl ethanethiolate has been demonstrated in sediments [10]. The origin of the acyl residues of methyl ethanethiolate and methyl propanethiolate cannot be predicted since both fatty acid and amino acid precursors could contribute to their formation. The acyl residues of the S- and O-esters found in addition to these may be derived from amino acids. However, precursor studies have only been performed for O-esters in banana slices [11]. If one

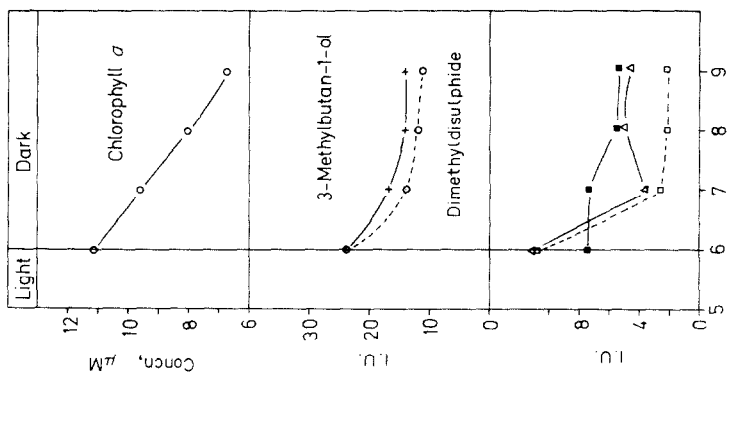

Fig. 2. Consumption of volatile compounds by *O. danica* after the transfer of a culture from the light to the dark thus producing fully anaerobic conditions. (△) Methyl ethanethiolate; (□) methyl propanethiolate; (■) dimethyltrisulphide.

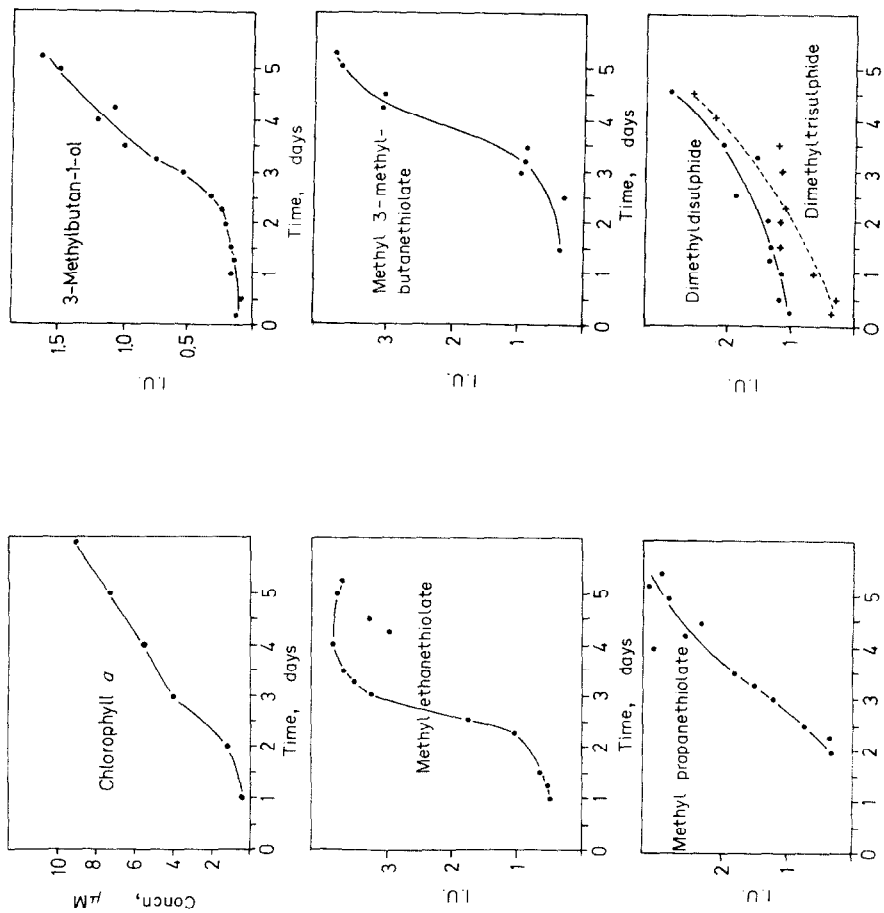

Fig. 1. Growth (chlorophyll *a* concentration) of *O. danica* in the light under microaerobic conditions and formation of volatile compounds (I.U. = integrator units).

Table 1. Volatile compounds excreted by *O. danica**

R_t (min)	Compound	Amount† (μg/l. suspension)
4.8	Butanone	tr‡
5.6	3-Methylbutan-2-one	tr
5.8	Methyl 2-methylpropanoate	tr
6.4	Pentan-2-one	tr
7.4	Methyl ethanethiolate	1160
7.6	Methyl 2-methylbutanoate	20
7.8	Methyl 3-methylbutanoate	20
8.1	Dimethyldisulphide	1520
8.7	2-Methylpropan-1-ol	tr
9.3	Methyl propanethiolate	130
10.0	Methyl 2-methylpropanethiolate	90
11.2	Heptan-2-one	tr
11.4	3-Methylbutan-1-ol	280
12.1	Methyl 3-methylbutanethiolate	10
13.4	2-Methylbut-2-en-1-ol	tr
14.3	Heptan-2-ol	tr
14.7	Dimethyltrisulphide	250
17.0	Oct-1-en-3-ol	tr
19.3	Octan-1-ol	tr
20.1	Oct-2-en-1-ol	tr
20.9	1, 2, 4-Trithiolate	tr
21.7	Dimethyltetrasulphide	tr

*The table lists only those compounds regarded as excretion products of the protozoon. Those compounds (e.g. xylene, toluene etc) which are ubiquitous pollution products are omitted.

†To determine absolute amounts, authentic substances were added to water samples in such concentrations as to give the same peak heights in a chromatogram as those isolated from the algal suspension.

‡tr, trace.

assumes the same origin of the acyl residues of the thioesters, valine may be the precursor of methyl 2-methylpropanoate, methyl 2-methylpropanethiolate and 2-methylpropan-1-ol; leucine of methyl 3-methylbutanoate, methyl 3-methylbutanethiolate and 3-methylbutan-1-ol, and isoleucine of the methyl 2-methylbutanoate present in trace amounts.

S-Methylthioacetate has been reported as an important aroma constituent of beers and wines [12] and cheese [13]. Strains of *Micrococcus* [14] and *Brevibacterium linens* [15] have been isolated that can synthesize this thioester. Although several *S*-methylthioalkylesters of longer chain length have been demonstrated in hop oil [16] and cheese, the organisms which produce these components have not been isolated.

O. danica was unable to use, in an exclusively fermentative way, the organic compounds present in the medium. Light was shown to be an essential requirement for the growth and the formation of all volatile products. A rapid uptake of the excreted volatile compounds was observed in the dark. It was most rapid for the thioesters and less for dimethyldisulphide and dimethyltrisulphide indicating that thioesters are good sources of reduced sulphur.

EXPERIMENTAL

Growth of algae. O. danica Pringsheim strain L 933-7 was obtained from the Sammlung von Algenkulturen des Pflanzenphysiologischen Instituts der Universität Göttingen and grown under axenic conditions at 27° and 1000 lx (fluorescent tube) in 300 ml Erlenmeyer flasks. A defined medium [17] was used from which NaCN and Tween 80 were omitted and 0.5 g L-histidine.HCl and 1 g trisodium citrate.$2H_2O$ added. 1 l. fermenters, each of which was stirred with a magnetic bar, were illuminated with six fluorescent tubes (9000 lx) and maintained at 28°.

Analysis of volatile organic compounds. 30 ml of algal suspension was removed under axenic conditions from the fermenter. The volatile compounds were stripped from the suspension for 15 min [18] after addition of 10.8 g NaCl, adsorbed on an odour trap, transferred thermically into a gas chromatograph and separated on a 25 m UCON 50 HB 5100 WCOT glass capillary column [4] with H_2 as the carrier gas. The same procedure and column were used for GC/MS (MAT 112 S), except He was used as the carrier gas and the temp. programme was run at 6°/min. Substances were identified by comparison of R_ts and mass fragmentation patterns with those of authentic substances. Integrator units were used for the quantitation of major compounds and peak heights for minor components.

Reference compounds. The methyl *O*-esters were synthesized from the corresponding chlorides with MeOH, the methyl *S*-esters from the chlorides with MeSH without solvent under ice cooling. GC/MS (80 eV) methyl ethanethiolate *m/z* (rel. int.): 43 (100), 90 $[M]^+$ (70), 47 (18), 45 (17), 42 (10), 48 (8), 46 (5), 75 (4); methyl propanethiolate: 57 (100), 104 $[M]^+$ (43), 47 (21), 45 (16), 75 (15), 48 (13), 46 (6), 61 (6), 56 (5), 58 (5); methyl 2-methylpropanethiolate: 43 (100), 41 (70), 71 (70), 48 (29), 118 $[M]^+$ (23), 39 (22), 42 (18), 47 (18), 45 (13), 75 (13), 49 (10); methyl 3-methylbutanethiolate: 57 (100), 85 (67), 41 (63), 43 (28), 75 (18), 39 (18), 47 (11), 132 $[M]^+$ (6), 117 (6). Dimethyltrisulphide was obtained from dimethyldisulphide and NaS [5].

Determination of chlorophyll a. Chlorophyll *a* was estimated from $A_{665\,nm}$ of an EtOH extract of the algae [19].

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