The production of hydrocarbons by *Botryococcus braunii*

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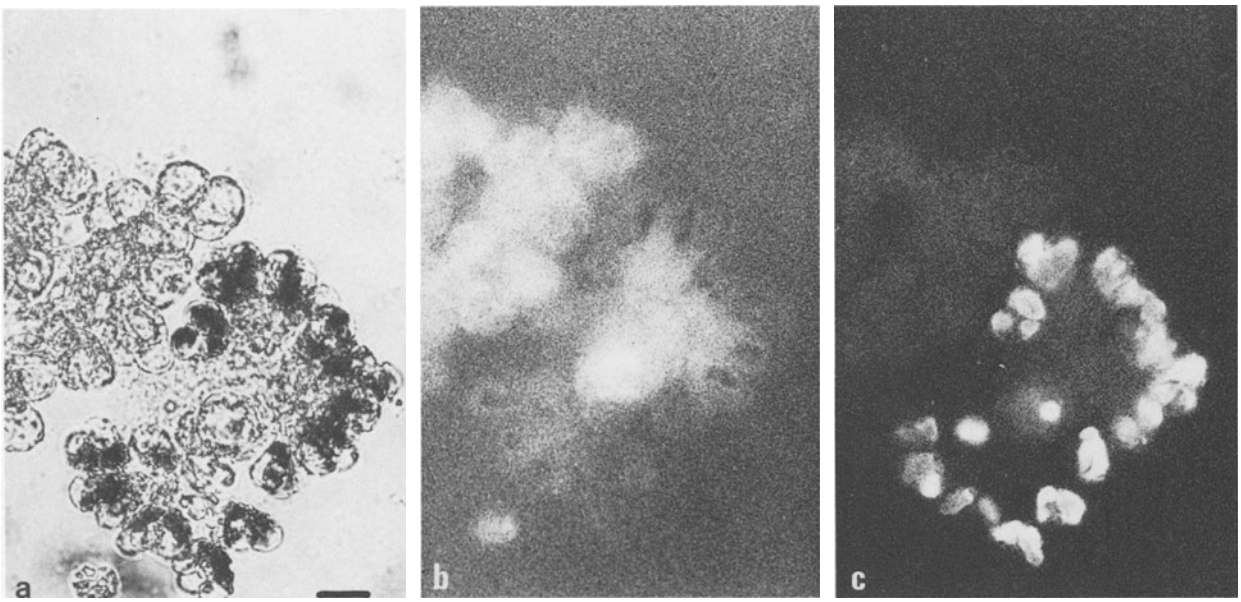
As Tornabene[1,2] has already discussed in this and in an earlier volume, many microorganisms - photosynthetic or not - are capable of producing hydrocarbons which can account for up to 1% of the dry mass (see tables I–V in ref. 1). At the moment, it seems that there is only 1 exception described in the literature which produces a larger proportion: the unicellular green alga *Botryococcus braunii.*

Botryococcus braunii was first described some 130 years ago by Kützing[3]. The alga is found widely, in fresh water lakes as well as in brackish water on all continents. In some rare cases water blooms of *Botryococcus* are noted[4], the algae float on the surface on the lake and form combustible sediments on the shore. Such sediments are regarded as the origin of the boghead coals and tar-like deposits found in different locations, known as torbanite, coorongite or balkaschite[5–9].

Botryococcus is unicellular, but the cells form aggregates of up to 0.5 mm in diameter. The single cells are embedded in a gelatinous mass containing oils and carotenoids[10]. This is seen in the different fluorescence properties of the chloroplast within the cells and the carotenoids in the gel between them (figure). 3 different growth states are known[11]. During exponential growth in cultures the alga is green having the chlorophylls a and b, and a hydrocarbon content of around 20%[11–15]. In algal blooms however, the cells change to a resting state of yellow-orange color due to massive accumulation of carotenoids. At the same time the lipid composition is drastically altered, the unsaponifiable lipids increase to up to 80% of the dry weight[11,16]. There is also a shift in chain length of the hydrocarbons produced from C_{27} to C_{31} during growth to mainly C_{34} (botryococcene and isobotryococcene) in the yellow state (tables 1 and 2)[17]. Furthermore, a green resting state exists in which the hydrocarbon production is very low, yielding less than 1% of the dry weight of the cells.

Growth in laboratory cultures as well as in natural environments is rather slow, with generation times of roughly 1 week[4,12]. Recently generation times of 2 days have been obtained in laboratory cultures[18].

With electron microscopy it can be seen that oil droplets accumulate predominantely outside the cell walls[14], containing around 95% of the hydrocarbons of the cells. Yet oil droplets are also found within the cytoplasm. Feeding experiments with radioactive precursors demonstrate that the 2 pools are not in equilibrium. The bulk of the hydrocarbons seems to be produced at the outer wall[19,20]. Interestingly enough the cells are not able to metabolize the stored hydrocarbons[19]. Both pools have a similar composition of hydrocarbons; however, in the internal pool



Micrographs of a colony of *Botryococcus braunii;* bar indicates 10 μm (photos made by Dr W. Egger). *a* in blue light: the light absorbing chloroplasts appear dark; *b* fluorescence visible through an optical filter transmitting green light after irradiation with light of 366 nm. The light areas indicate the fluorescence of the carotenoids which are solubilized in the hydrocarbons outside the cells; *c* fluorescence visible through an optical filter transmitting red light after irradiation with light of 366 nm. The light areas indicate the fluorescence of chlorophyll which gives an identical image as in *a.*

C_{27} and C_{29} chains are accumulated predominantely, in the external pool C_{29} and C_{31}. The C_{34} forms of the yellow resting state are not found during growth[14]. Unfortunately nothing is known about the factors responsible for the shift from the green growth state to the yellow resting state and so far this change has never been observed under laboratory conditions[12].

Table 1. Hydrocarbons from *Botryococcus braunii*[9]

Hydrocarbon	Gelpi et al.[13]	Maxwell et al.[15]	Brown et al.[11]	Belcher[12]
$C_{17}H_{34}$	1.52	–	–	–
$C_{23}H_{46}$	0.14	–	–	–
$C_{25}H_{46}$	0.10	–	–	–
$C_{25}H_{48}$	0.65	–	–	–
$C_{27}H_{52}$	11.10	–	7.2	–
$C_{28}H_{54}$	0.65	–	–	–
$C_{29}H_{54}$	5.54	–	23.0	–
$C_{29}H_{56}$	50.40	–	32.6	–
$C_{31}H_{60}$	27.90	–	25.1	–
$C_{34}H_{58}$	–	83.5	–	–
$C_{34}H_{58}$	–	83.5	–	–
$C_{34}H_{58}$ (iso)	–	8.2	–	–
other oils	–	8.3	12.1	–
Total hydrocarbons of dry weight	0.3%	75%	to 17%	15-22%
Source of alga	Laboratory culture 1-2 weeks	Wild from Oakmere Cheshire	Laboratory culture	Laboratory culture 16 weeks
State of algal development	Exponential green	Algal bloom resting state	Exponential green	Resting state green

Table 2. Dominant hydrocarbons from *Botryococcus*[9,15]

1 Heptacosa-1,18-dien ($C_{27}H_{52}$);
$CH_2=CH-(CH_2)_{15}-CG=CH-(CH_2)_7-CH_3$

2 Nonacosa-1,20-dien ($C_{29}H_{56}$);
$CH_2=CH-(CH_2)_{17}-CH=CH\cdot(CH_2)_7-CH_3$

3 Hentaconta-1,22-dien ($C_{31}H_{60}$);
$CH_2=CH-(CH_2)_{19}-CH=CH-(CH_2)_7-CH_3$

4 Botryococcen

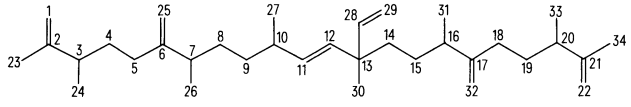


Table 3. Change of lipid content of algal cells by environmental manipulation (from Dubinsky et al.[21])

Environmental variable	Organisms	Variation	Change in lipid composition (in % of dry wt)
Light intensity	*Spirulina*	10 → 40 klux	4.2 → 6.2%
Temperature	*Ochromonas*	15 → 30 °C	39 → 53
Nitrogen depletion	*Chlorella*	With → without	10 → 70
Salinity	*Botryococcus*	0 → 6% NaCl	36 → 51
Senescence	*Cyanobacterium* strain 92	Young → old	9 → 26
Combination of factors	*Chlorella*	Normal → stress	4.5 → 86

Several environmental changes are known, however, to affect the lipid composition in the cell and to induce lipid synthesis in general. As seen in table 3, a variation of factors such as light intensity, temperature and salinity increase the lipid content in various algae tested so far[21].

The yields in hydrocarbons calculated from the water bloom in Oakmere[4] would amount to about 2.6 t/ha · year[9], a value similar to yields for terrestrial plants synthesizing hydrocarbons. If the growth rate of *Botryococcus* could be increased to the values obtained from *Chlorella* and *Scenedesmus*, hydrocarbon yield could be 10–15 times higher[9]. Studies on the growth and physiology of *Botryococcus* may lead to great improvements.

It seems rather unlikely that *Botryococcus* is the only organism with such a high hydrocarbon content. In screening other photosynthetic microorganisms producing hydrocarbons, species with similar capabilities as *Botryococcus* may be found. The first positive results in this direction have been reported by Lien[22] and Mitsui (personal communication).

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Glycerol production by *Dunaliella*

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Summary. Species of the unicellular alga *Dunaliella* possess outstanding tolerance of a wide range of salinities. They can adapt to grow in salt media which range from less than 0.5 M to saturated salt solutions and withstand enormous osmotic shocks through a unique osmotic adaptation. The osmoregulating mechanism depends on photosynthetic production of glycerol, whose intracellular concentration varies in direct proportion to the extracellular salt concentration and reaches values in excess of 50% of the total dry weight of the cells. *Dunaliella,* and another halotolerant glycerol producing alga, *Asteromonas gracilis,* osmoregulate biochemically by controlling glycerol biosynthesis and degradation. 3 new enzymes, NADPH-dihydroxyacetone-reductase, dihydroxyacetone kinase and glycerol-1-phosphatase seem to be involved in the osmoregulatory response via glycerol in *Dunaliella* and *Asteromonas.* A hypothetical scheme of glycerol metabolism in these algae utilizing these enzymes is presented. Growth studies of *Dunaliella* indoors and outdoors showed that salt concentrations favoring maximal glycerol productivity are not identical with those required for maximal algal productivity. Maximal yield of glycerol occurred around 2 M NaCl while maximal algal productivity occurred below 0.5 M NaCl. Observed yields of glycerol in *Dunaliella* culture outdoors are compared with theoretically calculated maximal yield.

1. *Introduction*

Utilization of the photosynthetic machinery for the production of energy, chemicals and food has a particular appeal because it is the most abundant energy storing and life-supporting process on earth. Starting with the photosynthetic reaction converting carbon dioxide and water into organic carbon and oxygen with solar irradiation as the energy source, photosynthetic plants and algae utilize intricate biochemical pathways to produce a variety of organic metabolites. Serveral potential crops have been suggested in recent years as possible candidates for converting solar energy via photosynthesis into biofuels and/or valuable organic compounds[1]. These include fast-growing tree species grown at high densities, conventional crops such as corn, sugar beet, sugarcane, and plants native to arid environments. Though each has its own specific advantages and disadvantages, there are several drawbacks common to almost all terrestrial plants, such as low solar conversion efficiency due to reflection and partial absorption, storage capacity limitations, investment of much of the photosynthate in nonrecoverable parts of the plant, competition with high economic value agricultural land and a high consumptive use of fresh water. The halotolerant unialga *Dunaliella*[2] has only a few of the drawbacks and in addition offers advantages not found in the other systems. It can be grown in a population density resulting in the presentation of an optimum absorbing surface area-to-unit land area ratio throughout the year. It can grow in salt water on arid land where there is maximum availability of solar energy and where the land is not utilizable for any other kind of potential crop. Lacking a typical polysaccharide cell wall, *Dunaliella* invests a much smaller fraction of the photosynthetic products in difficult to utilize structural constituents than do other algae and plants. Most significantly, the major photosynthetic end product in *Dunaliella* is glycerol, the concentration of which varies in direct proportion to the extracellular salt concentration reaching a maximum of around 80% of the algal dry weight[3,4]. The use of *Dunaliella* for direct conversion of solar energy into a useful chemical product is therefore of particular interest. Also of interest are the rather unique metabolic pathways which exist in this alga, permitting the synthesis and regulation of the massive intracellular concentrations of glycerol. The purpose of this manuscript is to examine the basic biochemistry of glycerol production and regulation in *Dunaliella* and to describe indoor and outdoor ex-