THE CHLOROCOCCALEAN ALGA *BOTRYOCOCCUS* AND ITS SIGNIFICANCE IN HYDROCARBON EXPLORATION

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Abstract. The chlorococcalean (Dictyosphaeriaceae), "oil-forming" alga *Botryococcus* and the significance it bears in the exploration process of various hydrocarbon types are considered. Morphological structure and characteristics of both the living and the fossil specimens, as well as the ecological requirements – as they all control the chain of hydrocarbon-forming mechanisms – are discussed. A concise review of its typical forms (physiological states), relative to the basic relationships with the corresponding hydrocarbons are presented.

Key words: Palynology, Chlorococcales, *Botryococcus*, morphology, (paleo)ecology, hydrocarbons

1. INTRODUCTION

The colonial alga *Botryococcus* has aroused interest for many years now due to its astonishing capability to synthesize different types of hydrocarbon compounds and, by doing so, having the essential precursory role in contributing to the formation of distinctive hydrocarbon categories.

Through time, there were quite some attempts to put it in a certain place inside the Plant Kingdom which it belongs to and this mainly because of its very peculiar status as compared with other fresh- and/or brackish water algae (Frémy and Dangeard, 1938; Kiss, 1939; Traverse1955; Chadfaud and Emberger, 1960; Gray, 1960; Nagy, 1967; Combaz, 1980; Pop *et al.*, 1983; Wingate, 1983; Alpern, 1987; Glikson *et al.*, 1989;).

Morphological and (paleo)ecological research, as well as experimental investigations tried to explain the morphological changes as response to specific modifications of the host habitat conditions, plus have been approached too (e. g., Blakburn, 1936; Temperly, 1936; Round, 1965; Potonié and Rehnelt, 1971; Cane, 1976; Tappan, 1980; Wake and Hillen, 1981; Wake, 1983; Kedves, 1986 a, 1988; Kelts, 1988; Guy-Ohlsson, 1992, Vér, 1994; Demetrescu, *in press*).

The basic relationships between different stages of morphological development of *Botryococcus* and the nature of resulted hydrocarbon products were also investigared (Brown, 1969; Douglas et al., 1969; Anders and Robinson, 1971; Demetrescu, *in press*).

In this study, the discussed specimens are assigned to Chlorophyta, Chlorococcales, Dictyosphaeriaceae, *Botryococcus* Kützing 1849, as treated in Pop *et al.* (1983).

2. MATERIAL AND METHOD

The material used in this study has been collected over the years and analyses have been performed during a series of laboratory stages as required by a particular theme that was in progress at a given time.

It consists mostly of dark grey to dark brown clay and coaly clay interbedded in the Pliocene coal-bearing sequences developed along the South Carpathians Depression. An important quantity came from the Miocene sediments of the Black Sea, offshore Romania.

The laboratory treatment followed the standard palynological procedure involving maceration with HCL 30% and HF 45%. Where necessary to put in evidence the humic content of certain samples, to emphasize some specific relationships, which *Botryococcus* shows with such chemical compounds, KOH 5% has been considered too, and the data processing used the Humic Concentration scale (HCS) (Demetrescu, *in press*).

3. MORPHOLOGIC STRUCTURE AND CHEMICAL CHARACTERISTICS

Blakburn (1936) was the first who has approached the morphologic background and has given a schematic view of this chlorococcalean alga. The basic structural elements are shown in Fig. 1; from base to top they are: pedunculus, cupula and mucilage.

Two or more branches form the pedunculus, each branch including one or two pairs of individuals whose growth took place during one season of development. The mother cell is caught in a cuticle formed of two parts: the cuticle of the mother cell, which has a basal position and emerges from each branch of the pedunculus (namely the "polypier" of Chadefaud and Emberger, 1960), and the cuticle of each individual cell developed inside the cupulae. Each cell contains several protoplasmic organelles such as nucleus, oil drops, starch, plastids, pigments, and other cytoplasm constituents, and has the capacity of storing kerogen. It appears that the mucilaginous structure representing the hydrocarbon matrix is *bi-functional* (*Fig. 2*). As an enveloping stratum it plays a protective role to the outer part of the colony branches, whereas in the form of extended strings it helps to link simple colonies to one another and produce a compound colony, either branched or unbranched. Cellulose plus pectinous

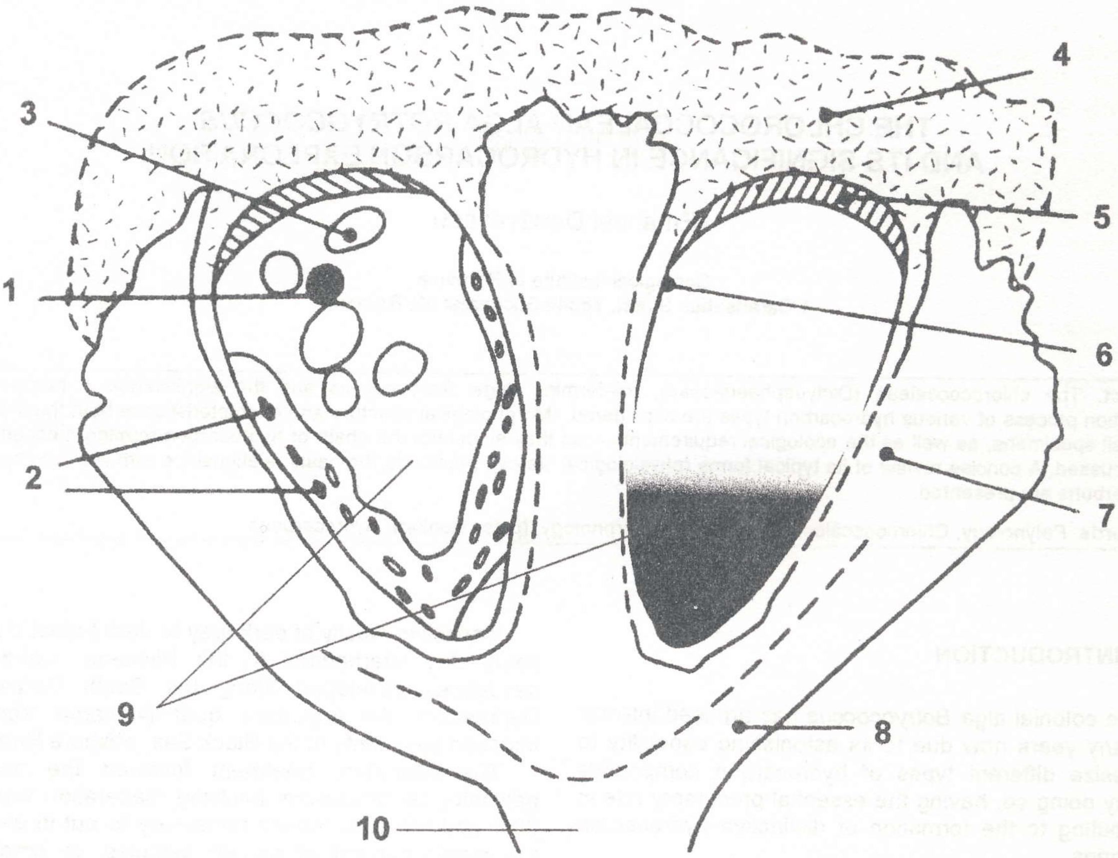

Fig. 1. Botryococcus branch of a simple colony: longitudinal section (modified after Blakburn, 1936). (1)nucleus; (2) oil drops; (3) starch; (4) hydrocarbon matrix (mostly white hyaline amorphous carbohydrates); (5) cell cap made up of cellulose and pectinoid substances; (6) cellulosic wall; (7) cuticle; (8) cuticle of the mother cell; (9) kerogen; (10) part of the proximal region of pedunculus.

substances form the cellular wall and confer the colony a high resistance to decay.

The alga dimensions range from several microns (usually 5 μm to 12 μm in the case of unicellular stage), to one millimeter for mature colony (commonly the colonies range between 40 and 100 μm). The main colour is brown but it changes to bright green in response both to seasonal changes – that may modify the light intensity and nitrate content – and age plus the reproductive stage of the colony (Guy-Ohlsson, 1992).

4. REQUIRED DEVELOPMENTAL CONDITIONS AND SEDIMENTARY CONTROLS

The habitat in which *Botryococcus* accommodates and develops is controlled essentially by climatic and depositional factors that co-operate with one another to enable a successful growth of the alga.

Inland, shallow and oxygenated freshwater lakes, ponds, pools or slowmoving waters plus ditches, bogs and puddle or plashy sites accompanied by a wide spectrum of climatic conditions through the year (Guy-Ohlsson, 1992) appear as the most common settings and surrounding influences, which together, strongly contribute to *Botryococcus* development. It may inhabit oligo- to mesotrophic waters with various pH (Wake and Hillen, 1981), but the most adequate is a eutrophic medium and slight acidic pH.

The salinity of most of the freshwater lakes is less than 5‰ (normally less than 3‰), unless more concentrated solutions are introduced from outside the lake. This value represents the apparent threshold of salinity tolerance of most fresh-water aquatic microorganisms (Collinson, 1978 b). It is considered that this limit may also be characteristic of deeper lakes where a low salinity level can be maintained by escape of more concentrated water as ground water through surrounding permeable soils. As an effect of reversed mechanism, for instance, salt water influxes entering the freshwater body through the bottom floor of the respective habitat (which increases the salinity of depositional environment beyond the tolerance level), a regression in the development of this alga may be inflicted, regardless the particular stage of growth that has to face this change. Conversely, a freshening of even slight saline ambience by high rainfall may trigger significant blooms (Cane, 1976). All these suggest that when found in sediments of marine environment (as a constitutive element of an assemblage dominated by marine palynomorphs), this clearly indicates an influx of freshwater inside the marine depositional setting, or re-deposition.

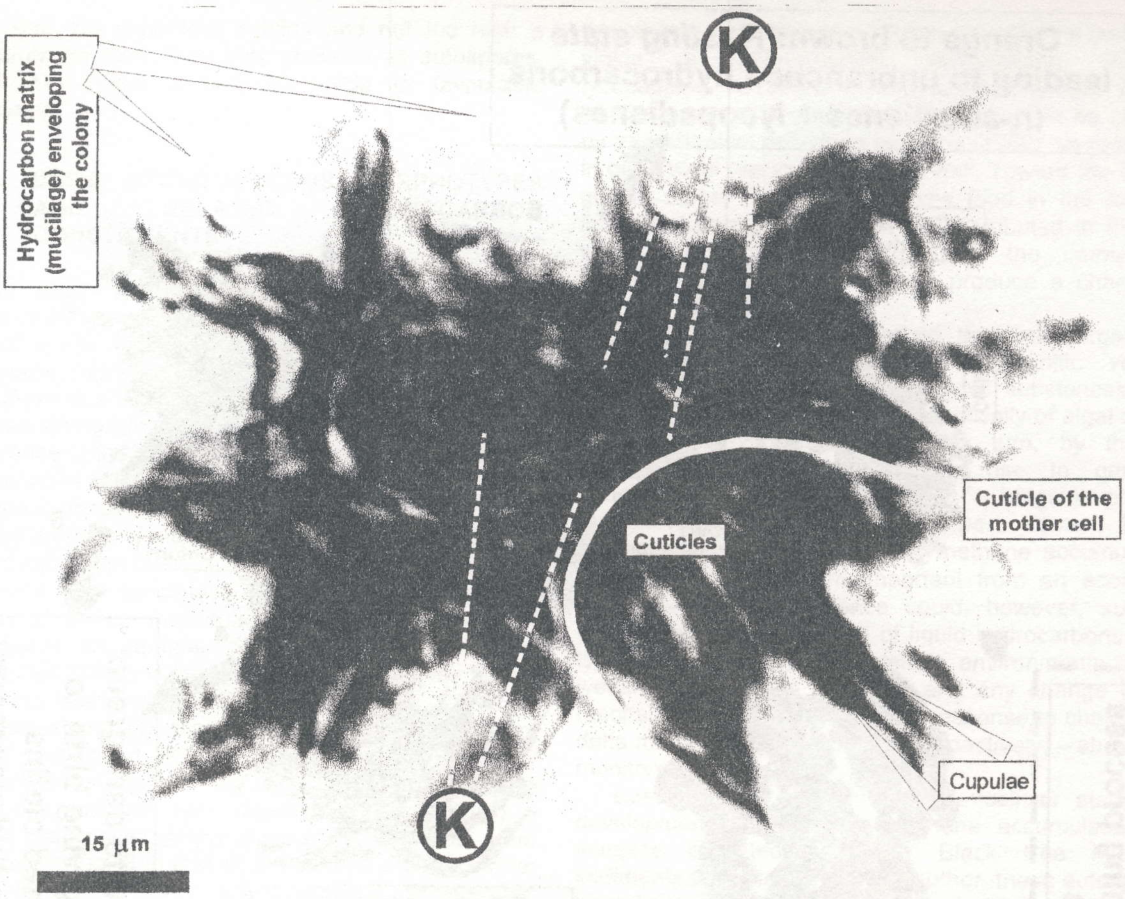

Fig. 2 Polar view of simple colony shown in Fig. 3/5; at high focus kerogen becomes visible toward the of colony (K)

The way different types reflecting various developmental stages appear suggests significant climatic and/or sedimentogenetic changes. Any change in the environment conditions, such as cold, moist conditions or, on the contrary, dry and hot conditions, may be related to corresponding modifications in form shown by the living alga (Blakburn, 1936).

The input of siliciclastic fraction by increased sedimentation rate (due to an accentuated fluvial erosion, or a significant inflow of waters carrying high amounts of humic substances), may drastically diminish or even interrupt a given stage of development (Demetrescu, *in press*). Although a humics forerunner at one given point of growth, *Botryococcus* cannot stand a dystrophic ambience and has never been found in sediments rich in humic compounds (*ibid.*)

It is quite obvious that under certain circumstances, different developmental stages may be related to certain sediments, their morphostructural status being strikingly influenced by specific sedimentary controls.

Well-preserved colonial specimens always reflect settling in a protected depositional site with calm waters and minimal climatic variations. When a given factor (such as wind, for instance) disturbs the habitat equilibrium, the colonies sink and reach again the water surface only after the calm status is restored.

Such conditions should last along the whole period during which the differing stages resulting from one another will complete the chain of morphologic developments and should be followed – after deposition – by rapid burial to avoid decomposition by microbial/bacterial activity. If the colonies are found in samples characterized by high quantities of amorphous organic matter (particularly of aquatic origin), this indicates thet the accumulation process took place in dysoxic-anoxic conditions.

During an entire developmental cycle the differing stages may occur together ranging from the unicellular stage to skeleton matrix, or even completely degraded, structureless and fluffy mass. This happens in cases when all basic morphotypes are found in one and the same sample, indicating that a series of seasonal changes have influenced their life cycle prior to the depositional process, as well as during the interval of time when this process was in progress. When, conversely, any of these morphotypes is characteristic of a particular sedimentary level, then each reflects specific depositional ambience at the time of deposition as shown in Fig. 3.

Deceased colonies, particularly complex aggregates made up of compound ones, may remain longer in a floating state and be displaced by wave or wind action toward the shoreline where they may accumulate in significant amounts (Kelts, 1988), this way participating to the formation of hydrocarbon source sediments.

It is worth mentioning that individual cells detach themselves of the lifeless colonies, and thereafter sink and settle on the bottom floor. If proper burial conditions

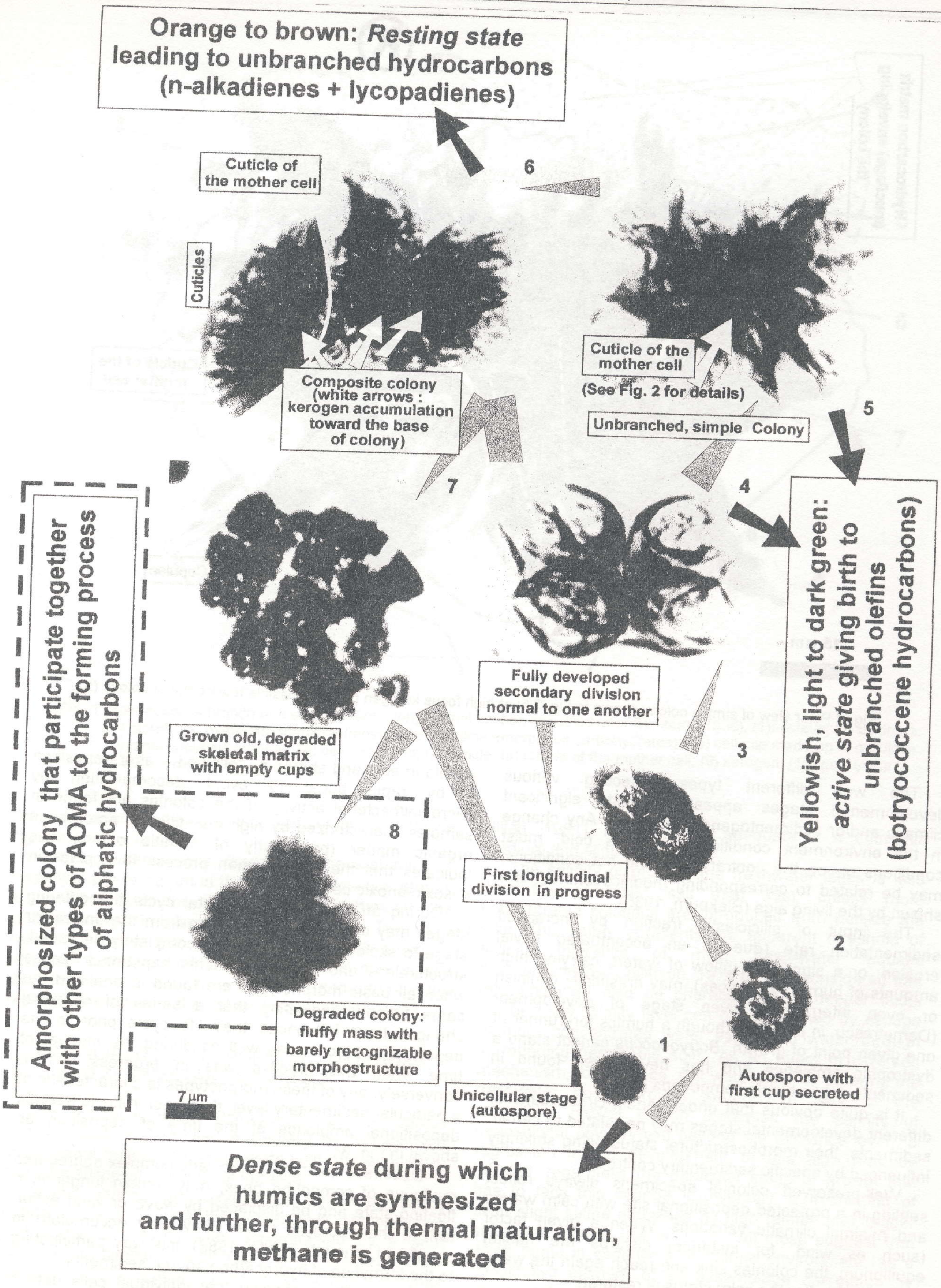

Fig. 3 *Botryococcus* physiological stages (or races) from 1 trough 7 and the corresponding hydrocarbon categories. The particular amorphous state is illustrated in sequences 8 (see text for details)

occur (not too superficial settling and not too high a sedimentation rate), they may preserve as autospores, which may begin a new life cycle in favourable conditions.

5. BASIC *BOTRYOCOCCUS* MORPHOTYPES AND RELATED HYDROCARBON CONSTITUENTS

The chain of *Botryococcus* main morphological stages of development is shown in Fig. 3. The starting point of a new life cycle begins with the moment the autospores representing the unicellular stage restore themselves due to new adequate conditions, consisting of a freshening ambience, specifically characterized by an increase of the oxygenation level. The autospores are dark brown in colour, of about 7 μm in size, and possess a compact, dense structure.

The process of morphologic growth is firstly tributary to the mechanism of secreting a cellulosic and pectinous membrane that constitutes the primary cup (primeval cupula) of a new individual of the future colony. By subsequent longitudinal divisions, a new, simple, unbranched colony is formed. Such morphotypes further give birth to compound colonies that may gather to constitute eventually a complex aggregate made up of several compound colonies tied to one another by mucilaginous strings. In an advanced stage, the alga grows old and the cells disperse leaving behind a skeletal matrix with empty cupulae. A particular state illustrated in Fig. 3 is that of amorphous organic matter of aquatic origin (AOMA), which suggests strong anoxic environment at the sediment-water interface and an extremely low value or total depletion of oxygen within the few meters of the overlying water column.

Beside differentiate causes which control the process of oil formation, the physiological state of living alga may also control the hydrocarbon nature (Brown *et al.*, 1969).

Various developmental stages show distinctive affinity to certain sediments and are controlled by certain environmental factors and, in turn, are responsible for producing different hydrocarbon constituents. Brown *et al.*, (1969) have assumed that three different "physiological states" are present in the modern *B. braunii*, two of them being known to produce various amounts of hydrocarbons: the green (active state), that yields about 32% (by weight) hydrocarbons, and the orange-brown (resting), which produces up to 75% hydrocarbons. However, the highest weight percentage of aliphatic biomacromolecules (up to 12%), related to total algal biomass, is that found in *Botryococcus* (Dubreuil *et al.*, 1989). Furthermore, it has also been thought that the green cells may produce unbranched, saturated olefins whereas during the resting stage the colonies produce branched, unsaturated hydrocarbons. The branching mechanism was initially considered as being tributary to the salinity level (Temperly, 1936). Actually, the new available data (Brown *et al.*, 1969) have demonstrated that it is the reproductive stage which controls the branching.

Recent investigations (Wolf *et al.*, 1985; Metzger *et al.*, 1990) have shown that the so-called "physiological" state are different races of this species (and also three in number). They also showed that the first state, which is yellow-green, produces botryococcene hydrocarbons, the other state produces n-alkadienes, and the last state yields a tetraterpenoid hydrocarbon, namely lycopadiene. The last two, which are resting cells, exhibit an orange-brown colour. This change might be caused by a mechanism described by Davis (1962), as occurring in other algal representatives as well. Toward the end of growth stage, the alga preserves food in the form of lipids and carotenoids which are distributed in the cell walls. While chlorophyll degrades, the carotenoids become dominant and this may produce a change in colour.

Pauli (1961) has remarked that microorganisms may synthesize humics from non-lignitic vegetal constituents. The opinion that humic substances may evolve from vegetational cells, specifically of algal origin, such as *Botryococcus*, which, in turn, by thermal maturation processes may give rise to gaseous hydrocarbons (mostly methane as a biogenic product that will migrate upward) (Baltes, *pers. comm.*, 1995), appears to be very plausible. If methane accumulation would not always seem important from an economic point of view, its presence could, however, suggest much deeper accumulation of liquid hydrocarbons. This is especially important in deltaic environments where wetland settings are common, and any change in the mechanism of gas emission – in response to changes of delta morphology and ecology, respectively, – should be monitored.

Based on the present data, unicellular stages of development may indicate methane accumulation. In samples collected from the Black Sea Miocene sediments and analyzed by the author, these autospores have been found in high amounts (more than 1000 per slide).Their presence may suggest both an improvement of developmental conditions and, if considered in terms of hydrocarbons-prone microorganisms, most probably the basic material for producing biogenic gaseous hydrocarbons owing to their higher dense state that cannot confer them the status of oil-prone cells. It is noted that occurrence of significant methane pouch implies the existence, up the way in a given succession, of a porous stratum, a compulsory condition to enable the gas accumulation process, as well as the presence of reliable impermeable levels to preserve it in good conditions. That is exactly the depositional pattern met with in the Black Sea Miocene sediments. As predicted on the base of distribution of both *Botryococcus* autospores and the humic increased content at certain levels, the methane deposit has been found well above the stratigraphic level dominated by *Botryococcus*, still below the level enhanced in humic substances.

6. CONCLUSIONS

The present study has outlined the most important stages of *Botryococcus* life cycle, as they were found at different sedimentary levels of the Pliocene-Miocene succession of Romania.

The basic relationships established between *Botryococcus* morphotypes and different hydrocarbon constituents emphasize the outstanding role this alga plays in hydrocarbon exploration.

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