View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by UNL | Libraries

University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Papers in Natural Resources | Natural Resources, School of

1995

Effects of bromide and iodide on stalk secretion in the biofouling diatom Achnanthes longipes (Bacillariophyceae)

Kyle D. Hoagland
University of Nebraska-Lincoln, khoagland1@unl.edu

Lisa M. Johnson
University of Nebraska-Lincoln

Michael R. Gretz

Follow this and additional works at: http://digitalcommons.unl.edu/natrespapers


Part of the Natural Resources and Conservation Commons, Natural Resources Management and Policy Commons, and the Other Environmental Sciences Commons

Hoagland, Kyle D.; Johnson, Lisa M.; and Gretz, Michael R., "Effects of bromide and iodide on stalk secretion in the biofouling diatom Achnanthes longipes (Bacillariophyceae)" (1995). *Papers in Natural Resources*. 736.
http://digitalcommons.unl.edu/natrespapers/736

This Article is brought to you for free and open access by the Natural Resources, School of at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Papers in Natural Resources by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

J. Phycol. **31,** 401–412 (1995)

EFFECTS OF BROMIDE AND IODIDE ON STALK SECRETION IN THE BIOFOULING DIATOM *ACHNANTHES LONGIPES* (BACILLARIOPHYCEAE)[1]

Lisa M. Johnson,[2] *Kyle D. Hoagland*

School of Biological Sciences and Department of Forestry, Fisheries & Wildlife, University of Nebraska, Lincoln, Nebraska 68588

and

Michael R. Gretz

Department of Biological Sciences, Michigan Technological University, Houghton, Michigan 49931

ABSTRACT

Extracellular polymeric substance (EPS) secretion was examined in the stalked marine diatom Achnanthes longipes *Ag. in defined medium. This common biofouling diatom exhibited an absolute requirement for bromide for stalk production and substratum attachment, whereas elevated iodide concentrations in the growth medium inhibited stalk formation and adhesion. Varying EPS morphologies resulted from altering bromide and iodide levels: pads, stalk-pads, stalks, and no EPS. Cells showed no differences in growth with bromide or iodide concentrations, indicating that they were not physiologically stressed under conditions that impaired EPS secretion. Cells grown in elevated iodide secreted significantly more soluble extracellular carbohydrate into the medium, suggesting that the EPS was soluble and unable to be polymerized into a morphologically distinct gel. By replacing sulfate with methionine, the diatom lost its ability to form stalks even in the presence of bromide, indicating that free sulphate may be required for proper cross-linking of stalk polymers.* Lotus-*FITC, a fluorescent-tagged lectin, preferentially labeled the EPS and, thus, was used to visualize and quantify EPS secretion along a bromide gradient in conjunction with an image analysis system. This technique demonstrated a direct correlation between the amount of bromide present in the medium and the specific EPS morphology formed.*

Key index words: Achnanthes longipes; *Bacillariophyceae; biofouling; bromide; diatom; EPS secretion; image analysis; inhibition of adhesion; iodide;* Lotus-*FITC; stalks*

Diatoms are ubiquitous fouling microorganisms (Bishop et al. 1974, Marszalek et al. 1979, Daniel et al. 1980, Caron and Sieburth 1981, Daniel and Chamberlain 1981, Characklis and Cooksey 1983, Callow 1993). They adhere to submersed structures by secreting insoluble mucilages combined with glycoproteins in the form of adhering films, capsules, pads, tubes, and stalks (Chamberlain 1976, Blunn and Evans 1981, Daniel et al. 1987, Callow 1993, Hoagland et al. 1993). This adherence usually is permanent and imparts an ecological advantage to the attached organism (Daniel et al. 1987). Diatom mucilages have been more appropriately defined as extracellular polymeric substances (EPS; Hoagland et al. 1993), a term that is widely used to describe polysaccharides secreted external to the plasma membrane and that relates to their diverse chemical and physical nature (Sutherland 1988).

Achnanthes longipes, grown under ideal culture conditions, produces a stalk 200–500 μm long and 7–9 μm wide, which elevates it above the substratum. It is a common marine fouling diatom that is highly resistant to toxic antifouling coatings (Hendey 1951, Callow et al. 1976, Blunn and Evans 1981) and, thus, a contributor to the biofouling problem by increasing the frictional drag on ocean-going vessels. Cleaning ship hulls costs millions of dollars each year, and frictional drag leads to excess fuel consumption, leading to additional unnecessary loss of revenue (Alberte et al. 1992). Thus, there is clearly a need for understanding the biology of EPS production in *A. longipes,* which results in an increase in slime film thickness and, therefore, contributes to the fouling problem. EPS secretion leads to adhesion; thus, study of the former will lead to a better understanding of the latter.

The nutritional requirements for the formation of permanent EPS morphological structures such as stalks, tubes, pads, and capsules by diatoms is not well documented (Hoagland et al. 1993). EPS produced by marine planktonic diatoms under varying physiological conditions has been investigated most extensively. These secretions consist of soluble polymers, not insoluble EPS. However, insoluble EPS production by *Navicula pelliculosa* (Lewin 1955) and the benthic diatom *Amphora coffeaeformis* have been examined (Bruno et al. 1993). Extracellular polysaccharide release increases under conditions of reduced salinity (Allan et al. 1972), nutrient depletion (Lewin 1955, Myklestad and Haug 1972, Myklestad 1977), elevated N/P ratios (Myklestad and Haug 1972, Myklestad 1977), and stationary phase of growth (Lewin 1955, Myklestad and Haug 1972, Myklestad et al. 1989, Bhosle et al. 1993, Bruno et al. 1993). There are also conflicting arguments

[1] Received 8 November 1994. Accepted 23 February 1995.
[2] Author for reprint requests.

whether EPS is generated by healthy photosynthesizing cells (Mague et al. 1980, Fogg 1983) or is a product of senescent cells (Sharp 1977).

Prior to this study, Johnson (1995) found that *Achnanthes* cells grown in f/2-enriched ASPM (Guillard 1975), a defined seawater medium, formed only pads, whereas in f/2-enriched natural seawater they always produced stalks. This indicated that some factor necessary for stalk formation was lacking in f/2-enriched ASPM. Subsequently, an array of factors was tested, including medium dilution, macronutrients (N, P, N:P ratios, Ca, Mg, S), micronutrients (Fe, V, Li, Ru, Al, Br, I, F, f/2-enrichment concentration and dilution), heavy metals (Cd, Sr, Pb, Cr, Ba, Sn), chelators, pH, dialysis, ion exchange, and buffers (Johnson 1995). All were unsuccessful in inducing stalk secretion except bromide. In addition, the presence of elevated iodide levels in the culture medium not only prevented stalks but also resulted in the absence of all insoluble EPS. The current study addresses bromide as a nutrient required for stalk formation in *Achnanthes longipes* as well as the effect of iodide on the prevention of stalk secretion.

MATERIALS AND METHODS

A unialgal culture of *Achnanthes longipes* (NIES 330) was obtained from the National Institute for Environmental Studies, Japan. Cultures (xenic) were maintained in 300-mL flasks containing 200 mL of f/2-enriched ASPM seawater, a synthetic seawater medium (Guillard 1975), or Alga-Gro® seawater medium (Carolina Biological Supply, Burlington, NC), an enriched natural seawater medium ("ESW") at 18° C and illuminated on a 12:12 h LD cycle with cool-white fluorescent bulbs (photosynthetically active radiation = 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ measured with a LiCor® quantum light sensor, Lincoln, NE). In this report, the media f/2-enriched ASPM and Alga-Gro® seawater will be referred to as f/2 and ESW, respectively.

Conditions for EPS production. Cells were harvested from flasks with a rubber policeman, rinsed once and suspended in ASPM seawater base (without f/2 nutrients), and homogenized for 1 min at 8000 rpm with an Ultra-Turrax T-25 Tissuemizer (Tekmar, Cincinnati, OH). Ten milliliters of sterile media and 0.5 mL of homogenized cell suspension were added to a series of 15-×-60-mm sterile polystyrene Petri dishes and examined for EPS secretion after 2 days (EPS secretion type can be determined at this time).

Optimum chloride, bromide, and iodide concentrations for stalk production and inhibition of insoluble mucilage secretion were obtained by modification of procedures by McLachlan and Craigie (1967). Chloride was replaced by bromide in a graded series, both on a weight/volume basis while maintaining the 2.3% sodium salt concentration in f/2 (Guillard 1975). NaI was added weight/volume to the optimum chloride/bromide concentration for stalks.

Once optimum chloride, bromide, and iodide concentrations were determined, a protocol for obtaining various EPS secretion types was established. Chloride was kept constant, as a dilution of 51 M NaCl and various concentrations of bromide were prepared from 1.0 M NaBr. Different concentrations of iodide were made from dilutions of 6.7 M NaI.

Growth measurements. For cell number and optical density determinations, approximately 1×10^3 $cells \cdot mL^{-1}$ were grown in 15 mL of the following media: 1) f/2, 2) ESW, 3) f/2 prepared with 340/30 mM Cl^-/Br^-, and 4) f/2 prepared with 340/30/0.067 mM $Cl^-/Br^-/I^-$). Cells were harvested, and cell counts (Palmer-Maloney cell) and optical density readings (at 660 nm with a Milton-Roy spectrophotometer) were recorded every 3 days.

Extracellular carbohydrate release. Total soluble extracellular carbohydrate as μg glucose equivalents $\cdot cell^{-1} \cdot mL^{-1}$ media extract was determined after the method of Hanson and Phillips (1981), except that cells were grown and counted as for growth studies and the phenol-sulfuric acid sample mixture was allowed to cool 15 min before reading at 490 nm in a Perkin-Elmer λ-3B UV-VIS spectrophotometer. Total soluble glucose equivalents and cell densities were measured in quadruplicate.

Substitution of methionine for sulfate. f/2 medium was made up as earlier, except equimolar amounts of sulfur as L-methionine, 20 mM, were substituted for sulfate sulfur in f/2 medium, with other constituents kept constant. One group of cells was rinsed 4× in f/2 medium substituted with methionine to remove excess sulfate from the growth medium before a gradient of bromide concentrations was applied: 0 mM (f/2), 2.5 mM, 10 mM, 30 mM, and 30 mM Br^-/0.067 mM I^-. Another group of cells was rinsed 4× in f/2 prepared with sulfate and then placed in f/2 with methionine and the same series of bromide and iodide concentrations. With sulfate present in the inoculum, the final sulfate concentration in each Petri dish was calculated to be 0.06 mM.

Stalk quantification. For stalk quantification, two NaBr gradients were employed: a low-range gradient with bromide concentrations of 0.01, 0.1, 0.5, 1.0, 5.0, 10, 20, 30, 40, and 50 mM Br^- and a low- to high-range gradient with 10, 30, 100, 300, 400, 500, and 600 mM Br^-. Cells were added to Petri dishes as earlier, except 0.30 mL of $4–5 \times 10^5$ $cells \cdot mL^{-1}$ were introduced. Two Petri dishes were established per treatment, each containing a single 18-mm glass coverslip cleaned with 10% HCL, and incubated for 2 days. The experiment was performed twice for each gradient, using fresh media.

For lectin labeling, cells grown on coverslips (described earlier) were rinsed twice in phosphate-buffered saline (10 mM PBS, pH 7.2) for 10 min each and labeled with 25 μL of 100 $\mu g \cdot mL^{-1}$ *Lotus* lectin-FITC conjugate (E-Y Labs, San Mateo, CA) in PBS for 3 h. *Lotus*-FITC preferentially stained stalks rather than cells, making it a useful tool to visually isolate stalks for image analysis. *Lotus* lectin (isolated from *Lotus tetragonolobus*) is specific for terminal α-L-fucose residues (Sharon and Lis 1972), a terminal and major monosaccharide found in *A. longipes* (Gretz et al. 1993). Blocking nonspecific binding sites with bovine serum albumin and fixing the cells did not improve labeling intensity; therefore, the simpler and less time-consuming direct method was sufficient. Samples were rinsed for 1 min in PBS and mounted in Aqua-Polymount (PolySciences, Inc.) to hinder fluorescence quenching. Labeled stalks were examined on a Nikon Diaphot-TMD inverted fluorescence microscope equipped with a dual band set fluorescence filter cassette containing a FITC filter cube (Nikon B-1E/B-1A): excitation 485 nm and emission 535 nm. Photographs of *Lotus*-FITC–stained material were taken with Kodak Ektachrome Ultra 400 film.

To acquire images, 10 fields of view per coverslip were collected by an Optronics TEC-470 CCD video camera (Optronics Engineering, Goleta, CA) equipped with a 0.55× relay lens and 1.5× photo eyepiece. Images from the microscope were relayed to a Gateway 2000 P5-60 computer and processed with Optimas Image Analysis software, Version 4.1 (Optimas Corporation, Edmonds, WA). Every image was collected at the same camera speed (¼ s), contrast (7), and brightness (6) and with red and blue thresholds set at 0, all controlled by the Optronics TEC-470 CCD video processor. For analysis, image files (TIFF) were displayed on a Sony Trinitron PVM-1344Q color video monitor.

Lengths and widths of stalks were measured using Optimas Data Collection Utilities by drawing lines along the lengths and widths of stalks, in micrometers, on the computer image and storing the data. Pads and amorphous EPS were excluded in the

measurements. These data were used to calculate area and volume of stalks. Total EPS was measured from the same images by setting threshold values such that green fluorescing *Lotus*-FITC was distinguished from unstained background. Area, in square micrometers, of the stained EPS was calculated by estimating total percentage of pixels per percentage of threshold in micrometers of the ROI (or region of interest). Total amorphous EPS production (in μm^2) was finally compared to stalk area (in μm^2) as percentage of EPS area not containing stalks: [(total EPS area − total stalk area)/total EPS area] × 100.

Scanning electron microscopy. For scanning electron microscopy, cells grown on 10-mm glass coverslips were fixed in 2% glutaraldehyde in f/2 and postfixed in 1% OsO_4 at 25° C, dehydrated in ethanol, critical point–dried with liquid CO_2, and then mounted directly on aluminum stubs. Specimens were Au sputter–coated and then observed using the Cambridge S4-10 stereoscan scanning electron microscope (SEM).

Statistics and data analysis. A completely randomized design with replicates was used for growth interactions and soluble carbohydrate release with an algal culture tube as the experimental unit and an aliquot per tube as the sampling unit. Analysis of variance (ANOVA) was used to test the treatment effects of cell density and total soluble carbohydrate release, respectively. For image analysis data (stalk volume and total EPS area), the experimental design was a generalized randomized (complete) block design (Steel and Torrie 1980), with a coverslip as the experimental unit, image as the sampling unit, and a block as a "run" assumed to be a random effect. ANOVA was used to test treatment effects of bromide concentrations, using log-transformed data to meet the assumptions of homogeneity of variance. A significance value of $P < 0.05$ was used for all experiments and, when tests were significant, least squares (LS) means were used to compare treatment means.

RESULTS

Optimum bromide and iodide concentrations. Varying halide ratios resulted in four types of EPS secretion: 1) stalks, long, upright 200–500-μm-long secretions (Figs. 1, 3); 2) pads, small "cushion-like" secretions that, when stalks are present, are the spatially distinct regions where stalks are attached to the substratum (Fig. 5); 3) stalk-pads, prostrate distorted half-stalk, half-pad material (Fig. 6); and 4) no insoluble EPS present, only raphe EPS released; cells were "clean" and virtually free of a mucilaginous coating (cells treated with iodide; Figs. 2, 4). "Amorphous EPS" was any secretion having no distinguishable form.

The optimum level of bromide and chloride for stalk secretion (i.e. in comparison to cells grown in ESW) was 340 mM Cl^- and 30 mM Br^-, respectively (Table 1). As Br^- concentrations were sequentially decreased, there was a gradation in EPS morphology from stalks to stalk-pads to solely pads. Only pads were produced in f/2 medium, which lacked Br^-.

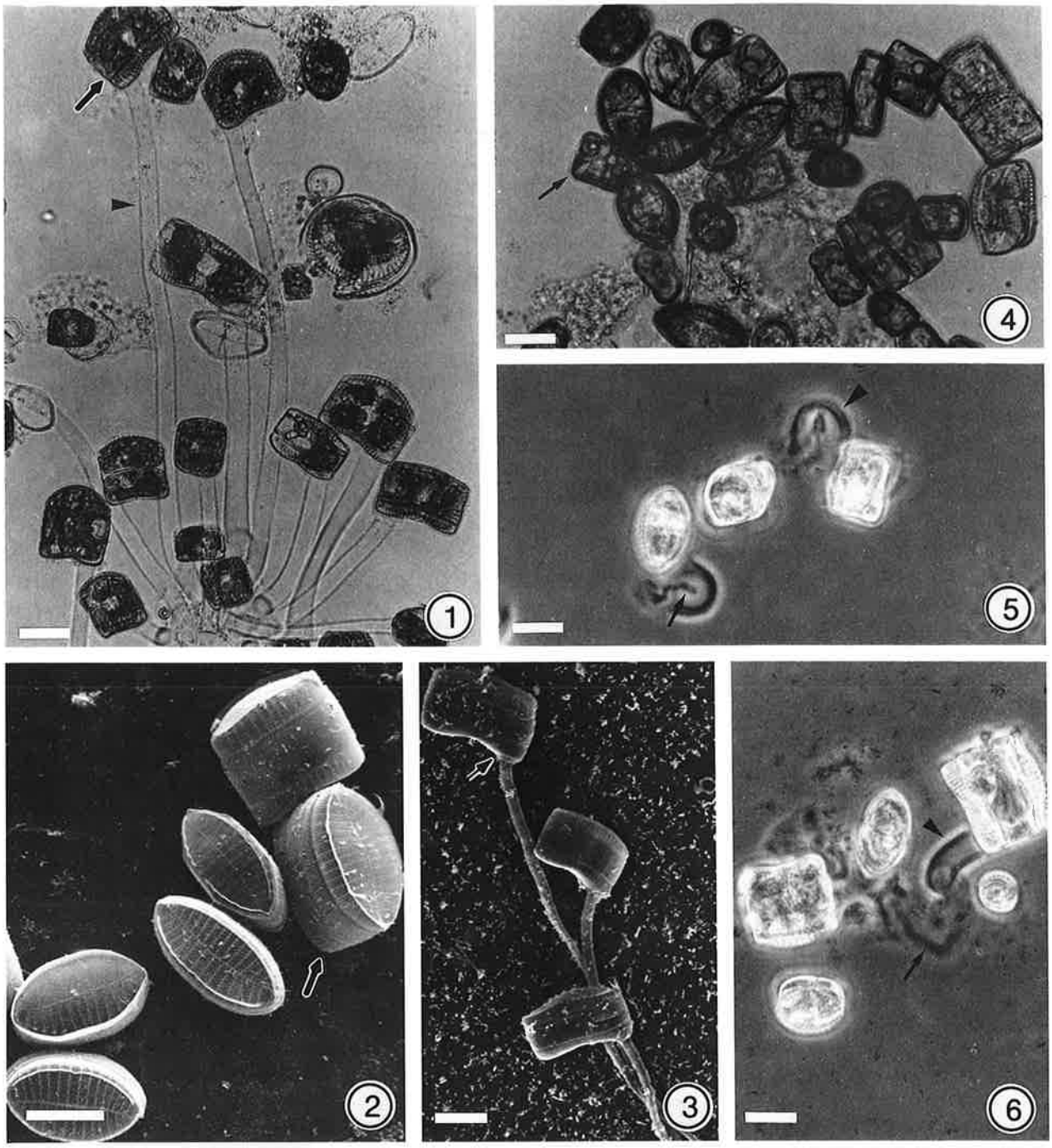
Increasing I^- levels resulted in a similar effect, with a change from a predominance of stalks to pads, until the molarity reached 0.067 mM, in which no insoluble EPS was produced even in the presence of Br^- and cells clung weakly to the substratum. This phenomenon was apparently irreversible because after repeated rinsing and adding stalk-inducing medium, cells still did not form stalks.

Growth study. The concentrations of Br^- and I^- required for optimum stalk production were much higher than those found in seawater, 0.84 mM and 0.5 μM, respectively (Brewer, 1975); consequently, growth measurements were performed to determine whether or not the concentrations of Br^- and I^- affected cell growth rates. Determination of growth rates was particularly critical for iodide, given the possibility that the production of no insoluble EPS by cells grown in elevated iodide concentrations was a result of physiological stress.

End-point analysis (one-way ANOVA) showed a significant difference in cell density among the four treatments ($P = 0.0001$, Fig. 7). Cells grown in defined media showed no significant difference in cell densities. However, the ESW treatment had a significantly lower density ($P = 0.0001$) than the other three, probably due to the much higher nutrient levels in the defined media.

Total soluble extracellular carbohydrate secretion. The growth study showed that elevated I^--treated cells grew at rates very similar to those grown in defined media lacking I^-, except that they did not produce insoluble EPS. Total soluble extracellular glucose equivalents were estimated for stalk-producing, pad-producing, and noninsoluble EPS–producing cells to determine whether or not iodide-treated cells secreted more soluble carbohydrate into the medium. Soluble carbohydrate release in cells having pads, stalks, and no stalks/pads culminated early and then dramatically decreased with time (Fig. 8). Total soluble carbohydrate secretion reached a maximum at 3 days, then decreased through 5 days, followed by a gradual decline up to 28 days. There was a significant treatment effect of total soluble carbohydrate secretion ($P = 0.0001$). Cells producing no stalks or pads released significantly more soluble carbohydrate into the medium than the other two treatments throughout the 4-week study period, except at 3 days, where LS means showed there was no significant difference among the three treatments ($P = 0.2496$). LS means also showed that at days 19 ($P = 0.2147$) and 26 ($P = 0.0656$) no significant difference in total soluble carbohydrate existed between pad-formers and stalk-formers.

Stalk volumes along a bromide gradient. Different amounts of stalk material were produced under different bromide concentrations (Fig. 9). At the lower concentration gradient (0.01–50 mM Br^-), an exponential increase in stalk volume occurred from 0.01 to 5.0 mM Br^-, then remained constant from 10 to 20 mM Br^-. Subsequently, an abrupt doubling in stalk volume at the optimum bromide level (30 mM Br^-) followed by an abrupt twofold decrease in stalk volume and a slight decline at 40–50 mM Br^- (see Fig. 9, inset) were observed. There was a significant treatment effect of bromide concentration on subsequent stalk formation ($P = 0.0142$), and LS means showed that 30 mM Br^- differed significantly from all other bromide levels. The second gradient (10–600 mM Br^-), which encompassed the upper



FIGS. 1–6. EPS secretion morphology with varying bromide levels. Scale bars = 20 μm. FIG. 1. *Achnanthes* cells with optimum stalks. Cells (arrow) are variable in size but average 20 μm in width by 30 μm in length. Widths and lengths of stalks (arrowhead) also vary, 7–9 by 200–500 μm, respectively. Treatment: 340/30 mM Cl^-/Br^-. Nonstained, live preparation. Bright field optics. FIG. 2. SEM of iodide-treated (0.067 mM I^-) cells. Note that no insoluble EPS has been secreted by the cells. Arrow indicates approximate region on the valve face, i.e., the raphe, where pads and stalks are normally secreted. FIG. 3. SEM of cells with stalks. Arrow indicates the collar region, which becomes separated from the pad as the stalk develops. ESW treatment. FIG. 4. Bright field micrograph showing iodide-treated cells. Again, no insoluble EPS has been secreted. Arrow points to area on cell where insoluble EPS is secreted. Cells lack firm adhesion and adhere to the substratum by only raphe mucilage. Black asterisk (bottom center) shows the biofilm residue normally associated with the bottom of the flask in which the cells were originally grown before transferring to media containing 0.067 mM I^-. FIG. 5. Phase-contrast micrograph of cells with pads (arrowhead). Note the highly distinctive core in the center of the pad. Pads are often elongated, showing an extended core (arrow). f/2 treatment. Unstained, live preparation. Phase-contrast optics allows visualization of the cores without staining. FIG. 6. Cell with stalk-pad, consisting of a short stalk portion (arrowhead) and an amorphous pad region with distorted "ladder-like" core region (arrow). Note that the stalk portion is normal, lacking a deformed core; however, the usual thin central core cannot be seen in this photograph. 340/70 mM Cl^{-1}/Br^- treatment. Unstained, live preparation. Phase-contrast optics.

TABLE 1. *Effect of bromide and iodide on EPS secretion morphology. + = present, − = absent. Stalk-pad = prostrate distorted half-stalk, half-pad material.*

Halide molarity (mM)			Secretion morphology		
Cl^-	Br^-	I^-	Stalk	Stalk-pad	Pad
340[a]	0	0	−	−	+
340[b]	30[b]	0	+	−	−
340	10	0	+	+	+
340	0.1	0	−	+	+
340	0.01	0	−	−	+
340	30	67×10^{-6}	+	−	−
340	30	67×10^{-5}	−	+	−
340	30	67×10^{-4}	−	−	+
340	30	67×10^{-3}	−	−	−

[a] Molarity of NaCl in f/2 medium.
[b] Optimum stalk production levels.

bromide level where no stalks formed, again showed a threefold increase, between 10 mM Br^- and the optimum level (30 mM Br^-), followed by a greater than threefold decrease at 100 mM Br^- (Fig. 9). A gradual leveling occurred between 100 and 400 mM Br^-, and there was an abrupt leveling of stalk production from 400 to 600 mM Br^-. No stalks were formed at the highest Br^- level (600 mM). Again, there was a significant treatment effect of bromide on stalk secretion ($P = 0.0057$), and LS means showed 30 mM Br^- to be significantly different from all other bromide concentrations.

Total EPS secreted along a bromide gradient. The trend seen for total EPS secreted and stalk area along the bromide gradient (Fig. 10) was similar to that

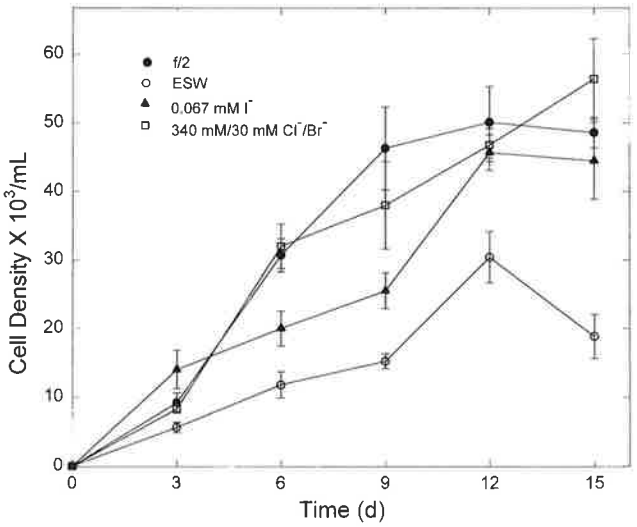


FIG. 7. Cell density (cells·mL^{-1}) as a function of time. Vertical bars = ±1 SE. There was a significant treatment effect on cell density ($P = 0.0001$); cells grown in ESW exhibited much lower growth than cells grown in defined media. There was no significant difference between cells grown in f/2, elevated iodide (0.067 mM I^-), and the optimum bromide level (340/30 mM Cl^-/Br^-), indicating elevated iodide levels were not physiologically stressful to cells.

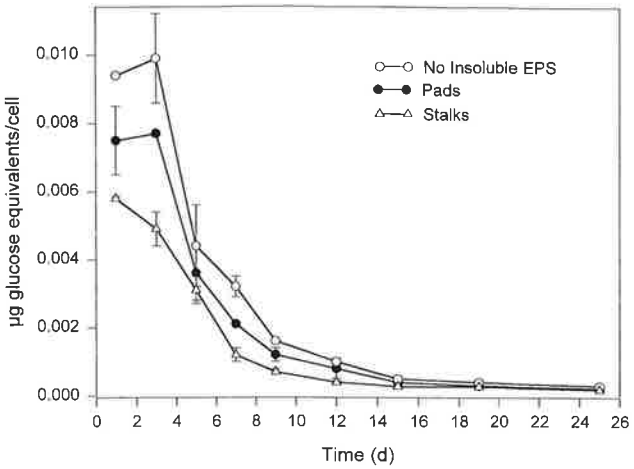


FIG. 8. Total extracellular carbohydrate (as glucose) for three types of EPS released over time. Secretion maxima at 3 days, followed by a sharp decline and leveling off after 2 weeks. Vertical bars = ±1 SE. Cells producing no insoluble EPS secrete significantly more carbohydrate than cells making stalks or pads ($P = 0.0001$), except at day 5, where there was no significant difference among the treatments ($P = 0.2496$).

observed for stalk volumes. At the lower bromide gradient, an exponential increase occurred from 0.01 to 30 mM Br^-, followed by an exponential decrease to 50 mM Br^- (Fig. 10B, inset). However, in this case no twofold increase occurred at the optimum level of 30 mM Br^-. A significant difference between bromide treatments was observed ($P = 0.0219$). However, LS means showed no significant effect of bromide concentration between 20 mM bromide and the optimum bromide level, 30 mM ($P = 0.1352$). The threshold spanning the lower to upper range, 10–600 mM Br^-, showed a twofold increase to the optimum level of 30 mM Br^-, followed by a three-

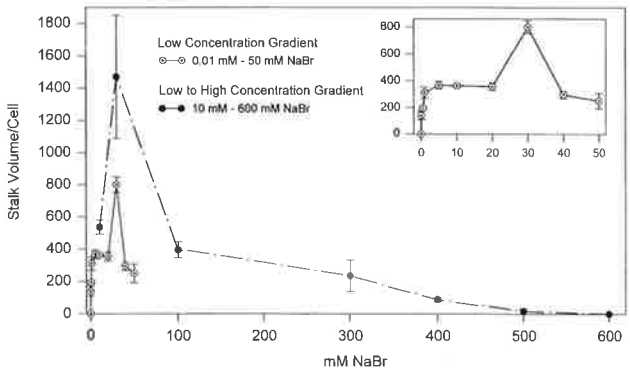


FIG. 9. Stalk volume expressed as a function of molar NaBr concentration. Vertical bars = ±1 SE. The low concentration gradient shows an exponential increase in stalk volume from 0.01 to 5 mM Br^-, then a constant response between 10 and 20 mM, followed by an exponential rise to the optimum bromide level (30 mM) and a gradual decline from 40 to 50 mM ($P = 0.0142$). Inset shows expanded curve. The low to high concentration gradient from 10 to 600 mM Br^- shows a threefold maximum in stalk volume at 30 mM, followed by a leveling off at higher concentrations ($P = 0.0057$).

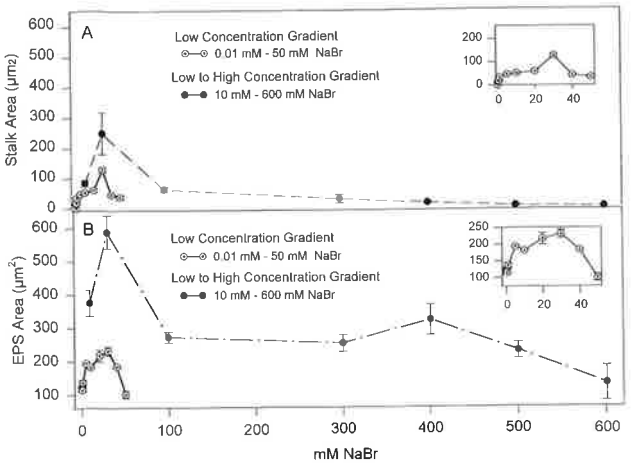


FIG. 10. Molar NaBr concentration along the same two bromide gradients as for stalk volume vs. A) stalk area and B) total EPS area. Vertical bars = ±1 SE. Both curves show the same general shape as for stalk volume, particularly the peak at 30 mM Br^-. This peak is not so pronounced for the low concentration gradient in the Total EPS area graph (see inset) and $P = 0.1352$ for 30 and 10 mM Br^-. Note the general increase in EPS area vs. stalk area, especially at the very low and very high Br^- concentrations.

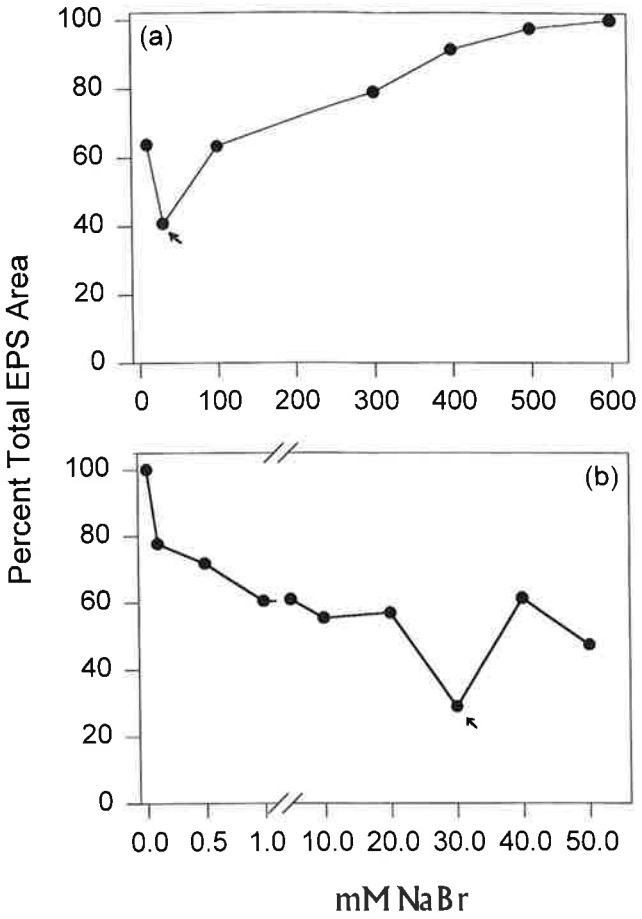


FIG. 11. Percentage of EPS area not containing stalks; produced in response to a) the high-range and b) the low-range bromide gradients. Arrow = optimum bromide concentration, 30 mM Br^-. Treatments with lower stalk volume tend to show larger areas without stalks, indicating much of the total EPS is not polymerized into stalks. Also, note that the lowest area of amorphous EPS occurs at the optimum bromide concentration because most of the polymer exists as stalks.

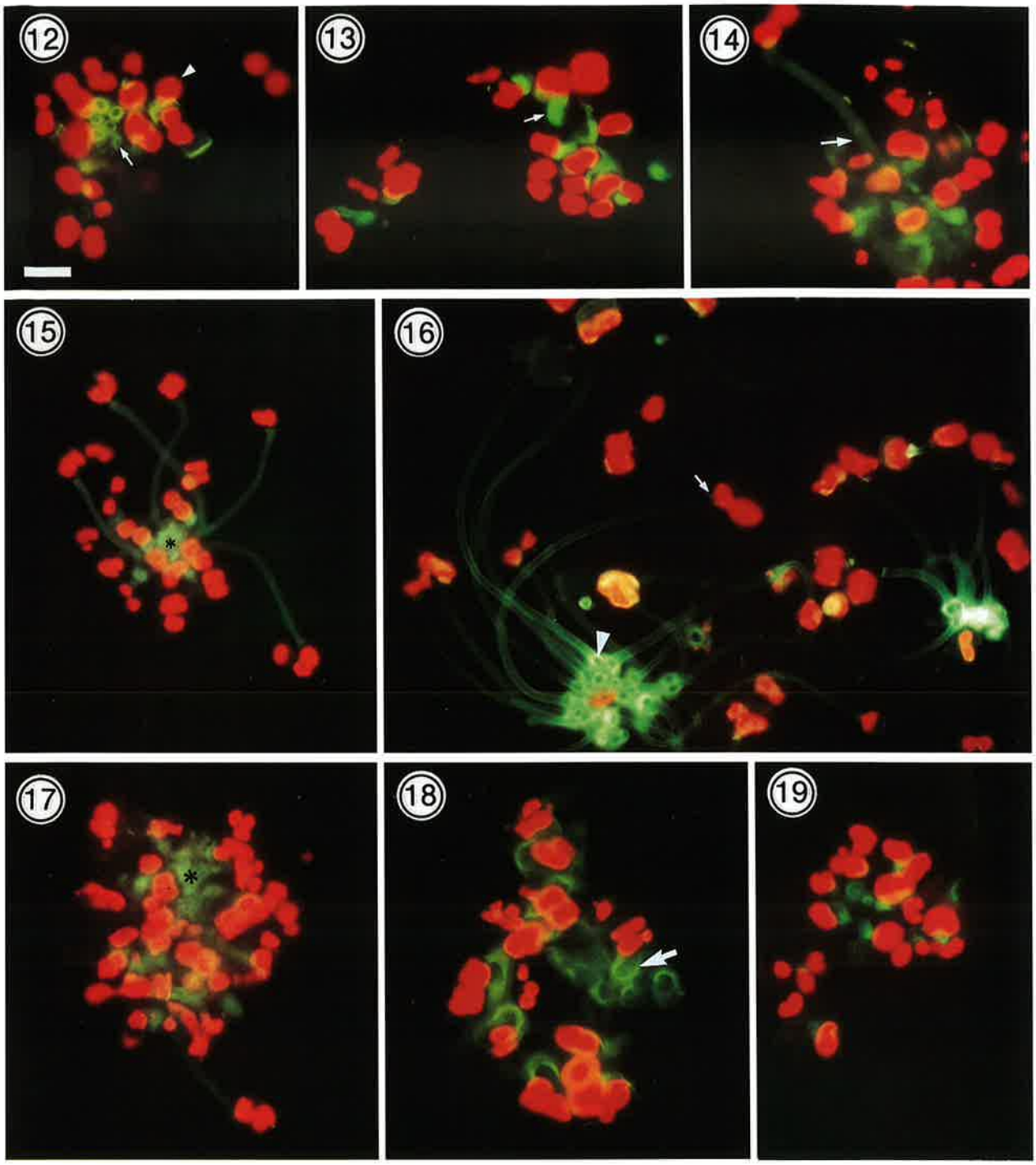
fold drop to 100 mM Br^-. This was followed by a leveling off at 300 mM Br^- and an increase at 400 mM Br^-, followed by a linear decrease to 600 mM Br^- ($P = 0.0028$); LS means showed that the EPS area at 30 mM Br^- was significantly different from the other concentrations.

EPS was produced at all levels of bromide. Total EPS area was at least twice that of stalk area (Fig. 10B). Those bromide levels resulting in production of decreased stalk areas (i.e. fewer or shorter stalks) tended to have a larger percentage of increase in EPS area (Fig. 11), reflecting a higher quantity of amorphous EPS. The lower the stalk area, the greater the corresponding total EPS area. Total EPS areas increased about 100-fold for those bromide levels where no stalks were formed, 0.01 and 600 mM Br^-. Conversely, cells grown at the optimum bromide level (30 mM) had the lowest percentage of total EPS area increase, reflecting that most of the secreted EPS occurred as stalks.

The trend in stalk and EPS secretion along a bromide gradient progressed from cells possessing only pads (Fig. 12), to stalk-pads or elongated pads (Fig. 13), to a few thick stalks, stalk-pads, and amorphous EPS (Fig. 14), to many small stalks and amorphous EPS (Fig. 15), and finally to stalks (Fig. 16). At higher concentrations of bromide, beyond the optimum threshold, a reversal in the trend is observed: from few short stalks, stalk-pads, and amorphous EPS (Fig. 17), to stalk-pads and pads (Fig. 18) and to only pads at the highest bromide concentration (Fig. 19).

Substitution of methionine for sulfate. Cells rinsed 4× in f/2 prepared with methionine, and subsequently placed in media of varying concentrations of bromide substituted with methionine, appeared healthy. However, they lacked adhesion and seemed to have reduced division rates. No motile cells, even those positioned on their valve face, were observed until 4 days and only in 30 mM Br^-. Normally, cells become motile within a few hours after inoculation.

Cells that were rinsed 4× in f/2 prepared with magnesium sulfate (20 mM) and then distributed to media containing methionine and differing bromide concentrations were healthy, rapidly dividing and actively motile within several hours. The final sulfur concentration in each of the Petri dishes was 0.06 mM sulfur. No insoluble EPS, as pads, stalk-pads, or stalks, were produced at any bromide concentration. Iodide + methionine–treated cells appeared no different from bromide + methionine–treated cells. Cells were weakly adherent, apparently attached to the substratum only by raphe mucilage.



FIGS. 12–19. Cells and EPS labeled with *Lotus*-FITC. The label is EPS-specific; EPS is green while cells appear red due to chlorophyll autofluorescence. Scale bars = 50 μm. FIG. 12. 0.01 mM Br^-, lowest bromide concentration where no stalks form. Only pads are present (arrow). The label is more specific for outer regions of the pads making them appear ring-like. Arrowhead = single cell. FIG. 13. 0.1 mM Br^-. Note elongation of pad mucilage into stalk-pad morphology (arrow). FIG. 14. 5 mM Br^-. Few stalks are present at this concentration (arrow). Stalks formed at suboptimum bromide levels tend to be short and stout. FIG. 15. 10 mM Br^-. Well-developed thin stalks, although relatively short. Amorphous EPS, not polymerized into stalks, still present (small black asterisk). FIG. 16. 30 mM Br^-. Bromide concentration optimum for stalk secretion. Stalks well defined and extremely elongate. The collar region (arrow) stained more intensely than the stalks. It appears ring-like as do the pads (arrowhead), suggesting a similar origin and polymer composition. FIG. 17. 100 mM Br^-. Increasing the concentration of bromide above the optimum level resulted in a similar trend as that seen for the suboptimum levels. Note similarity of morphology to that seen in Figure 14. Stalks are short and wide. The amount of amorphous EPS dramatically increased. FIG. 18. 300 mM Br^-. At elevated levels of bromide, EPS is formed as elongate pads (arrow). EPS secreted at high bromide concentrations was less defined than that at very low concentrations; compare to Figures 12 and 13. FIG. 19. 600 mM Br^-. Extreme bromide level at which no stalks are produced. Pads stain uniformly, more weakly specific for the outer region.

DISCUSSION

Growth requirements for bromide and iodide. This investigation demonstrates that bromide is essential for stalk secretion in the fouling diatom *Achnanthes longipes,* while elevated iodide concentrations inhibit insoluble EPS production and limit adhesion. The physiological requirement for bromide is sparsely documented in algae with reports confined to the red and brown algae. *Polysiphonia urceolata* requires bromide for optimal growth (Fries 1975), and the refractile inclusions in vesicle cells of members of the Bonnemaisoniaceae fail to form in bromide-limiting conditions (Wolk 1968). Pedersén (1969) found that low levels of bromide stimulated growth in several brown algal species, the effect being reversed at high levels (100 μM). Eliminating bromide from the culture medium resulted in diminished growth of *Fucus edentatus* embryos (McLachlan 1977). The demand for iodide has been found for several red and brown algae (Fries 1966, Iwasaki 1967, Pedersén 1969, Woolery and Lewin 1973), whereas no iodide requirement was found for green algae or chrysophytes (von Stosch 1961).

This study represents the first account of bromide required for a developmental process in diatoms. However, contrary to reports on the red and brown algae, bromide is not required for maintenance of optimal growth rates in *Achnanthes longipes,* because optimal growth rates were observed in the presence or absence of bromide. This phenomenon suggests that the possession of stalks is not crucial to the growth of these organisms; however, *A. longipes* was grown under nutrient-rich conditions without competition. In nature, *A. longipes* competes for space, light, and nutrients. In a biofilm where many sessile and motile diatoms occupy the substratum and compete for nutrients, the possession of a securely attached stalk that raises the cell into a region where there may be more nutrients and optimal light intensity may confer an advantage (Blunn and Evans 1981, Hoagland et al. 1982, Hudon and Legendre 1987, Jönsson et al. 1994).

Although this investigation reports the requirement of bromide for stalk secretion in *Achnanthes longipes,* the role of bromide in stalk induction and the mechanism of iodide inhibition is unknown. Nevertheless, these potential roles will be discussed based on limited evidence, primarily from studies of macroalgae.

Varying EPS quantity reflects lack of polysaccharide polymerization. Different quantities of stalk and EPS material were produced under varying bromide concentrations, implying incomplete crosslinking or polymer gelation except at the optimum bromide concentration. All treatments produced pads, except the elevated iodide treatment. Normal stalk ontogeny begins as the EPS, secreted through one end of the raphe, is first secreted as a basal pad (Daniel et al. 1987). Subsequently, additional material is added, and the stalk raises the cell above the substratum (White and Chamberlain 1982, Daniel et al. 1987). It is unknown whether or not there is cell polarity and whether or not the stalk is secreted from a particular raphe end. The duration of stalk secretion in *A. longipes* was briefly recounted by Hendey (1951), who described initial pad formation followed by "elongation of this mucus pad into a stalk or stipe" as occurring in a "few hours." More investigation of this very basic phenomenon is needed to corroborate biochemical and physiological data. According to White and Chamberlain (1982) and Daniel et al. (1987), some of the basal pad material is carried along with the elongating stalk and develops into a ring-like structure called the collar (Fig. 12), which envelopes the stalk at the proximal end. The presence of a collar also has been described by Blunn and Evans (1981). The similar nature of pad and collar material has been suggested based on histochemical results, in that a strong periodic acid–Schiff reaction was seen in both structures; however, the pad showed traces of sulfation, whereas the collar gave a pure carboxylated reaction (Daniel et al. 1987). They attribute this to the extension of stalk material, growing away from the pad. The pad and collar stain more intensely than the stalk region with fucose-specific lectins *Lotus,* (Figs. 12–14), *Anguillia* (Johnson, unpubl.), and, more so, *Ulex* (Wustman, unpubl.), also suggesting a similar nature of the pad and collar. At the same time, this implies that the stalk may be quite different in chemical composition and, thus, in physical properties, i.e. gel quality, as well. The stalk may be composed of a polymer matrix with much more rigid properties than pads; thus, when cells are grown in suboptimum bromide concentrations as well as very high levels of bromide, there may be an inhibition of stiff gel polymerization or crosslinking limiting the ability to form an upright stalk. However, we presently are unable to distinguish between the suppression of gelation or crosslinking in this system. *Achnanthes* stalks are composed predominantly of polysaccharides. Neutral monosaccharide analysis shows that the stalk consists primarily of terminal and 3-linked fucosyl residues (Gretz et al. 1993). Methylation analysis provided evidence for sulfated and carboxylated polymers, and protein was found to comprise 5.0% of the total stalk fraction (Gretz et al. 1993). This analysis is inclusive of the entire insoluble stalk structure secreted by *A. longipes,* including both the pad and collar region, and thus may be misrepresentative of these potentially discrete regions. The chemistry of *Achnanthes* pad polymers is presently under investigation and should reveal whether the pads and stalks are chemically distinct.

As demonstrated by the current study, cells producing no insoluble EPS secrete significantly more total soluble extracellular carbohydrate into the medium. This suggests that they are unable to secrete polymer which can form molecular associations leading to an insoluble matrix; thus, soluble com-

ponents are released into the surrounding medium. Stalk formers release significantly less soluble extracellular carbohydrate, suggesting that much of the manufactured photosynthate is packaged into stalk material. Pad-producing cells secrete an intermediate amount of soluble extracellular polysaccharide because they do form some insoluble EPS. This is also supported by the results of measuring total EPS area along a bromide gradient. Total EPS area (with the exception of 20 mM; see Fig. 10B) for the optimum bromide concentration (30 mM) was greater than all other bromide levels. Some EPS underwent gelation or crosslinking forming amorphous EPS; however, some of the polymer must have been soluble and released into the medium, without association within the matrix. The physical properties, such as gel formation, of polysaccharides are influenced by polymer shape, which depends on basic chemical structure and solvent properties (Christensen 1989, Christensen and Characklis 1990). That we see variation in EPS secretion type implies that the chemical composition or solvent properties are altered with varying bromide and iodide levels. Polymer matrix properties of EPS in other marine diatoms change when grown in different media, although the exact mechanism is unknown. The diatom *Nitzschia frustulum,* grown in high salinity media, produced EPS that was soluble whereas in low salinity media it tended to form EPS in the form of flocculent precipitates (Allan et al. 1972).

Soluble extracellular carbohydrate release decreased dramatically with time; i.e. cells in the log phase secreted significantly more glucose equivalents than during the stationary phase (Fig. 8). This has also been demonstrated for the planktonic diatom *Chaeotoceros affinis,* in which rapidly dividing cells released more extracellular carbohydrate than stationary phase cells (Myklestad et al. 1989). However, when photosynthetic rate is accounted for, as total cell carbohydrate, its marked decrease during the stationary phase contradicts this finding. Relative extracellular release was much more pronounced in senescent cells (Claus 1988, Myklestad et al. 1989). Total cell carbohydrate was not measured in this investigation but should be considered in future studies.

Possible role of sulfate in gel polymerization. Achnanthes stalks consist of four concentric layers; histochemical work indicated negatively charged polysaccharides, with stains for localized uronic acids, in the outer three layers and neutral sugar localization in the outer two layers (Daniel et al. 1987). Neutral polysaccharides were also localized in the outer layers of the stalk of *A. subsessilis* by Blunn and Evans (1981). The inner layer and the interface between the third and inner layers stained intensely for sulfate groups (Daniel et al. 1987). This has also been confirmed by x-ray microanalysis on thin sections of *A. longipes,* where a strong sulfur peak occurred in the dense fibrillar region of the stalk (Johnson 1995). Sulfur has also been detected in the stalk central core by SEM x-ray analysis. (Novarino 1993). Highly sulfated stalk centers have been documented in other fouling diatoms such as *Achnanthes subsessilis* (Blunn and Evans 1981) and *Licmophora flabellata* and *L. abbreviata* (Daniel et al. 1987). Examination of normal stalks and distorted stalk-pads showed that normal stalks have a long, thin central core, whereas the stalk-pad has a thickened "ladder-like" core (Fig. 6). These stalk-pads are prostrate, growing along the surface of the substratum. We suggest that without the optimum bromide concentration, cells may be unable to adequately incorporate sulfate linkages between neighboring polymer chains and consolidate them into a rigid core matrix. In addition, substituting methionine for sulfate in the growth medium resulted in no stalk formation despite the presence of an optimum bromide level. No insoluble EPS was produced, and cells lacked firm adhesion to the substratum. This implies that sulfate plays an integral role in polymer assembly and/or secretion and gel formation, not only in stalk secretion but also in insoluble EPS production. Sulfation of fucan is required for adhesion (Crayton et al. 1974) and localized insertion of polysaccharides into the rhizoid wall of *Fucus* zygotes (Hogsett and Quatrano 1978, Quatrano 1982). Binding between neighboring sulfated oligosaccharide side chains on glycoproteins is attributed to cell wall assembly or attachment to the cell membrane in *Chlamydomonas reinhardii* and *Lobomonas piriformis* (Roberts et al. 1980). *Achnanthes* stalks contain trace amounts of protein (Gretz et al. 1993). The addition of methionine results in no insoluble EPS production, suggesting that the integrity of the stalks and pads is not sustained by disulfide bridges in the protein component but that free sulfate groups are essential for polymer structure.

Possible bromide requirement for enzyme catalysis. The necessity for bromide may involve the action of a haloenzyme that uses bromide in catalysis, such as a bromoperoxidase. This function is currently under investigation. Haloenzymes are responsible for the ability of marine algae to extract halides, Cl^-, Br^-, and I^-, from the water and synthesize many different halometabolites (Fenical 1975, Neidleman and Geigert 1986, Kirk 1991).

Bromometabolite synthesis has been documented in some marine diatoms; however, nearly all halometabolites have been characterized for macroalgae. Recently, bromoform and bromoalkane emission by ice diatoms *Nitzschia stellata* and *Porosira pseudodenticulata* (Sturges et al. 1992, 1993) has been measured. A brominated phenolic benzaldehyde synthesized by *Navicula* sp. has also been reported (Manley and Chapman 1978). Haloperoxidases have not yet been characterized for diatoms thus far; however, the presence of bromometabolites in diatoms suggests that they may be present.

Although haloperoxidases have not been directly implicated in the process, peroxidases are enzymes involved in cell wall biosynthesis in higher plants through their oxidation of phenolic compounds (Fry

1982a, b, Cooper et al. 1984, Northcote 1985). It is believed that phenolic compounds play a role in wall synthesis and stabilization through crosslinking between glycoproteins by means of covalent bonds or chelated complexes (Smith and O'Brien 1979, Cooper et al. 1984, Fry 1988). However, a similar process is now believed to occur in brown algae (Vreeland and Laetsch 1990, Vreeland et al. 1993).

Vreeland et al. (1993) found that both carbohydrate and phenolic substances were secreted during initial adhesion of *Fucus* zygotes. Based on experimental evidence such as vanadate peroxidase activity in secretory vesicles during zygote attachment and evidence that phenolics crosslink with carbohydrate "fibers" in the presence of this enzyme, the authors confirmed that a vanadate-requiring haloperoxidase catalyzes the formation of phenolic crosslinks between wall carbohydrates. The finding that *Fucus* zygotes secrete numerous phenolic vesicles immediately following zygote fertilization led Clayton and Ashburner (1994) to consider the possibility that phenolics may also have a role in zygote wall formation in *Durvillaea.*

Achnanthes stalks subjected to ultraviolet light (300–400 nm) did not autofluoresce (Johnson, unpubl.), a preliminary indicator of the presence of phenolic compounds (Fry 1979, Smith and O'Brien 1979). Other methods for detecting phenolics will be used in future work. Although the presence of phenolic compounds and haloperoxidase activity in *A. longipes* has yet to be demonstrated, it is conceivable that the process of stalk secretion is similar to that of wall synthesis and adhesion in *Fucus.*

Vanadate added to or deleted from the growth medium of *A. longipes* did not affect stalk production (Johnson 1995). However, it was introduced at levels much higher (0.5–500 μM) than that found in seawater, 50 nM (Brewer 1975). The effects of lower concentrations of vanadate should be examined; nevertheless, the influence of trace contamination in other media salts cannot be overlooked.

Inhibition by bromide and iodide. Stalk secretion was suppressed by both very low and extremely high bromide concentrations (Figs. 9, 10). As with any required nutrient, very low bromide levels were insufficient to elicit the physiological process of stalk secretion, while too much bromide in the medium was inhibitory. Although we presently have no evidence of enzyme involvement in *Achnanthes* stalk secretion, elevated bromide levels inhibit haloenzyme activity in a number of marine algae (Manthey and Hager 1989, Soedjak and Butler 1991, Sheffield et al. 1993, van Schijndel et al. 1993).

Results of the soluble extracellular carbohydrate release experiment suggest that EPS is synthesized under elevated iodide concentrations but is soluble and released into the surrounding medium. The action of elevated iodide may prevent gelation of stalk polymers, possibly by inhibiting the action of bromide. That iodide may irreversibly prevent the action of bromide is supported by the observation that once *Achnanthes* cells have been cultured in iodide-treated media, they no longer form stalks when this media is replaced by a stalk-promoting one, and growth is not impaired. Although we do not yet know the mechanism in *A. longipes,* there are reports of iodide inhibition from other marine algal systems: 1) iodide acts as an enzyme inhibitor of selected haloperoxidases (Pedersén 1976, Neidleman and Geigert 1986), and 2) iodide prevents gelation and assembly of polymers (Grasdalen and Smidsrød 1981, Watase et al. 1990).

Variability among bromide treatments. The basis for the large variability seen among treatments of the same bromide concentration, despite the consistency in the general trend along the bromide gradient, is not known. Ideal stalks are invariably obtained in ESW, while in defined media optimum stalks (200–500 μm in length) were not consistently produced. This suggests that some other factor was involved in maximal stalk secretion other than bromide.

Concluding remarks. Because this is the first report on the role of bromide in *Achnanthes* stalk secretion, we have drawn on existing evidence from other algal groups in an attempt to hypothesize on the bromide requirement in this fouling diatom. In short, the mechanisms of the stimulatory function of bromide and inhibitory role of iodide in *A. longipes* stalk secretion are unknown. However, this study sets the stage for subsequent work investigating the roles these halides have in the regulation of stalk development in *Achnanthes longipes.* Further investigation should involve elucidation of the following: 1) peroxidase or bromoperoxidase activity, 2) localization of alleged phenolic compounds in the stalk or cell of the diatom, 3) localization of bromide and iodide in the cell or stalk using x-ray microanalysis, and 4) degree of sulfation in stalks versus amorphous EPS.

This research was funded by Office of Naval Research grant N00014-91-J-1108 to KDH and MRG, and the UNL Water Center (KDH). The authors thank Professors James R. Rosowski for conceptual input and support and Kent M. Eskridge for statistical advice and help with SAS. This work is part of a dissertation by LMJ submitted in partial fulfillment of the requirements for a Ph.D. degree from the School of Biological Sciences, University of Nebraska, Lincoln.

Alberte, R. S., Snyder, S., Zahuranec, B. J. & Whetstone, M. 1992. Biofouling research needs for the United States navy: program history and goals. *Biofouling* 6:91–5.

Allan, G. G., Lewin, J. & Johnson, P. G. 1972. Marine polymers. IV. Diatom polysaccharides. *Bot. Mar.* 15:102–8.

Bhosle, N. B., Evans, L. V. & Edyvean, R. G. J. 1993. Carbohydrate production by *Amphora coffeaeformis,* a marine fouling diatom. *Biofouling* 7:81–91.

Bishop, J. H., Silva, S. R. & Silva, V. M. 1974. A study of microfouling on antifouling coatings using electron microscopy. *J. Oil Col. Chem. Assoc.* 57:30–5.

Blunn, G. W. & Evans, L. V. 1981. Microscopical observations on *Achnanthes subsessilis,* with particular reference to stalk formation. *Bot. Mar.* 24:193–9.

Brewer, P. G. 1975. Minor elements in sea water. *In* Riley, J. P. & Skirrow, G. [Eds.] *Chemical Oceanography,* Vol. 1. Academic Press, New York, pp. 417–9.

Bruno, M., Coccia, A. & Volterra, L. 1993. Ecology of mucilage production by *Amphora coffeaeformis* var. *perpusilla* blooms of Adriatic sea. *Water, Air Soil Pollut.* 69:201–7.

Callow, M. E. 1993. A review of fouling in freshwaters. *Biofouling* 7:313–27.

Callow, M. E., Evans, L. V. & Christie, A. O. 1976. The biology of slime films. *Shipp World Shipbuild.* 3923:949–51.

Caron, D. A. & Sieburth, J. M. 1981. Disruption of the primary fouling sequence on fiber glass-reinforced plastic submerged in the marine environment. *Appl. Environ. Micro.* 41:268–73.

Chamberlain, A. H. L. 1976. Alga settlement and secretion of adhesive materials. *In* Sharpley, J. M. & Kaplan, A. M. [Eds.] Proceedings of the 3rd International Biodegradation Symposium. Applied Science Publ., London, pp. 417–32.

Characklis, W. G. & Cooksey, K. E. 1983. Biofilms and microbial fouling. *Adv. Appl. Micro.* 29:93–138.

Christensen, B. E. 1989. The role of extracellular polysaccharides in biofilms. *J. Biotechnol.* 10:181–202.

Christensen, B. E. & Characklis, W. G. 1990. Chemical and physical properties of biofilms. *In* Characklis, W. G. & Marshall, K. C. [Eds.] *Biofilms.* John Wiley & Sons, New York, pp. 93–130.

Claus, W. 1988. Einfluss des Wachstums auf die Bildung extrazellulärer Kohlenhydrate und Proteine von zwei marinen Plankton-Algen. *Mitt. Inst. Allg. Bot. Hamburg* 22:63–78.

Clayton, M. N., & Ashburner, C. M. 1994. Secretion of phenolic bodies following fertilisation in *Durvillaea potatorum* (Durvillaeales, Phaeophyta). *Eur. J. Phycol.* 29:1–9.

Cooper, J. B., Chen, J. A., Varner, J. E. 1984. The glycoprotein component of plant cell walls. *In* Dugger, W. M. & Bartnicki-Garcia, S. [Eds.] *Structure, Function, and Biosynthesis of Plant Cell Walls.* Waverly Press, Baltimore, Maryland, pp. 75–88.

Crayton, M. A., Wilson, E. & Quatrano, R. S. 1974. Sulfation of fucoidan in *Fucus* embryos. II. Separation from initiation of polar growth. *Dev. Biol.* 39:164–7.

Daniel, G. F. & Chamberlain, A. H. L. 1981. Copper immobilization in fouling diatoms. *Bot. Mar.* 24:229–43.

Daniel, G. F., Chamberlain, A. H. L. & Jones, E. B. G. 1980. Ultrastructural observations of the marine fouling diatom *Amphora. Helgol. Wiss. Meeresunters.* 34:123–49.

——— 1987. Cytochemical and electron microscopical observations on the adhesive materials of marine fouling diatoms. *Br. Phycol. J.* 22:101–18.

Fenical, W. 1975. Halogenation in the Rhodophyta, a review. *J. Phycol.* 11:245–59.

Fogg, G. E. 1983. The ecological significance of extracellular products of phytoplankton photosynthesis. *Bot. Mar.* 26:3–14.

Fries, L. 1966. Influence of iodine and bromine on growth of some red algae in axenic culture. *Physiol. Pl.* 19:800–8.

——— 1975. Requirement of bromine in a red alga. *Z. Pflanzenphysiol. Bd.* 76(Suppl.):366–8.

Fry, S. C. 1979. Phenolic components of the primary cell wall and their possible role on the hormonal regulation of growth. *Planta (Berl.)* 146:343–51.

——— 1982a. Phenolic components of the primary cell wall. Feruloylated disaccharides of D-galactose and L-arabinose from spinach polysaccharide. *Biochem. J.* 203:493–504.

———. 1982b. Isodityrosine, a new cross-linking amino acid from plant cell-wall glycoprotein. *Biochem. J.* 204:449–55.

——— 1988. *The Growing Plant Cell Wall: Chemical and Metabolic Analysis.* Longman Scientific & Technical, New York, pp. 224–49.

Grasdalen, H. & Smidsrød, O. 1981. Iodide-specific formation of κ- carrageenan single helices. ^{127}I NMR spectroscopic evidence for selective site binding of iodide anions in the ordered conformation. *Macromolecules* 14:1842–5.

Gretz, M. R., Hoagland, K. D. & DeNicola, D. M. 1993. Diatom adhesion mechanisms: characterization of stalks of *Achnanthes longipes* and *Cymbella cistula. J. Phycol.* 29(Suppl.):11.

Guillard, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates. *In* Smith, W. L. & Chanley, M. H. [Eds.] *Culture of Marine Invertebrate Animals.* Plenum Press, New York, pp. 29–60.

Hanson, R. S. & Phillips, J. A. 1981. Total carbohydrates by phenol reaction. *In* Gerhardt, P., Murray, R. G. E., Costlow, R. N., Nester, E. W., Wood, W. A., Kreig, N. R. & Briggs Phillips, G. [Eds.] *Manual of Methods of General Bacteriology.* American Society for Microbiology, Washington, D.C., pp. 333–4.

Hendey, N. I. 1951. I.—Littoral diatoms of Chichester harbour with special reference to fouling. *Trans. J. Roy. Micr. Soc.* 1–86.

Hoagland, K. D., Roemer, S. C. & Rosowski, J. R. 1982. Colonization and community structure of two periphyton assemblages, with emphasis on the diatoms (Bacillariophyceae). *Am. J. Bot.* 69:188–213.

Hoagland, K. D., Rosowski, J. R., Gretz, M. R. & Roemer, S. C. 1993. Diatom extracellular polymeric substances: function, fine structure, chemistry, and physiology. *J. Phycol.* 29:537–66.

Hogsett, W. E. & Quatrano, R. S. 1978. Sulfation of fucoidin in *Fucus* embryos. III. Required for localization in the rhizoid wall. *J. Cell Biol.* 78:866–72.

Hudon, C. & Legendre, P. 1987. The ecological implication of growth forms in epibenthic diatoms. *J. Phycol.* 23:434–41.

Iwasaki, H. 1967. Nutritional studies of the edible seaweed *Porphyra tenera.* II. Nutrition of *Conchocelis. J. Phycol.* 3:30–4.

Johnson, L. M. 1995. Physiology of *Achnanthes longipes* Ag.: Nutritional requirements for the secretion of extracellular polymeric substances. Ph.D. thesis, University of Nebraska, Lincoln, 174 pp.

Jönsson, B., Sundbäck, K. & Nilsson, C. 1994. An upright lifeform of an epipelic motile diatom: on the behavior of *Gyrosigma balticum. Eur. J. Phycol.* 29:11–5.

Kirk, K. L. 1991. *Biochemistry of the Elemental Halogens and Inorganic Halides.* Plenum Press, New York, 293 pp.

Lewin, J. 1955. The capsule of the diatom *Navicula pelliculosa. J. Gen. Microbiol.* 13:162–9.

Mague, T. H., Friberg, E., Hughes, D. J. & Morris, I. 1980. Extracellular release of carbon by marine phytoplankton; a physiological approach. *Limnol. Oceanogr.* 22:570–3.

Manley, S. L. & Chapman, D. J. 1978. Formation of 3-bromo-4-hydroxybenzaldehyde from L-tyrosine in cell-free homogenates of *Odonthalia floccosa* (Rhodophyceae): a proposed biosynthetic pathway for brominated phenols. *FEBS Lett.* 93: 97–101.

Manthey, J. A. & Hager, L. P. 1989. Characterization of the catalytic properties of bromoperoxidase. *Biochemistry* 28: 3052–7.

Marszalek, D. S., Gerchakov, S. M. & Udey, F. R. 1979. Influence of substrate composition on marine microfouling. *Appl. Environ. Micro.* 38:987–95.

McLachlan, J. 1977. Effects of nutrients on growth and development of embryos of *Fucus edentatus* Pyl. (Phaeophyceae, Fucales). *Phycologia* 16:329–38.

McLachlan, J. & Craigie, J. S. 1967. Bromide, a substitute for chloride in a marine algal medium. *Nature (Lond.).* 214:604–5.

Myklestad, S. 1977. Production of carbohydrates by marine planktonic diatoms. II. Influence of the N/P ratio in the growth medium on the assimilation ratio, growth rate, and production of cellular and extracellular carbohydrates by *Chaetoceros affinis* var. *Willei* (Gran.) Hustedt and *Skeletonema costatum* (Greve.) Cleve. *J. Exp. Mar. Biol. Ecol.* 29:161–79.

Myklestad, S. & Haug, A. 1972. Production of carbohydrates by the marine diatom *Chaetoceros affinis* var. *Willei* (Gran.) Hustedt. I. Effect of the concentration of nutrients in the culture medium. *J. Exp. Mar. Biol. Ecol.* 9:125–36.

Myklestad, S., Holm-Hansen, O., Vårum, K. M. & Volcani, B. E. 1989. Rate of release of extracellular amino acids and carbohydrates from the marine diatom *Chaetoceros affinis. J. Plankton Res.* 11:763–73.

Neidleman, S. L. & Geigert, J., Eds. 1986. *Biohalogenation: Prin-*

ciples, Basic Roles and Applications. Ellis Horwood, Chichester, 203 pp.

Northcote, D. H. 1985. Control of cell wall formation during growth. *In* Brett, C. T. & Hillman, J. R. [Eds.] *Biochemistry of Plant Cell Walls.* Cambridge University Press, Cambridge, pp. 177–97.

Novarino, G. 1993. Presence of minerals in the mucilage stalk of the diatom *Achnanthes longipes. Diatom Res.* 8:199–202.

Pedersén, M. 1969. The demand for iodine and bromine of three marine brown algae grown in bacteria-free cultures. *Physiol. Plant.* 22:680–5.

——— 1976. A brominating and hydroxylating peroxidase from the red alga *Cystoclonium purpureum. Physiol Plant.* 37:6–11.

Quatrano, R. S. 1982. Cell-wall formation in *Fucus* zygotes: a model system to study the assembly and localization of wall polymers. *In* Brown, R. M. [Ed.] *Cellulose and Other Natural Polymer Systems.* Plenum Press, New York, pp. 45–59.

Roberts, K., Gay, M. R. & Hills, G. J. 1980. Cell wall glycoproteins from *Chlamydomonas reinhardii* are sulphated. *Physiol. Plant.* 49:421–4.

Sharon, N. & Lis, H. 1972. Lectins: cell agglutinating and sugar-specific proteins. *Science (Wash. D.C.)* 177:949–59.

Sharp, J. H. 1977. Excretion of organic matter by marine phytoplankton: do healthy cells do it? *Limnol. Oceanogr.* 22:381–400.

Sheffield, D. J., Harry, R., Smith, A. J. & Rogers, L. J. 1993. Purification and characterization of the vanadium bromoperoxidase from the macroalga *Corallina officinalis. Phytochemistry* 32:21–6.

Smith, M. M. & O'Brien, T. P. 1979. Distribution of autofluorescence and esterase and peroxidase activities in the epidermis of wheat roots. *Aust. J. Plant. Physiol.* 6:201–19.

Soedjak, H. S. & Butler, A. 1991. Mechanism of dioxygen formation catalyzed by vanadium bromoperoxidase from *Macrocystis pyrifera* and *Fucus distichus. Biochim. Biophys. Acta* 1079: 1–7.

Steel, R. G. D. & Torrie, J. H. 1980. *Principles and Procedures of Statistics. A Biometrical Approach,* 2nd ed. McGraw-Hill, New York, pp. 215–20.

Sturges, W. T., Cota, G. F. & Buckley, P. T. 1992. Bromoform emission from Arctic ice algae. *Nature (Lond.)* 358:660–2.

Sturges, W. T., Sullivan, C. W., Schnell, R. C., Heidt, L. E., & Pollock, W. H. 1993. Bromoalkane production by Antarctic ice algae. *Tellus* 45B:120–6.

Sutherland, I. W. 1988. Bacterial surface polysaccharides: structure and function. *Int. Rev. Cytol.* 113:187–231.

van Schijndel, J. W. P. M., Vollenbroek, E. G. M. & Wever, R. 1993. The chloroperoxidase from the fungus *Curvularia inaequalis*; a novel vanadium enzyme. *Biochim. Biophys. Acta* 1161: 249–56.

von Stosch, H. A. 1961. Wirkungen von jod und arsenit auf meeresalgen in cultur. *In* DeVirville, Ad. D. & Feldmann, J. [Eds.] Proceedings of the Fourth International Seaweed Symposium. Pergamon Press, New York, pp. 142–53.

Vreeland, V. & Laetsch, W. M. 1990. A gelling carbohydrate in algal cell wall formation. *In* Adair, W. S. & Mecham, R. P. [Eds.] *Organization and Assembly of Plant and Animal Extracellular Matrix.* Academic Press, San Diego, pp. 137–71.

Vreeland, V., Parungo, C. & West, J. A. 1993. Vanadate peroxidase in the development of wall adhesive in *Fucus* zygotes. *Mol. Biol. Cell* 4(Suppl.):147a.

Watase, M., Nishinari, K., Williams, P. A. & Phillips, G. O. 1990. Effect of ammonium salts on rheological and thermal properties of kappa-carrageenan gels. *Food Hydrocolloids* 4:227–37.

White, R. J. & Chamberlain, A. H. L. 1982. Attachment of epiphytic freshwater diatoms. *Microscopy* 34:470–6.

Wolk, C. P. 1968. Role of bromine in the formation of refractile inclusions of the vesicle cells of the Bonnemaisoniaceae (Rhodophyta). *Planta (Berl.)* 78:371–8.

Woolery, M. L. & Lewin, R. A. 1973. Influence of iodine on growth and development of the brown alga *Ectocarpus siliculosus* in axenic cultures. *Phycologia* 12:131–8.