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The Influence of Low and High Oxygen Concentration on the Yield and Spectrum of Fatty Acids in *Porphyridium cruentum*

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Summary

The influence of O_2-concentration on the growth rate of the red alga *Porphyridium cruentum*, on the fatty acid spectrum, as well as on the content of the individual fatty acids was subject of investigations. The O_2-concentration was changed at different times of the batch cultivation. The influence of increased as well as of decreased O_2-concentrations was investigated.

It was evident that high oxygen concentration inhibited the biomass production. Nevertheless, this stress factor was connected with increasing EPA and AA contents as a short-time effect. This timely limited increase of EPA and AA was connected with a corresponding increase of the content of total fatty acids and at the expense of a decreasing content of palmitic acid. In the absence of oxygen the biomass production increased and the content of most of the fatty acids decreased for a short period of 6 h. After an adaption period of 24 h the fatty acid content increased also in this case. The effect of O_2-caused EPA increase is most evident if the oxygen concentration is increased on the 5th day of batch cultivation.

Key words: Porphyridium, arachidonic acid, biomass, eicosapentaenoic acid, fatty acids, oxygen.

Abbreviations: PUFA = polyunsaturated fatty acids; FA = fatty acids; AA = arachidonic acid; EPA = eicosapentaenoic acid; dm = dry matter.

Introduction

Many scientists are concerned with the investigation of the influence of cultivation conditions on the fatty acid composition of the red alga *Porphyridium cruentum*, Klyachko-Gurvich et al. (1985), (Cohen, 1990), Springer et al. (1994). This is due to the high pharmacological value of PUFA and the possibility of using this culture in biotechnology as producer of fatty acids, polysaccharides and phycobiliproteins. Despite some minor differences in the fatty acid content, which may be explained by the use of various strains and the application of different methods, the problem may be regarded as well known.

Beside cultivation conditions such as light, temperature, etc., oxygen plays an important role in numerous processes of cell metabolism, participating in the lipid exchange, i.e. in desaturation of fatty acids (Mazliak, 1994) and in autoxidation, Watanabe et al. (1993). Further, oxygen is involved in the formation and functioning of the photosynthetic cell apparatus (Geider and Osborne, 1992), (Akiyev and Tsoglin, 1992). Our previous work was concerned with the investigation of the gas exchange during the cultivation of microalgae at different oxygen conditions. The oxygen concentration proved to be of influence on the synthesis of important vital compounds, such as proteins, chlorophylls and lipids, Akiyev et al. (1994). It is natural to expect that different concentrations of O_2, dissolved in the nutritive solution, may influence the lipid and fatty acid synthesis in *Porphyridium* which is of

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interest to the mass cultivation of this alga as a source of PUFA. Subject of this work was the investigation of the effect of changes in oxygen concentrations on the production of biomass in different periods of cultivation as well as on the fatty acid synthesis in the unicellular red alga *Porphyridium cruentum*.

Material and Methods

The investigations were performed on the unicellular red alga *Porphyridium cruentum*, strain 1932/107, of the collection of the Institute for Cereal Processing, Ltd., Germany. The algae were cultivated on a modified Jones medium, Jones et al. (1963) in the 2 L laboratory reactor, type Braun Biostat MD, Germany.

Cultivation temperature was at 25 °C lighting was with day light lamps at an intensity of 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$, using a batch cultivation method.

Different gas regimes were used for the aeration of the suspension:

a) Control: air during the whole experiment;
b) Treatment 1: oxygen-free, on the 2nd day air was substituted for nitrogen;
c) Treatment 2: high oxygen, 3rd day increase of O_2-concentration on the 3rd day to approx. 70 %; and
d) Treatment 3: high oxygen, 5th day increase of O_2-concentration on the 5th day to approx. 70 %.

In the control as well as in treatments 1–3 the CO_2-concentration amounts to 2 %.

The oxygen partial pressure was measured with a Clark electrode (Ingold). The O_2-concentration amounted to 21 % in the control and to 4–6 % in treatment 1, which obviously is due to the photosynthetic activity of the cells.

The initial density of the suspension in all the variants amounted to approx. 0.4 $g L^{-1}$ (dm).

The biomass dry matter was measured gravimetrically. The lipids were extracted by mechanical disruption in chloroform/methanol in a Retsch swing-mill; the further procedure was as already described

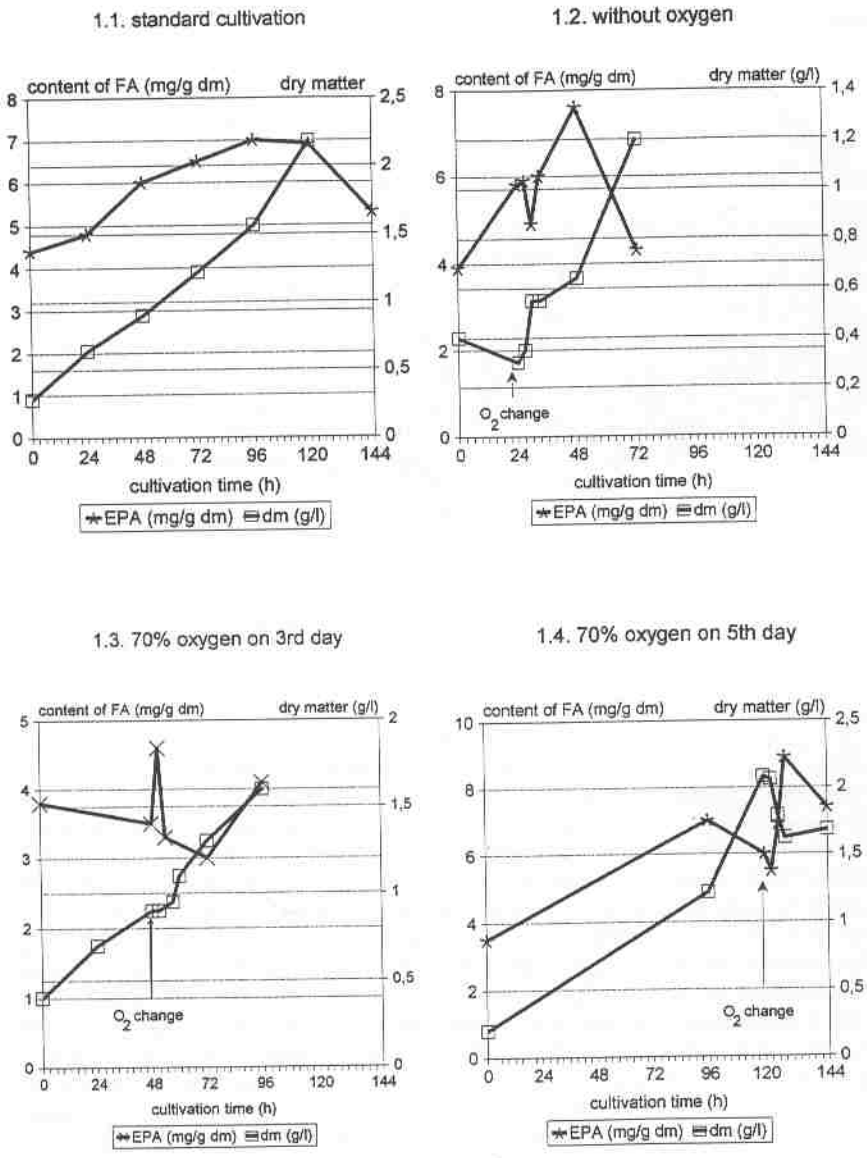


Fig. 1: The influence of oxygen on the yield of biomass and EPA (1.1.: standard cultivation, 1.2.: without oxygen, 1.3.: 70 % oxygen on 3rd day, 1.4.: 70 % oxygen on 5th day).

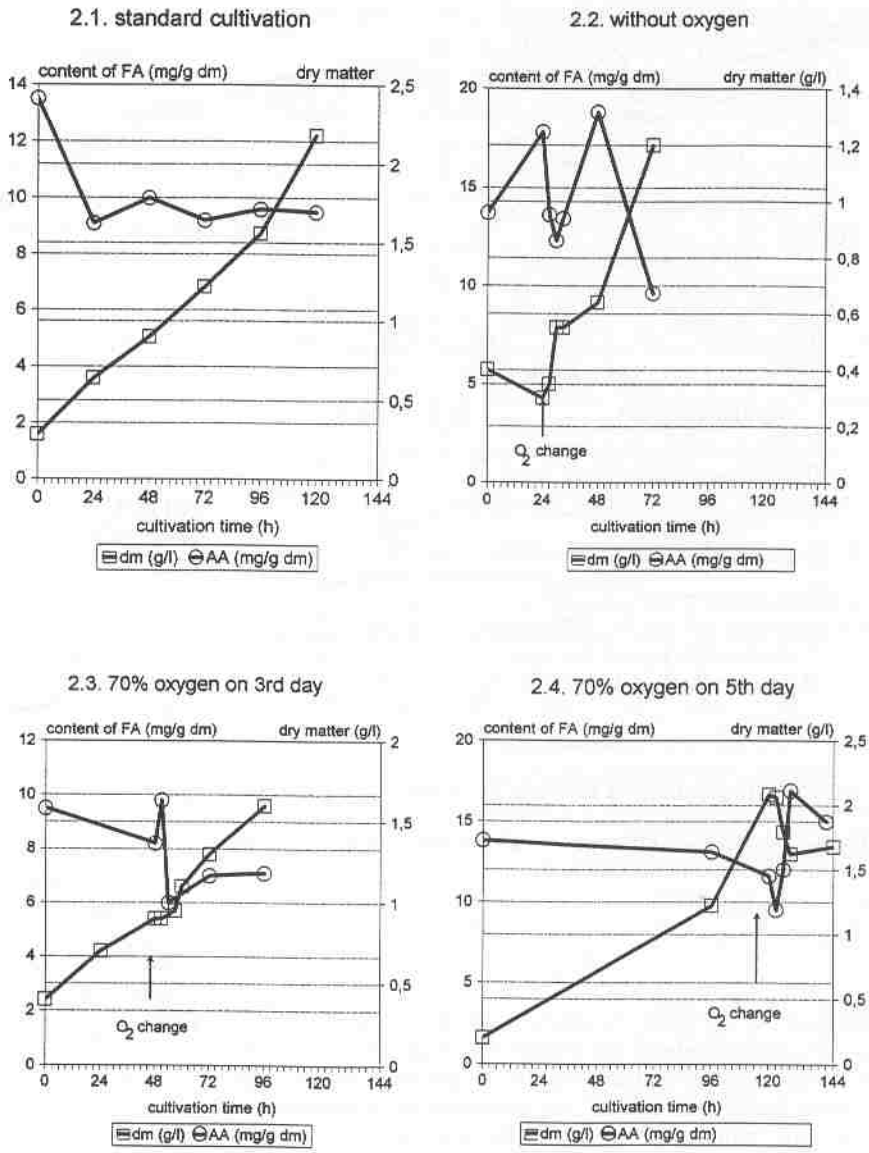


Fig. 2: The influence of oxygen on the yield of biomass and AA (2.1.: standard cultivation, 2.2.: without oxygen, 2.3.: 70 % oxygen on 3rd day, 2.4.: 70 % oxygen on 5th day).

Table 1: Fatty acid content of *Porphyridium cruentum* at standard cultivation conditions.

Fatty acids		mg g^{-1} dm	%/total FA
16:0	palmitic	14.4	39.1
16:1	palmitoleic	tr.	tr.
18:0	stearic	0.3	1.3
18:1	oleic	1.4	3.8
18:2n6	linoleic	2.3	6.3
20:3	eicosatrionic	tr.	tr.
20:4	arachidonic	10.0	28.2
20:5	eicosapentaenoic	7.0	19.5

in Springer et al. (1994). The qualitative and quantitative contents of fatty acids were determined by GLC of methyl-esters in a gas-chromatograph (Hewlett-Packard). The statistical analysis of the method of lipid determination resulted in a standard deviation of about 0.3.

Results

Changes of the biomass dry weight and fatty acid content and spectrum of *Porphyridium cruentum* were investigated. Table 1 shows that palmitic, linoleic, arachidonic and eicosapentaenoic acids predominate in this alga. At standard cultivation conditions biomass increases during the whole batch cultivation time of 5 days. On the 5th day the optimal EPA concentration was achieved (Fig. 1.1.) whereas the highest AA concentration was recorded at the beginning of the cultivation (Fig. 2.1.).

Figure 1. (1.1.–1.4.) shows that the growth rate of biomass was nearly uniform in all variants, amounting to approx. 0.35 g L^{-1} d^{-1}. The dry weight of biomass increased from 0.4 g L^{-1} to 2.0–2.2 g L^{-1}. The change-over to oxygen-free cultivation conditions after an adaptation phase of 3 h accelerates the growth. The highest growth acceleration is attained 6 to 9 h after the reduction of the concentration of dissolved

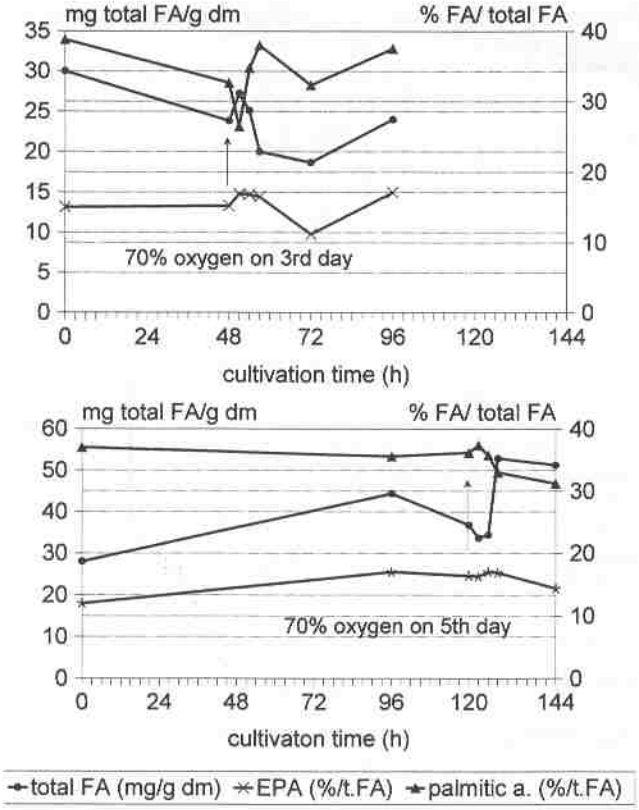


Fig. 3: The influence of oxygen on the content of total fatty acids and the relation EPA : palmitic acid.

oxygen (Fig. 1.2.). On the other hand, an increase of O_2-concentration will reduces the growth rate. The extent of growth retardation by O_2-increase depends on the time of O_2-change during batch cultivation. If O_2-concentration is increased in the early exponential growth phase (treatment 2, 3rd day) there is only insignificant growth retardation (Fig. 1.3.). If O_2-concentration is increased in the late exponential growth phase (treatment 3, 5th day) growth is retarded significantly (Fig. 1.4.).

For the evaluation of the influence on fatty acid synthesis the EPA-content is illustrated in Fig. 1 and the AA-content in Fig. 2. At reduced O_2-concentration the general trend of increased biomass production and the short-time effect (up to 6 h) of a decrease of the total fatty acid content as well as the content of EPA and AA is observed. After an adaptation period of about 24 h the total fatty acids exceeded their initial level, and especially the content of EPA increased.

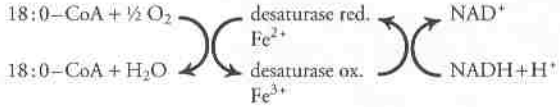
In the other case an increase of the oxygen content caused a retardation of biomass production (Figs. 1.3. and 1.4.) and an increase of total fatty acids, EPA and AA.

These adaptation processes are demonstrated even more clearly in the case of changing over to increased oxygen concentrations in 5 days after the beginning of cultivation. The biomass production is reduced from 2.1 to 1.6 g/L and was nearly constant during 24 h. The total concentration of fatty acids at the start of change of O_2-concentration decreased insignificantly, and already after 6 h the quantity of fatty acids exceeded the initial concentration and achieved a level of 53 mg g^{-1} dm. This high level of fatty acid content was constant during 24 h.

The trend of the change of the acid concentrations 20 : 4 and 20 : 5 is analogous to the changes of the total fatty acid content of *Porphyridium cruentum* in each variant, but simultaneously at the expense of decrease of palmitic acid on the other side (Fig. 3). The concentration of AA increased from 8.2 to 9.8 mg g^{-1} during 2 h in variant 2, and from 9.5 to 16.8 mg g^{-1} during 6 h in variant 3. The concentration of EPA increased from 3.5 to 4.6 and from 5.5 to 8.9 mg g^{-1}, respectively. As may be seen from the figures, the highest rate of formation of 20 : 4 and 20 : 5 acids as short-time effect (9 h) was observed at an increase of the O_2-concentration, 5 days after the beginning of cultivation. However, high AA- and EPA-contents also are achieved after a 24 h adaptation to a reduced oxygen content.

Discussion

It was of interest to study the changes of algal lipids at different O_2-concentrations. High oxygen in our case has the effect that the quantity of the total fatty acids is increased during a short period after the change of O_2-concentration. The quantity of 20 : 4 and 20 : 5 acids increases after 3 h in variant 2 and after 6 to 9 h in variant 3. During this time, the concentration of palmitic acid, one of the precursors in the synthesis chain of PUFA, decreased (Fig. 3). This may be explained by the involvement of O_2 in the desaturation of fatty acids. O_2 is used as co-factor of this reaction, as well as $NADH_2$ and $NADPH_2$. Desaturases catalyze the following reactions (Voet and Voet, 1992):



In the presence of oxygen, stearyl-CoA is desaturated to oleyl-CoA. In our experiments we observed a reduction of the total quantity of fatty acids immediately after having increased the oxygen concentration and a subsequent increase.

During the work with *Chlorella* Akyiev et al. (1994) observed that under the influence of low oxygen the total quantity of fatty acids increases after 2 h, compared with the control treatment (21 % O_2) in *Chlorella*. This obviously is due to the fact that *Chlorella* cells have a higher range of photosynthesis and oxygen evolution as compared with *Porphyridium cruentum*. It produced sufficiently high intracellular O_2-concentration to maintain biochemical reactions. The reduction of the oxygen concentration of the cultivation medium probably has activated the photosynthesis and oxygen evolution rates. As a result of this, synthesis was increased to its initial level.

These changes are expressed more clearly in treatment 3, which may be explained by the fact that the retardation of the growth rate by increased O_2-concentration 5 days after

the beginning of cultivation will practically stop the biomass production, and the increased fatty acid synthesis may be connected with the changes to lipid accumulation. At the same time, high energy losses of the cells are compensated by decreasing biomass production and by the promotion of desaturation.

On the basis of the results achieved it is possible to conclude that changes in the gas content of the cultivation medium of *Porphyridium cruentum* may be utilized in the mass cultivation of this red alga to increase PUFA production.

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