

Carbohydrate Metabolism in Suspended and Attached Cells of a Marine Fouling Diatom, *Navicula* sp.

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A marine fouling diatom, *Navicula* sp., was cultured in the laboratory under 12-hour light : 12-hour dark conditions. Cells in suspension and attached to the flask walls were analyzed for chlorophyll *a*, cell organic carbon, total cell carbohydrate, and carbohydrate fractions. Maximum chlorophyll *a* concentrations were obtained on the fifth and eighth days of cultivation for suspended and attached cells, respectively. For both cell types, total carbohydrate production increased with the cultivation period. The total carbohydrate : cell carbon ratio also increased with cultivation, being marginally higher for the attached cells. Throughout the experiment, the suspended cells had more acid soluble carbohydrate, whereas the carbohydrate group composition varied with cultivation period for the attached cells. During the initial four-hour dark cultivation period, the attached cells had more alkali-soluble carbohydrates. Following this period, the acid-soluble carbohydrate was the major component of the total carbohydrate of the attached cells. In general, more acid-soluble carbohydrate was observed for attached cells, whereas alkali-soluble and residual carbohydrates were more abundant in the suspended cells.

INTRODUCTION

Carbohydrates are common storage and structural compounds in marine and terrestrial organisms. The production and chemical composition of these compounds in microorganisms may be influenced by several factors including nutrient availability in the growth medium and the growth phase of the organism. While colonizing surfaces, microorganisms generally produce polysaccharides (Costerton et al. 1988, Marshall 1988), which have been implicated in biofouling and biocorrosion processes (Geesey et al. 1986).

Each adherent microorganism may produce chemically and structurally unique polysaccharides (Decho 1990). Several functions including maintenance, protection, and detoxification have been ascribed to the polysaccharides of biofilms. Despite this, very little is known about polysaccharide production by microfouling organisms.

In this paper, data on carbohydrate production by the suspended and attached cells of a marine fouling diatom, *Navicula* sp., are presented and discussed.

METHODS

Culture and Growth Conditions: *Navicula* sp. were isolated from panels immersed in the coastal waters of the Arabian Sea. A stock culture of the diatoms was maintained as a uni-algal culture in Ehrlenmeyer flasks under 12-hour light : 12-hour dark conditions in Guillard's F_2 medium (Guillard 1975) prepared from seawater. Cultures were transferred into fresh F_2 medium at seven-day intervals.

Monitoring Growth and Carbohydrate Production: Experiments to assess growth, carbohydrate production, and group composition were carried out in Ehrlenmeyer flasks (two liter) under 12-hour light : 12-hour dark conditions. Experimental flasks containing 200 ml F_2 medium were inoculated with known quantities of diatom cells. The flasks were removed at two-day intervals over a period of 16 days. Their contents were decanted to collect the suspended cells, the flasks washed twice with 10 ml of growth medium, and the washings and decanted spent medium combined for analyses of the suspended cells. The cells attached to the walls of the flasks were scraped using a soft brush (Bhosle et al. 1989) and made to volume (100 ml). Aliquots of both suspended and attached cells were filtered using a GF/C glass filter (47 mm, 1.0 μ m pore size). The filters were then analyzed for chlorophyll *a*, cell carbon, total cell carbohydrate, and carbohydrate fractions.

Chlorophyll *a* and cell carbon were measured spectrophotometrically following Parsons et al. (1984). The carbohydrate fractions [dilute acid (0.1 N H_2SO_4), dilute alkali (0.1 N NaOH)] and residual carbohydrate were isolated as described earlier (Bhosle and Dhople 1988). Total carbohydrate and carbohydrate fractions were estimated by the phenolsulphuric acid method (Dubois et al. 1956). The analytical precision of these methods based on six replicates was $\pm 10\%$ or less.

RESULTS

The algal biomass measured as chlorophyll *a* increased, with the cultivation

periods reaching maxima on the sixth and eighth days of cultivation for the suspended and attached cells, respectively (figure 1). Both cell types also showed increased production of total cell carbohydrate as a function of the cultivation period (figure 2a, b). Dilute acid soluble-, dilute alkali soluble-, and residual carbohydrates showed trends similar to that obtained for total carbohydrate (figure 3). When expressed as a percentage of the total carbohydrate, the attached algal cells had more alkali-soluble and residual carbohydrates compared to acid-soluble carbohydrate, especially during the first four days of cultivation. By contrast, the suspended algal cells had more acid-soluble carbohydrate throughout cultivation (figure 4). Compared to the suspended cells, the attached cells had more acid-soluble carbohydrate and less alkali solubles and residual carbohydrates, especially after the initial four-day cultivation. Somewhat higher values for the total cell carbohydrate/cell carbon ratios were observed for the attached cells, whereas the total cell carbohydrate/chlorophyll *a* ratios were higher for the suspended cells (figure 5).

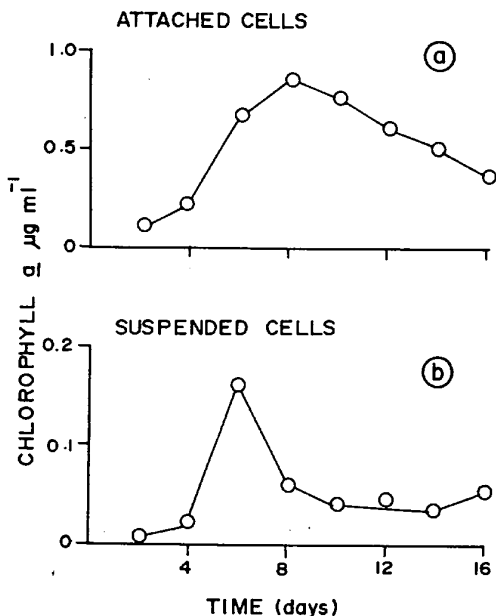


Figure 1. Growth pattern of *Navicula* sp. as a function of cultivation period when grown under 12-hour light : 12-hour dark conditions in medium. (a), attached cells; (b), suspended algal cells.

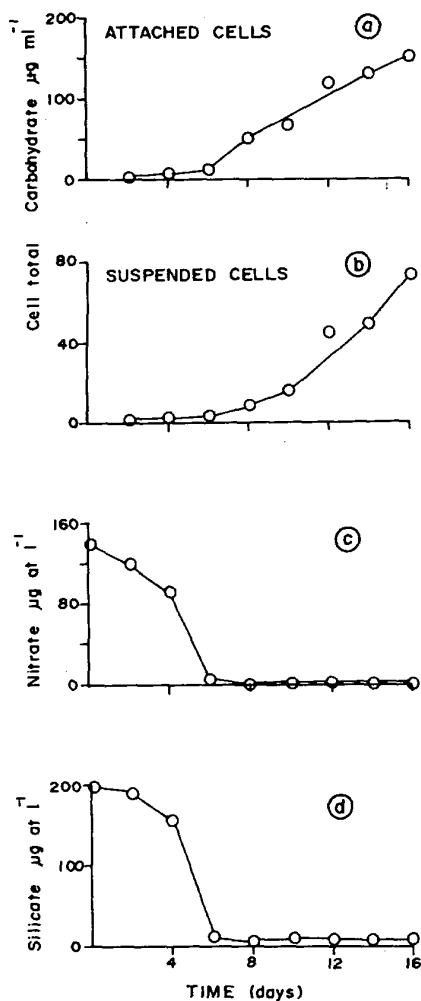


Figure 2. Total cell carbohydrate production by attached (a) and suspended (b) cells of *Navicula* sp. and changes in nitrate (c) and silicate (d) contents as a function of cultivation period when grown in F_2 medium.

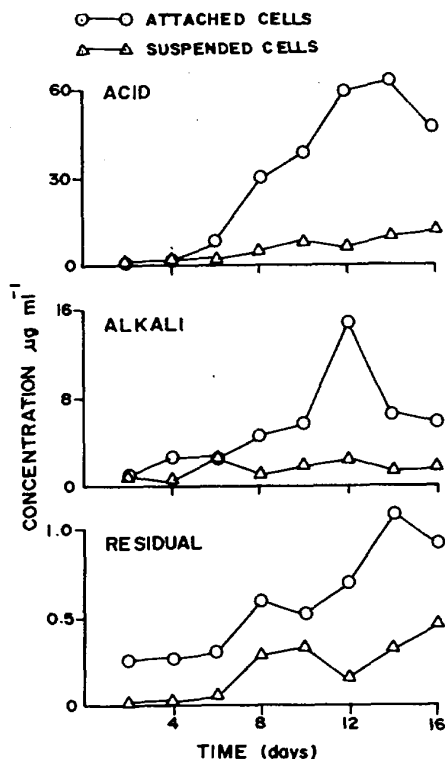


Figure 3. Variations in carbohydrate fractions of attached and suspended cells of *Navicula* sp. as a function of cultivation period when grown in F_2 medium.

DISCUSSION

When grown under 12-hour light : 12-hour dark conditions, the highest biomasses were obtained on the fifth and eighth days of cultivation for the suspended and attached cells of *Navicula* sp., respectively, as evident from changes in chlorophyll *a*. These observed differences in the development of the algal biomass imply that the suspended cells were growing faster than the attached cells. The apparent decrease in biomass, i.e., chlorophyll *a* for both types of cells, was perhaps associated with nonavailability of nutrients. Of the various nutrients measured in the growth medium, nitrate and silicate decreased with cultivation (figures 2c, d). Therefore, the

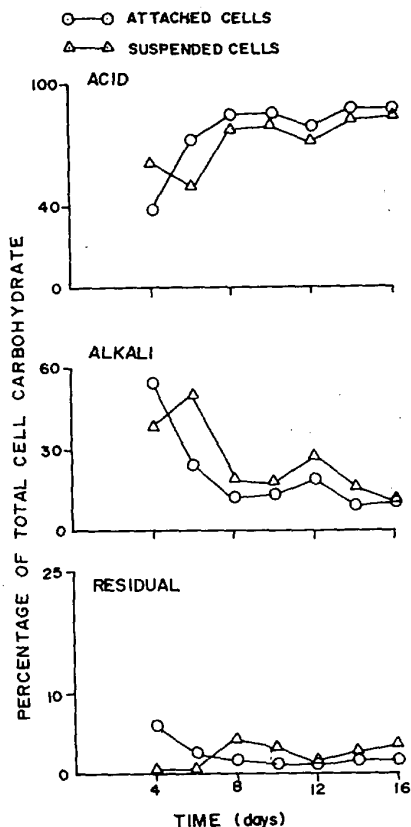


Figure 4. Changes in carbohydrate fractions when expressed as percentages of total carbohydrate of attached and suspended cells of *Navicula* sp. grown in F_2 medium.

observed decrease in chlorophyll *a* for both types of cells was due to the nonavailability of these essential nutrients and/or some other limiting factors.

Marine diatoms appear to have a relatively high production rate of carbohydrate in culture (Handa 1969, Mykkestad 1977, Mykkestad et al. 1989). This is also true for the cells of *Navicula*. Furthermore, carbohydrate production was especially high during the stationary phase of the algae.

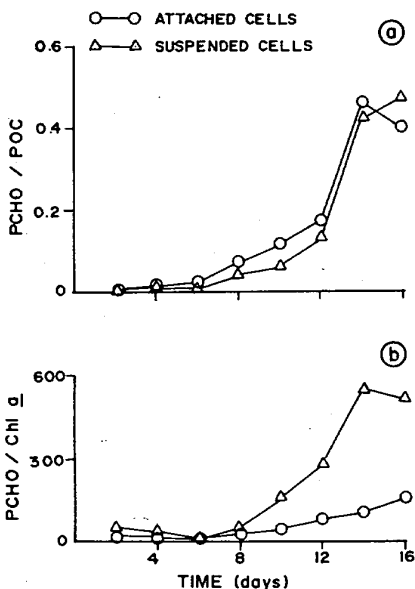


Figure 5. Total cell carbohydrate : cell carbon (a) and total cell carbohydrate : chlorophyll *a* (b) ratios of attached and suspended cells of *Navicula* sp. when grown in F_2 medium.

Environmental conditions, particularly availability of nitrogen, affect the carbohydrate and protein contents of algae. Moreover, nitrogen starvation enhances carbohydrate production (Myklestad 1977, Hama 1988). Thus, algae in a nutrient-deficient culture will increase their carbohydrate and/or lipid production at the expense of protein. It appears that both types of *Navicula* cells produced large amounts of total carbohydrate during the growth phase when nutrient-deficient especially nitrogen conditions prevailed, as evident from the decrease in nitrate content of the growth medium (figure 2c).

Cellular carbohydrates from diatoms can be divided into two groups: storage and structural carbohydrates (Handa 1969, Haug and Myklestad 1976). The structural carbohydrates can be further divided into dilute-alkali soluble- and residual carbohydrates (Haug et al. 1973). Thus, the storage and structural carbohydrates of *Navicula* sp. were isolated by sequential extraction of algal cells with dilute acid and dilute alkali, respectively.

The carbohydrate composition for both types of algal cells indicates that the acid-soluble carbohydrate was the major component and the dilute-

alkali-soluble- and residual carbohydrates were the minor components of the total carbohydrates. Nevertheless, differences in the relative distribution of these carbohydrates were observed for the attached and suspended cells. For example, acid-soluble carbohydrate was less ($< 40\%$) than alkali-soluble carbohydrate ($> 52\%$) in the attached cells during the initial four-day cultivation period. It is suggested that while colonizing surfaces, diatoms may synthesize adhesive polymers that are acidic in nature (Characklis and Cooksey 1983). Such polymers can be extracted with dilute alkali. Therefore, it is possible that the higher amount of alkali-soluble carbohydrate was produced by the attached cells in order to attach to the glass surface. Alternatively, diatom cells during respiration utilize dilute acid-soluble carbohydrate (Handa and Yanagi 1969). Such selective removal of acid-soluble carbohydrate will result in higher relative abundance of alkali-soluble and residual carbohydrates. After this initial attachment period, the attached *Navicula* cells initiated the synthesis of acid-soluble carbohydrate as seen by the increasing abundance of this compound. These data were further supported by the increasing trends observed for total cell carbohydrate : cell carbon ratios. From this it can be concluded that the attached cells, compared to suspended cells, produced a higher amount of carbohydrate, especially acid-soluble carbohydrate. Thus, the observed carbohydrate abundance in the attached algal cells was perhaps associated with a higher metabolic activity of these cells or in response to their association with the glass surface. Further differentiation of carbohydrate metabolism was also evident in the type of carbohydrate produced. This suggests that the attached cells produced different types of carbohydrates during their association with the glass surface.

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