Indices biochimiques et milieux marins. Journées du GABIM, Brest, 18-20 Nov. 1981
Publi. CNEXO (Actes Colloq.) n. 14, 1982, p.147 à 154

CHANGES IN THE POLYSACCHARIDE MATRIX OF CALCAREOUS GREEN ALGAE DURING GROWTH

by

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ABSTRACT

The organic matrix of three species of calcareous green algae (family Caulerpales, order Udoteaceae) was analysed during different growth stages for its carbohydrate content.

In all cases investigated a decrease with age in the relative content of structural polysaccharides paralleled by an increase in reserve polymers was observed.

A dynamic carbonate deposition model is suggested based on consideration of the carbohydrate composition.

RESUME

Les hydrates de carbone de la matière organique de trois espèces d'algues calcaires vertes (famille de Caulerpales, ordre de Udoteaceae) ont été analysées au cours de différentes phases de croissance.

Dans tous les cas étudiés, une décroissance avec l'âge du contenu relatif en polysaccharides de structure a été observée parallèlement à une augmentation des polymères de réserve.

Un modèle de la dynamique du dépôt du carbonate, faisant intervenir la composition en hydrates de carbone est suggéré.

KEY WORDS

Calcareous green algae, matrix polysaccharides, carbonate deposition

MOTS CLEFS

Algues calcaires vertes, polysaccharides de structure, dépôt du carbonate

INTRODUCTION

Calcareous green algae deposit carbonates during growth upon an organic matrix primarily consisting of polysaccharides. Iriki et al. (1960) and Böhm et al. (1980) reported β-1,3-xylans to be the main structural polymers of the green algal species Halimeda sp., Penicillus sp. and Udotea sp. (family Caulerpales, order Udoteaceae).

Recently, Wefer (1980) determined growth rates of 1 - 2 mm d^{-1} for Penicillus sp. whereas Halimeda sp. was found to grow at a rate of 1 segment per day. Udotea sp. showed growth rates similar to Penicillus sp. (Wefer, pers. comm., 1980). By adoption of a staining technique it became possible to positively identify the age of individual segments of the plants.

MATERIALS AND METHODS

Algae were collected in August 1979 in Bermuda after in situ staining with alizarin sulphonic acid as described by Wefer (1980). During growth the dye is incorporated into the carbonate deposited thus necessitating a 10 - 15 min treatment with 15% sodium hypochlorite solution before the staining marks become visible.

Briefly, natural algal communities are enclosed within polythene bags. The enclosures are removed 24 hours after introduction of the dye and the algae allowed to grow for a further 14 - 21 days prior to collection.

After lyophilisation, the plants were segmented according to Fig. 1.

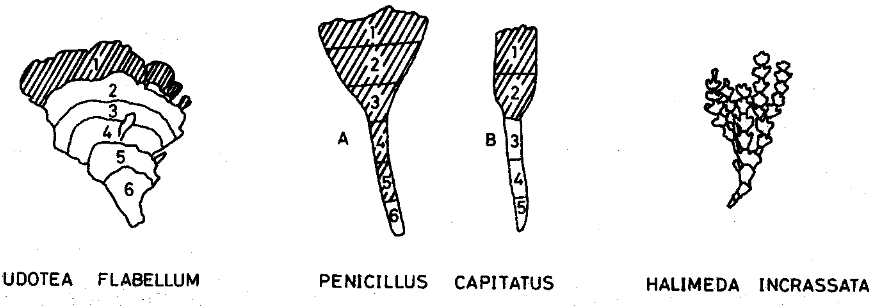

Fig. 1 Segmenting of the Udotea and Penicillus samples. An example for Halimeda is given. Shaded parts developed after staining.

In the case of Halimeda incrassata segments of seven different plants were analysed assuming a growth rate of 1 segment day^{-1}.

The individual segments were weighed into Teflon-lined screw cap vials and sufficient 4M HCl was added for complete dissolution of the carbonate. Additional 4M HCl was added to adjust the final acid molarity to 2M HCl. After hydrolysis under nitrogen at 110 °C for 3 h, calcium was removed with cation exchange resin (Dowex 50 W X8, H^+ form). The hydrolysate and distilled water washings were combined and lyophilised after the addition of 100 µl glycerin/ethanol 1:1 (Dawson and Mopper, 1978). The residue was taken up in 20% aqueous ethanol (v/v) and an aliquot injected into a self-constructed sugar analyser. Separation of the monosaccharides was achieved by anion exchange of their borate complexes. Details of the construction and operation of the analyser have been given elsewhere (Dawson and Liebezeit, 1981; 1982).

Calcium was removed from the cation exchange resin with 2.5M NaCl solution and determined according to Bisque (1961).

RESULTS AND DISCUSSION

Böhm et al. (1980) showed for sediment samples that polysaccharides not intimately associated with the carbonate matrix i.e. non-structural polymers could be removed by treatment with 15% sodium hypochlorite solution. A similar approach was adopted to distinguish between the different polymers in *Halimeda* and *Penicillus*. The results are given in Table 1.

Table 1 Relative molar composition of the polysaccharide matrices of *Halimeda* and *Penicillus*. - denotes untreated specimens, + indicates treatment with 15% NaOCl solution for 90 minutes. Whole plants were analysed.

	Halimeda sp.			*Penicillus* sp.			
oxidation	-	+	-	+	-	+	
glucose	28.3	9.3	41.9	15.7	44.6	11.0	mole %
xylose	65.1	88.2	50.1	79.5	49.7	84.1	
galactose +							
arabinose +							
fucose	3.8	0.4	4.7	2.7	2.4	0.3	
mannose	1.8	1.5	2.1	-	0.9	-	
rhamnose +							
ribose	0.3	0.6	0.5	-	0.3	0.1	
others	0.7	-	0.7	2.1	2.1	4.4	
μmol g^{-1} carbonate	273.2	66.0	364.8	162.6	754.0	282.1	

Both species showed a considerable change in relative molar composition before and after oxidative treatment. Whereas the analysis of untreated samples indicates that glucans are present as reserve polymers, xylans clearly dominate the spectrum after removal of labile components. Small amounts of other sugars are present which seem to be associated with the matrix polymers.

The considerable decrease of the total sugar content indicates that structural polymers are degraded to a certain extent. Here, *Penicillus* seems to be more resistant than *Halimeda* probably due to the fact that carbonate deposition takes place via different pathways in the two plants, as discussed by Böhm et al. (1978), resulting in an increased protection of the organic matrix in the case of *Penicillus*.

Udotea flabellum

The sample was collected at a water depth of 1.7 m. Segment 1 (Fig. 1) developed in 7 days and thus the approximate age of the whole plant is 42 days. A characteristic feature of this algal species is the occurrence of "growth rings". Segments were cut along the more obvious markations.

The mean $CaCO_3$ content was 49.6% and is higher than the 39.1% and 38.4% reported by Böhm (1972; 1973a). These discrepancies may arise from differences in environmental conditions, since the above author found the degree of calcification in various Udoteaceae to be dependent upon solar insolation.

Results are depicted in Fig. 2. In younger parts of the plant, the total sugar content is relatively high with a high proportion of xylans. With increasing age, $CaCO_3$ content increases paralleled by a decrease in total sugars. In the oldest segments, this trend reverses.

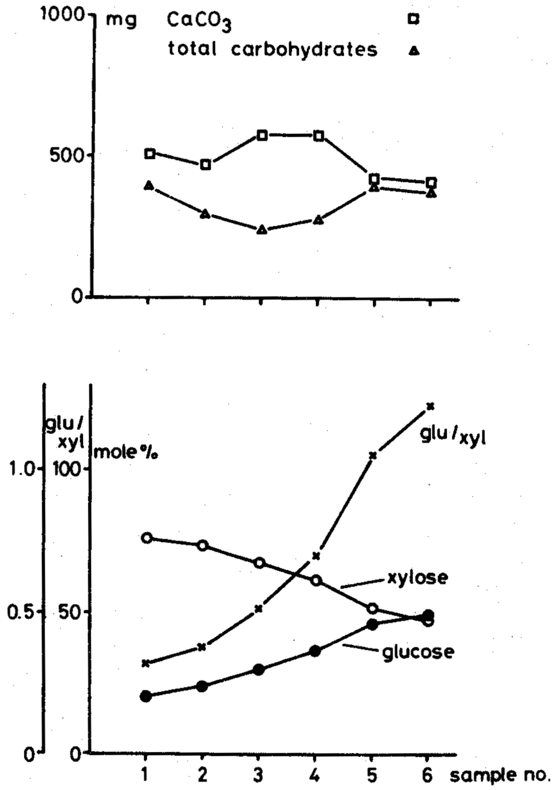

Fig. 2 $CaCO_3$ and sugar contents in different segments of Udotea flabellum

Since no data on nutrient concentrations or weather conditions are available for the entire growth period of 42 days, it can only remain speculative as to whether environmental factors are reflected in both carbonate and total sugar contents.

The relative molar composition exhibits a decrease in xylans and increasing glucan content and glucose/xylose ratios with age.

Penicillus capitatus

Both plants were collected at a water depth of 8 m. The shaded portions (Fig. 1) developed in 21 days corresponding to growth rates of 4.3 (Pen A) and 1.7 mm (Pen B) d^{-1}.

The mean $CaCO_3$ content of Pen B is 54.7% being in good agreement with data giben by Böhm (1972; 1973a). Pen A has a mean carbonate content of 76.8%. This relatively high value together with a growth rate exceeding the range given by Wefer (1980) makes it likely that Pen A is a species other than *P. capitatus*.

Since considerable widthwise growth accompanies an increase in length, the results obtained (Fig. 3) are more ambiguous than those for the flat, leaflike *U. flabellum*. Nevertheless, there are similarities in several of the analysed characteristics of the two plants.

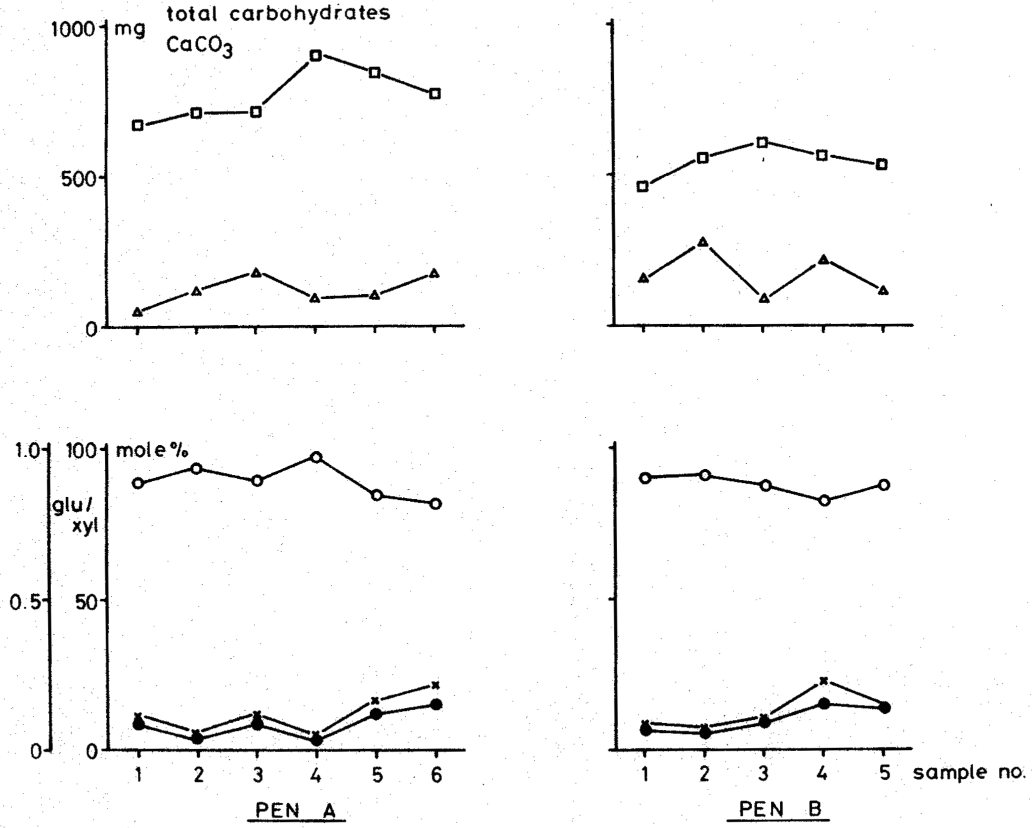

Fig. 3 $CaCO_3$ and total carbohydrate contents in different parts of *Penicillus capitatus*. Notations as in Fig. 2.

Total sugar contents are lower than in *U. flabellum*, the major portion being xylans (83 mole%) which is in accordance with findings of Iriki et al. (1960) who reported 76 - 79% of the organic fraction of *Penicillus* sp. to consist of this polymer.

At the branching point, a maximum in $CaCO_3$ together with a carbohydrate minimum is recognisable. Wefer (pers. comm., 1980) occasionally found staining at this part of the plants which lends support to the assumption that the branching point is a centre of activity during growth.

Again, as in the case of *U. flabellum* an increase in relative glucose content at the expense of xylose is to be noted with increasing segment age. Glucose/xylose ratios also increase with age. In the growing parts, a parallel decrease of both $CaCO_3$ and total sugars is observed, giving rise to the assumption that organic polymers other than xylans or glucans are involved in carbonate deposition (see below).

Halimeda incrassata

Samples were collected at a water depth of 2 m.

The carbonate material grown in one day was combined from the segments of seven plants to ensure a sufficient amount of material for analysis. As a consequence the results show a wide scatter reflecting the biological variations between plants (Fig. 4). Segments of days 5, 9 and 13 were all multiply branched.

The mean CaCO$_3$ content was determined to be 71.3% and falls within the range given by Böhm (1972; 1973a).

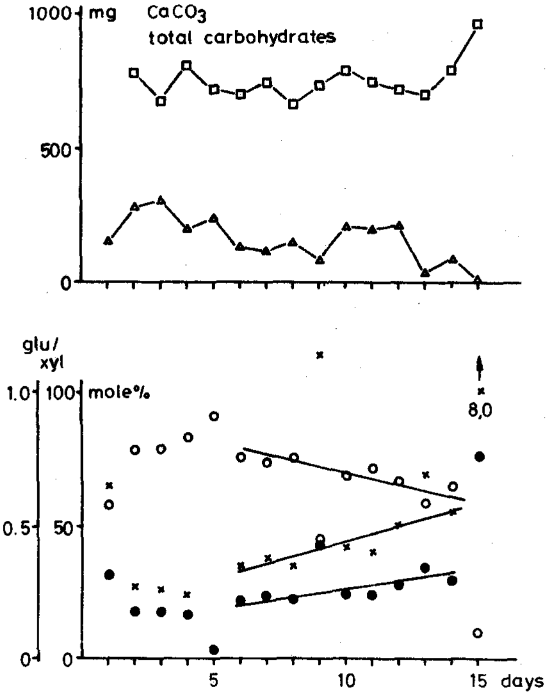

Fig. 4 CaCO$_3$ and total sugar contents in different parts of Halimeda incrassata. Notations as in Fig. 2.

Both total carbohydrate and carbonate contents varied widely, however, the trends discussed above, namely a decrease in xylose content paralleled by an increase in glucose with increasing age of the individual segments remain evident (Fig. 4).

In all species analysed, the sum of organic and inorganic constituents determined ranges from 60 to 100% (Figs. 2 - 4), in most cases a considerable portion escapes identification. Inorganic compounds such as strontium, magnesium, iron, silicate or sulphate are present only in minor quantities (Böhm, 1972; 1973a).

Böhm (1973b) found 6 - 9% water insoluble and 8 - 17% water soluble protein in H. opuntia employing a spectrophotometric technique.

The presence of calcium-binding polysaccharides in H. opuntia was reported by Böhm (1973b) and Böhm and Goreau (1973) occurring in a water-soluble form containing carboxyl and sulphate ester functions. Our own preliminary analyses with U. flabellum exhibited the presence of galacturonic and glucuronic acids in minor quantities. With increasing age of the segment, a decrease in these acids was observed. Due to the hydrolysis procedure employed (detrimental to these compounds) no quantitative treatment was possible. It can, however, be assumed that proteins and uronic acid residues of polysaccharides -especially in younger parts of the algae- are involved in the deposition of calcium carbonate.

Although the effect of an increasing degree of calcification accompanying a decrease in total sugars with increasing age of the algal segments could only partly be established, it is evident from the data presented above that reserve polysaccharides (glucans) are synthesised at the expense of structural polymers (xylans) with increasing age.

The above data together with literature evidence lead to the suggestion of a hypothetical pathway of carbonate deposition. As a first step xylans are synthesised during growth. These polymers are extremely resistant and were shown to retain their original structure even after extensive treatment with hydrochloric acid and hypochlorite solution (Iriki et al., 1960; Liebezeit, unpublished data). This fibrous support is associated with a polymer containing uronic acid and amino acid residues possibly being linked to the xylan via sulphate esters. Calcium binding then occurs as a result of chelation with e.g. aspartic acid residues (Matheja and Degens, 1968). The growing carbonate layer is protected by this polymer and/or by a glucan from redissolution. After a certain degree of calcification has been reached probably dependent on environmental conditions the mediating polymers are either degraded or transported to actively growing parts of the plant leaving the back bone skeleton of the xylan and the protecting glucan behind. This glucan may serve as an additional reserve polysaccharide. A transport mechanism of the calcium binding polymer is supported by the fact that no arabinose could be observed in the residual fraction as a result of galacturonic acid decarboxylation.

ACKNOWLEDGMENTS

Skilled technical assistance of Miss M. Eilers is gratefully acknowledged. G. Wefer kindly supplied the algal material.

This work was supported by the Deutsche Forschungsgemeinschaft and carries the contribution No. 378 of the Sonderforschungsbereich 95.

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ÉCOLOGIE ET PHYSIOLOGIE DES PRODUCTEURS SECONDAIRES ET TERTIAIRES