

THE EFFECT OF CATHODIC POLARISATION ON MONOSACCHARIDES OF *AMPHORA COFFEAIFORMIS*, A MARINE FOULING DIATOM

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The composition of monosaccharides and their variation in concentration in *Amphora coffeaeiformis* cells on non-polarised and cathodically polarised 304 stainless steel were examined when cells were grown under continuous illumination at 18°C for 8 d in medium prepared in synthetic seawater. Lower concentrations of monosaccharides were found in *A. coffeaeiformis* cells attached to cathodically polarised steel. Under these conditions, the abundance of monosaccharides decreased with time. In contrast, concentrations of monosaccharides were higher by a factor of 8 to 40 in *A. coffeaeiformis* cells attached to non-polarised steel. The abundance of monosaccharides increased over the period of cultivation in the cells on non-polarised steel. Eight individual monosaccharides viz. rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose were identified in cells from both non-polarised and cathodically polarised steel. Amongst these, hexoses such as mannose, glucose and galactose were most abundant in cells from both non-polarised and cathodically polarised steel; the concentration of these hexoses increased in the former and decreased in the latter.

KEYWORDS: cathodic polarisation, *Amphora coffeaeiformis*, monosaccharides, stainless steel, microfouling

INTRODUCTION

Steel structures immersed in seawater are protected by several methods including cathodic protection, non-toxic paint coatings or a combination of both. Cathodic protection is effected by the introduction of an electrical current to counteract the normal electrochemical reaction. The steel is forced to become cathodic and thus prevented from corrosion. Electrochemical polarisation may change the chemical nature of the surface and thus may influence the attachment, growth and activity of microfouling organisms. Earlier studies have considered the effect of cathodic polarisation on the attachment of microorganisms, especially bacteria, to metal surfaces (Gordon *et al.*, 1981; Dhar & Bockris, 1981; Dhar *et al.*, 1982; Edyvean & Moss, 1986; Little *et al.*, 1988; Mollica *et al.*, 1989; Guezennec, 1991).

Carbohydrates are common structural and storage products of marine organisms. Microorganisms synthesise polysaccharides while colonizing surfaces (Corpe, 1974; Costerton *et al.*, 1987; Ford & Mitchell, 1990; Neu & Marshall, 1991). Moreover, the involvement of polysaccharides in biofouling and corrosion processes has been documented (Geesey *et al.*, 1986; Jolly *et al.*, 1988; Bhosle & Wagh, 1992). Despite their importance in these processes, little is known about the influence of cathodic polarisation on carbohydrate metabolism and composition in fouling organisms including

diatoms, although it has been reported that carbohydrate metabolism is influenced in *A. coffeaeformis* cells from cathodically polarised steel (Bhosle *et al.*, 1993b).

In a continuation of studies on the effect of cathodic polarisation on diatoms, data on the concentration of monosaccharide and their composition in *A. coffeaeformis* cells from cathodically polarised and non-polarised 304 stainless steel are presented and discussed in this paper. Although cathodic polarisation is not generally used on stainless steel, the use of this metal provides a relatively inert surface for settlement studies in comparison with other metals which do not have the same passivity.

MATERIALS AND METHODS

Test Panels

Before use test panels (10 × 7.2 cm) were cleaned following the procedure described previously (Bhosle *et al.*, 1993b).

Culture and Growth conditions

Amphora coffeaeformis var. *perpusilla* (Grunow) Cleve was isolated and cultured as described by Bhosle *et al.* (1993a).

Monosaccharides of Algal cells from Non-polarised 304 Stainless Steel

Experiments to assess monosaccharide concentrations and composition in cells of *A. coffeaeformis* from non-polarised steel were carried out as reported earlier (Bhosle *et al.*, 1993b). After inoculation, tanks were kept under continuous illumination at 18°C for 2, 4 and 8 d.

Monosaccharides of Algal Cells from Cathodically Polarised 304 Stainless Steel

The test cells consisting of 5 dm³ PVC tanks equipped with a graphite counter electrode, Standard Colomel Electrode (SCE) and working electrodes in 1 dm³ of *f/2* medium have been described previously (Bhosle *et al.*, 1993b). After inoculation, metal panels were polarised using a potentiostat set to maintain a predetermined potential of the working electrode (WE) to within ±0.2 mV. The potential applied to the 304 stainless steel panels was fixed at -700 mV/SCE over a period of 2, 4 and 8 d.

Sampling For Analysis

At each sampling time *viz.* 2, 4 and 8 d, four cathodically polarised and four non-polarised stainless steel panels were removed from the PVC tanks and scraped using a nylon brush and Instant Ocean artificial seawater (Bhosle *et al.*, 1990a, b; 1993a, b). The volume of scraped material was adjusted and a known volume (5–35 ml) was filtered through pre-ignited (450°C, 12 h) GF/C glass filters (1 µm pore size). Two replicate samples were then analysed for monosaccharide composition. Based on 5 replicate analyses, the precision of the gas chromatographic analysis for glucose, galactose, mannose, xylose, arabinose, ribose, fucose, and rhamnose was 1.5, 4.8, 6.02, 7.55, 8.77, 5.23, 6.07 and 8.38% respectively. The standard deviation for duplicate analyses varied from 10 to 20%.

Monosaccharide Composition

The filters containing algal cells were hydrolysed with 1.2 M H₂SO₄. Monosaccharides were converted to alditol acetate derivatives and analysed by capillary gas chromatography (Bhosle *et al.*, 1990b; 1993a).

RESULTS

Capillary gas chromatographic analysis revealed the presence of eight neutral sugars *viz.* rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose and glucose in the algal cells obtained from both the cathodically polarised and non-polarised steels (Table 1, 2). The sum of these identified sugars has been defined as the total monosaccharides.

Monosaccharides of Algal Cells from Non-polarised Steel

The total monosaccharide concentrations of the algal cells from non-polarised steel increased over the period of cultivation (Fig. 1). Hexoses (mannose, galactose and glucose) were the most abundant constituents of total monosaccharides (Table 3).

Table 1 The monosaccharide composition of *A. coffeaeformis* cells attached to non-polarised stainless steel panels when grown in *f/2* medium under continuous illumination at 18°C for 8 d

Monosaccharide ($\mu\text{g}\cdot\text{dm}^{-2}$)	Time (d)		
	2	4	8
Rhamnose	9.88 \pm 1.77	34.14 \pm 4.83	56.63 \pm 10.70
Fucose	12.77 \pm 1.92	22.48 \pm 2.34	215.41 \pm 32.92
Ribose	9.44 \pm 1.51	17.21 \pm 1.89	184.32 \pm 29.58
Arabinose	4.74 \pm 0.91	23.87 \pm 3.07	215.69 \pm 36.76
Xylose	6.88 \pm 0.81	16.93 \pm 1.86	154.90 \pm 18.58
Mannose	21.76 \pm 3.39	119.36 \pm 23.87	668.73 \pm 132.40
Galactose	51.44 \pm 9.42	99.93 \pm 18.98	326.45 \pm 46.70
Glucose	312.02 \pm 35.88	505.23 \pm 80.87	547.42 \pm 53.66

Table 2 The monosaccharide composition of *A. coffeaeformis* cells attached to cathodically polarised 304 stainless steel panels when grown in *f/2* medium under continuous illumination at 18°C for 8 d

Monosaccharides ($\mu\text{g}\cdot\text{dm}^{-2}$)	Time (d)		
	2	4	8
Rhamnose	16.10 \pm 1.70	5.27 \pm 1.05	1.94 \pm 0.36
Fucose	16.93 \pm 3.00	1.66 \pm 0.19	1.38 \pm 0.20
Ribose	12.77 \pm 1.40	3.33 \pm 0.53	5.93 \pm 1.10
Arabinose	11.93 \pm 1.67	18.60 \pm 3.16	9.71 \pm 1.74
Xylose	11.38 \pm 0.90	1.66 \pm 0.31	11.66 \pm 2.33
Mannose	125.19 \pm 23.77	3.05 \pm 0.57	7.49 \pm 1.42
Galactose	36.08 \pm 7.23	3.33 \pm 0.53	4.21 \pm 0.75
Glucose	71.34 \pm 12.80	89.10 \pm 15.14	17.21 \pm 3.10

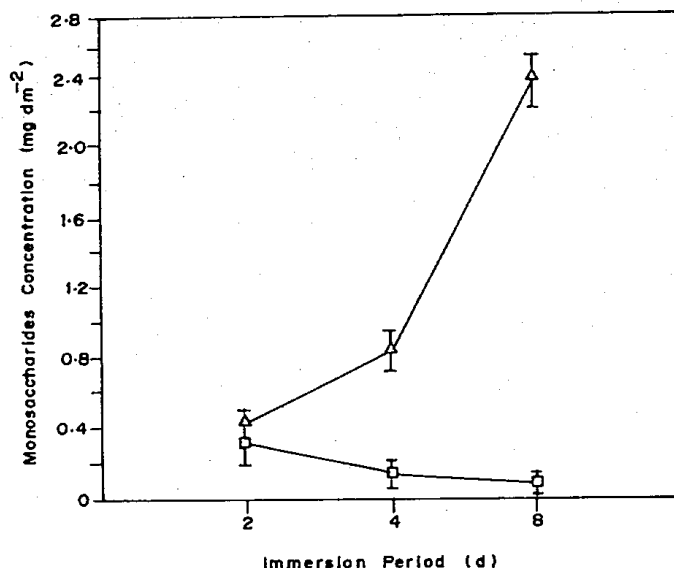


Fig. 1 Changes in the total monosaccharides of *A. coffeaeformis* cells attached to non-polarised (Δ-Δ) and cathodically polarised (□-□) 304 stainless steel.

Table 3 The monosaccharide group composition of *A. coffeaeformis* cells attached to non-polarised and cathodically polarised 304 stainless steel panels when grown in *f/2* medium under continuous illumination at 18°C for 8 d

Time (d)	Non-polarised			Cathodically polarised		
	Hex	Pent (% of total)	Deoxh	Hex	Pent (% of total)	Deoxh
2	89.81	4.91	5.14	77.10	11.95	10.94
4	86.45	6.91	6.74	75.77	18.72	5.50
8	65.10	23.41	11.48	48.65	45.76	5.58

Hex = Hexoses, Pent = Pentoses, Deoxyh = Deoxyhexoses

Increasing trends were also observed for pentoses (ribose, arabinose and xylose) and deoxyhexoses (rhamnose and fucose) (Figs 3, 4). Individual monosaccharides of the algal cells also exhibited considerable variation (Table 1), but generally, glucose, mannose and galactose were the most abundant. The concentration of other monosaccharides also increased over the period of cultivation.

Monosaccharides of Algal Cells from Cathodically Polarised Steel

The total abundance of monosaccharides decreased over the period of the cultivation (Fig. 1). Hexoses were the most abundant constituents of the total monosaccharides (Fig. 2, Table 3). The concentration of hexoses and deoxyhexoses decreased whereas that of pentoses did not show much variation (Figs 2 to 4). The abundance of individual monosaccharide did not show any particular trend (Table 2).

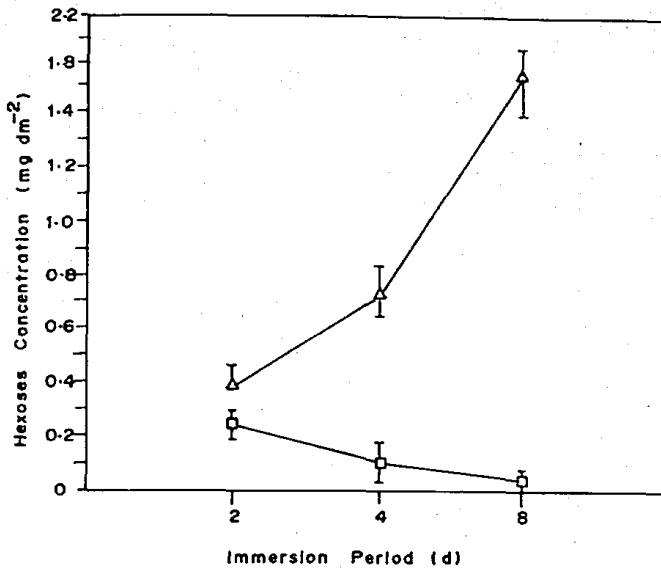


Fig. 2 Variations in hexose concentrations in *A. coffeaeformis* cells attached to non-polarised (Δ - Δ) and cathodically polarised (\square - \square) 304 stainless steel.

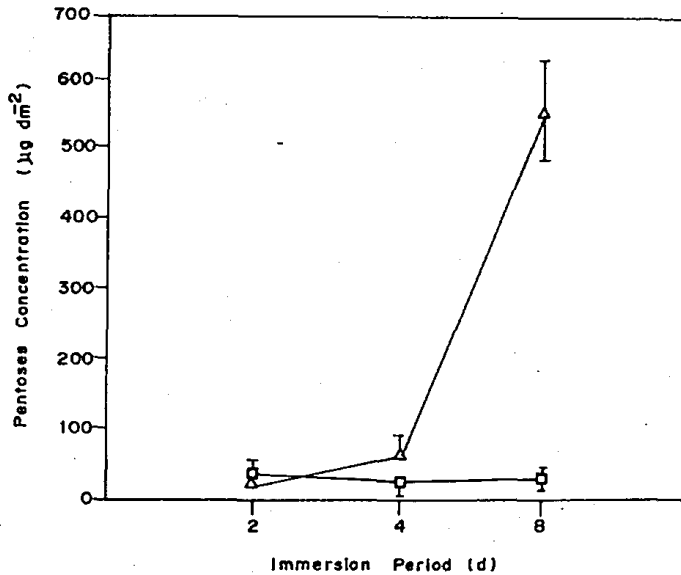


Fig. 3 Pentose concentrations in *A. coffeaeformis* cells attached to non-polarised (Δ - Δ) and cathodically polarised (\square - \square) stainless steel.

DISCUSSION

The total monosaccharide concentrations reported here are relatively lower than those reported earlier for total carbohydrates in *A. coffeaeformis* cells from non-polarised steel (Bhosle *et al.*, 1993b). This may be the result of over estimation of total carbohydrates by

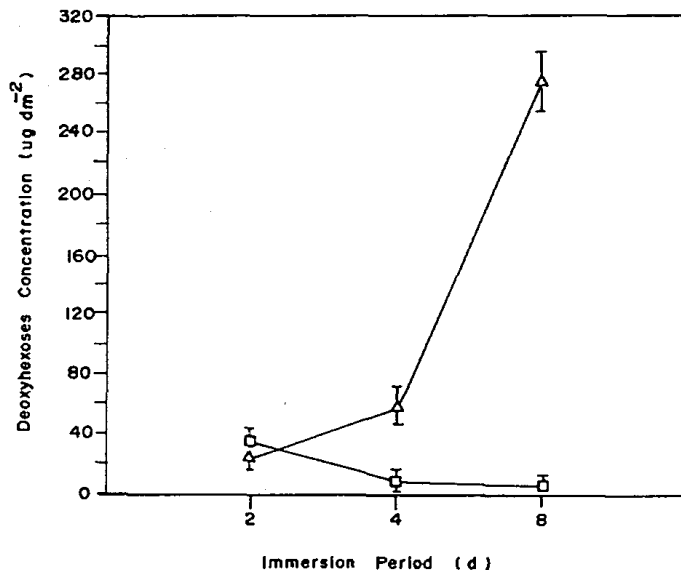


Fig. 4 Deoxyhexoses of *A. coffeaeformis* cells attached to non-polarised (Δ - Δ) and cathodically polarised (\square - \square) 304 stainless steel.

the phenol-sulphuric acid method. Thus comparison of the values reported earlier and in the present study may not be advisable.

The increasing trends observed for monosaccharide concentrations were similar to those obtained for cell carbon, chl *a*, protein and total carbohydrates of *A. coffeaeformis* from non-polarised steel (Bhosle *et al.*, 1993b). The increase in total monosaccharides as a function of time suggests net synthesis of these sugars in cells from non-polarised steel.

When the steel was cathodically polarised using a potential of -700 mV, the total monosaccharide concentration in *A. coffeaeformis* cells was relatively less. This is in agreement with earlier studies where lower values of cell carbon, protein, chl *a* and total carbohydrates were observed for *A. coffeaeformis* cells grown under similar experimental condition. From the consistent decrease over the period of incubation, it appears that these cells were unable to synthesise monosaccharides probably due to the effect of cathodic polarisation. The latter may influence interfacial pH, surface charge and conditioning processes thereby controlling attachment, growth and metabolism (Littauer & Jennings, 1968; Marshall, 1973; Corpe, 1974; Fletcher & Loeb, 1979; Gordon *et al.*, 1981; Dhar *et al.*, 1982; Little *et al.*, 1988). Some of these factors may have been responsible for the observed decrease in total monosaccharides of *A. coffeaeformis* cells on cathodically protected steel.

The decreased contribution of hexoses to the total monosaccharides of *A. coffeaeformis* cells from non-polarised steel suggests either a decrease in their production or an increase in the production of other monosaccharides. The decrease in hexoses was accompanied by an increase in concentration and abundance of pentoses and deoxyhexoses, especially after the fourth day of cultivation. This implies that the latter was the case. Hexoses and deoxyhexoses on the other hand showed a consistent decrease in the cells from cathodically polarised steel, implying that these compounds were utilised by the algal cells. Selective removal of hexoses and deoxyhexoses resulted in higher abundance of pentoses. From the consistent increase in pentoses (as % of total monosaccharides) in the

cells from cathodically polarised steel, it is clear that they were not utilised by the algal cells.

Eight monosaccharides were identified in *A. coffeaeformis* cells obtained from both the cathodically polarised and non-polarised steel (Tables 1 & 2). These monosaccharides are of common occurrence in the carbohydrates of marine diatoms (Handa, 1969; Haug & Myklestad, 1976; Hecky *et al.*, 1973; Ittekkot *et al.*, 1982; Parsons *et al.*, 1984; Liebezeit, 1984; Hama & Handa, 1992; Bhosle *et al.*, 1993a). The contribution of individual monosaccharides, however, varied in algal cells grown on non-polarised and polarised steel.

The glucose concentration increased with cultivation period and this was generally the most abundant monosaccharide in cells from non-polarised steel. However, when expressed as a percentage of the total monosaccharides, the relative contribution of glucose decreased. This was associated with an increase in other monosaccharides such as rhamnose, fucose, ribose, arabinose, xylose, mannose, and galactose. This implies that predominantly glucose-containing carbohydrates were synthesized by algal cells from non-polarised steel. These data are in agreement with earlier studies on the carbohydrates of flask grown cells of *A. coffeaeformis* (Bhosle *et al.*, 1993a).

Galactose, mannose, rhamnose, fucose, xylose, arabinose and glucose have been reported to be common components of heteropolysaccharides and cell walls of algae (Hecky *et al.*, 1973; Mackie & Preston, 1974; Haug & Myklestad, 1976; Hama & Handa, 1992). The production of these sugars by algal cells from non-polarised steel increased and their relative distribution varied during the period of cultivation. This suggests either differences in the proportions of the monosaccharide residues in a single carbohydrate and/or a change in the nature of the polymers produced with time by algal cells on non-polarised steel. It has been reported that the monosaccharide composition of the cell wall material in *Navicula pelliculosa* and in natural phytoplankton changed during the process of growth and was influenced by the cell age (Coombs & Volcani, 1968; Hama & Handa, 1992). Thus the observed variability in monosaccharide composition was probably due to physiological change in the alga during the period of cultivation.

In cells from cathodically polarised steel a marked decrease in the concentration of mannose and other sugars, especially after the second day following inoculation, was associated with an increase in glucose concentration. It has been reported that heteropolysaccharides containing mannose, galactose, fucose, rhamnose, xylose and arabinose are also removed during alga cell respiration and/or cell degradation, resulting in increased abundance of glucose and an overall decrease in total monosaccharides (Thanoue & Handa, 1987; Bhosle *et al.*, 1992). On the eighth day of cultivation, although glucose was the most abundant sugar, its concentration decreased in the cells grown on cathodically polarised steel. Such selective removal of glucose resulted in the higher abundance of mannose, galactose, xylose and ribose in these cells.

CONCLUSIONS

A. coffeaeformis cells grown on cathodically polarised and non-polarised steel showed large variations in monosaccharide composition and concentration. The observed changes in monosaccharides were due to growth of the algal cells on non-polarised steel. However, the concentration of monosaccharides generally decreased in algal cells from cathodically polarised steel perhaps as a result of utilization by the cells and/or cell decomposition due to the chemical conditions on the steel surface under cathodic protection.

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