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1 **Biochemical characterization of Extracellular Polymeric Substances**
2 **extracted from an intertidal mudflat using a cation exchange resin.**

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12

13 **ABSTRACT**

14 The biochemical characterization of Extracellular Polymeric Substances (EPS) excreted in a
15 European intertidal mudflat (Marennes-Oléron Bay) was performed. Experiments were
16 carried out for the first time *in situ*, by using an improved extraction recently developed. This
17 innovative procedure, using a cation exchange resin (Dowex), allows separating precisely
18 different fractions of EPS, especially pure bound EPS. Moreover, it avoids the contamination
19 of EPS fractions by residual and intracellular polymers, enabling to properly estimate
20 polymeric contents in each fraction. The results were partly similar to conventional results
21 described in the literature and the amount of colloidal carbohydrates (146µg/g of dry
22 sediment) extracted by the Dowex method fitted well with different EPS estimation in

23 European mudflats. Colloidal carbohydrates were essentially composed of glucose (>50%), a
24 carbon source rapidly consumed by the various communities in the sediment. Pure bound
25 carbohydrates were composed of specific carbohydrates (28% rhamnose, 22% xylose).
26 Residual fractions, considered as containing some refractory bound EPS and mostly other
27 internal polymeric substances, presented a more varied composition rich in carbohydrates:
28 galacturonic acid (20%), mannose (19.5%), glucose (19%), arabinose (15%), xylose (8%),
29 galactose (7%).

30

31 *Keywords: Extracellular compounds, biochemical characterization, biofilm, in situ*
32 *quantification, benthic ecology*

33

34

35 **1. Introduction**

36 The benthic biofilms which developed during the emerged periods in intertidal mudflats are
37 widely studied for various reasons. Considering an ecological perspective, the main reason is
38 to understand the influence of benthic biofilms in intertidal ecosystems, by analyzing their
39 compositions and their changes, depending on environmental parameters. Numerous studies
40 of the last decades have allowed determining their composition and highlighted the presence
41 of microalgae (microphytobenthos), bacteria and fungi, tangled in a complex mixture of
42 polymeric compounds that they produce (Frølund et al., 1996). These Extracellular Polymeric
43 Substances (EPS) are rich in polysaccharides, proteins, proteoglycans, lipids and many other
44 compounds expressed at different levels (Stoodley et al., 2002; Stal, 2003), related to the
45 location or environmental conditions which affect both food web and primary production of
46 this ecosystem (Underwood and Paterson, 2003). EPS are involved in the mobility system of

47 epipelagic diatoms (Stal and Défarge, 2005) and can be used as carbon sources by the bacterial
48 community (van Duyl et al., 1999; Hofmann et al., 2009). EPS also affect the
49 microenvironment of biofilms by varying physico-chemical parameters like porosity or
50 mechanical stability of the sediment (Orvain et al., 2003; Perkins et al., 2004; Spears et al.,
51 2008). On the other hand, benthic EPS can present interesting structures and functions, which
52 can be used in many biochemical fields. The extraction of sulfated polysaccharides for
53 medicinal applications is one example (Witvrow et al., 1997). Many works have already
54 proposed extraction protocols allowing the collection of particular EPS, having specific
55 biochemical properties (Stats et al., 1999; de Brouwer et al., 2001; Azerado et al., 2003;
56 Bellinger et al., 2005, Comte et al., 2006). All the data obtained have been each time
57 criticized and authors have agreed that there was no universal extraction method for EPS.
58 Recently, Takahashi et al. (2009) have optimized a protocol for EPS extraction and proposed
59 an innovative method, using a cation exchange resin, to extract cultured diatoms EPS without
60 any contamination by internal compounds. Furthermore, the method allowed the extraction of
61 bound EPS, a fraction poorly studied and heavily contaminated in other previous studies (de
62 Brouwer and Stal, 2004; Chiovitti et al., 2004).

63 The aim of the present investigation was to characterize the biochemical composition of EPS
64 collected from benthic biofilms during emerged periods on a macrotidal bay (Marennes-
65 Oléron Bay, France), using for the first time *in situ* the Dowex-resin method (Takahashi et al.,
66 2009).

67 **2. Methods**

68 *2.1. Intertidal mudflat samples*

69 The mud samples used in this study were collected from Marennes-Oléron Bay (Atlantic
70 Coast of France) in February 2008 (winter) at low tide (Fig. 1). Two hours after the beginning

71 of the emersion, sediment cores were sampled for three different squares, to take into account
72 spatial heterogeneity. Sediment samples were collected using core diameter of 20 cm, and the
73 top 1cm was collected three times and pooled to give a main sediment core, for each square.
74 After sampling, sediment was brought back on from the field by using a watercraft for an
75 immediate EPS extraction on fresh sediments on the upper shore. The colloidal, bound and
76 residual fractions were extracted through the Dowex-resin method then biochemical analyses
77 were performed, all in triplicate.

78 *2.2. Materials*

79 Dowex Marathon C, BicinChoninic Acid (BCA) Protein Assay Kit, Azure A, N,O-
80 bis(trimethylsilyl)trifluoroacetamide: trimethylchlorosilane (BSTFA: TMCS) (99: 1) were
81 obtained from Sigma-Aldrich. Standard carbohydrates (dextran, dextran sulfatate, heparin,
82 fucoïdan, glucose, galactose, rhamnose, fucose, fructose, xylose, arabinose, mannose, myo-
83 inositol, glucuronic and galacturonic acid) and a protein standard (Bovine Serum Albumin,
84 BSA) were obtained from Sigma-Aldrich. The DB-1701 J&W Scientific column (30m,
85 0.32mm, 1µm) for Gas Chromatography-Mass Spectrometry analysis (GC/MS) was obtained
86 from Agilent.

87 *2.3. EPS Extraction in situ*

88 The extraction was done immediately after sampling and sediment mixing (Takahashi et al.
89 2009). 20 mL of fresh mudflat was mixed with 20 mL of Artificial Sea Water (ASW 30
90 Practical Salinity Units) during 1 h in darkness at 4°C and then centrifuged at 3500 g and 4°C
91 for 10 min. The supernatant (a) containing colloidal EPS was collected and stored at 4°C. 20
92 mL of ASW and 1 g of activated Dowex (Marathon C, activated in Phosphate Buffer Saline
93 for 1 h in the dark) was added to the cap (b). The samples were mixed gently at 4°C for 1 h in
94 the dark and then centrifuged at 3500 g and 4°C for 10 min. A supernatant containing the

95 bound EPS (c) and a cap containing intracellular and residual polymers (d) were obtained.
96 The cap was then frozen. The residual polymers were extracted from the frozen samples, by
97 sonication at 100W for 3 min on ice after resuspension in 20 mL in ASW.

98 For each fraction (colloidal, bound and residual polymers), absolute ethanol at -20°C was
99 added to the sample (a) to obtain a final ethanol concentration of 75 % (v/v). The solution was
100 gently mixed and stored overnight at -20°C. The solution was then centrifuged at 3500 g and
101 4 °C for 15 min to obtain a supernatant (Low Molecular Weight, LMW fraction) and a cap
102 (High Molecular Weight, HMW fraction). Finally, the fractions were dried under air flow and
103 stored at -20 °C.

104 *2.4. EPS Composition*

105 Total sugar content was determined using the phenol-sulfuric acid assay, developed by
106 Dubois, using glucose as a standard (Dubois et al., 1956). Protein content was determined
107 using the bicinchoninic acid (BCA) assay, using bovine serum albumin (BSA) as a standard
108 (Smith et al., 1985). Uronic acid content was determined using the meta-hydroxydiphenyl
109 method (MHDP), using galacturonic and glucuronic acids as standards (Blumenkrantz and
110 Asboe-Hansen, 1973; Filisetti-Cozzi and Carpita, 1991). The sulfate content was measured by
111 the Azure A (Jaques et al., 1968) and the Ba/Cl₂ gelation method (Craigie et al., 1984), using
112 Dextran sulfate as a standard.

113 *2.5. Sugar Characterization*

114 Prior to carbohydrate characterization by GC/MS, EPS fractions were solubilized in 5 mL of
115 ultra-pure water, dialyzed (6-8 KDa) and freeze-dried (Bellinger et al. 2005). EPS were then
116 dissolved in 2M HCl at 50 mg/mL and heated at 90°C for 4 h. The preparation (which
117 contained mostly carbohydrates monomers) was then freeze-dried and stored at -20°C.
118 Analysis of the carbohydrate fractions were carried out by GC/MS using a Varian CP-3800

119 GC/Varian Saturn 2000 (Fig. 2). Operating conditions have been determined and optimized in
120 the laboratory (data not shown). 400 μ L of pyridine and 400 μ L of BSTFA: TMCS (99:1) was
121 added to 2 mg of purified polysaccharides. The solution was mixed for 2 h at room
122 temperature, then injected into a DB-1701 J&W Scientific column (30 m, 0.32 mm, 1 μ m) at
123 a flow of 1mL/min. The helium pressure was 8.8psi. The temperature of the injector was set at
124 250 °C. The rise in temperature in the oven was programmed for a first step at 150°C for
125 0min, then an increment of 10°C/min up to 200°C with a final step at 200°C for 35 min. The
126 ionization was performed by Electronic Impact (EI, 70 eV), the trap temperature was set at
127 150°C and the target ion was fixed at 40-650 m/z.

128 **3. Results and discussion**

129 *3.1. Type and composition of EPS*

130 Despite the fact that common practice is to freeze sediments at -80°C to eliminate the
131 consumption of EPS by bacteria, Takahashi's extraction method focus on the use of fresh
132 sediments to avoid cells lysis (Takahashi et al., 2009), which supports other studies
133 concerning the contamination of EPS fractions by internal storage compounds, as glucans,
134 proteins and chrysolaminaran (de Brouwer et al., 2001, Hanlon et al., 2006). The addition of
135 Dowex resin to a classical procedure clearly defines pools of carbohydrates, depending on
136 their properties and localization in the matrix complex (Bellinger et al., 2005; Abdullahi et al.,
137 2006).

138 Overall, 1g of dry sediment is composed of 1618 μ g of carbohydrates and 383 μ g of proteins
139 (Table 1-2). Although this concentration may seem low, EPS are extracted from crude
140 samples. Different authors have shown that natural mudflats samples may contain large
141 quantities of mineral impurities and salt (de Brouwer et al., 2001, Underwood and Paterson,
142 2003). The amounts of carbohydrates were slightly higher than those measured at the same

143 station in 1998 (Stal and Défarge, 2005). These authors had worked from the first 5 mm of
144 sediment, which represent more accurately the microphytobenthic biofilm. Our approach (first
145 10 mm) could overestimate diatom EPS production due to the contamination from other
146 sediment EPS sources (Perkins et al., 2003).

147 Colloidal fractions were rich in carbohydrates ($\pm 50/50$ % LMW/HMW) (Table 1-2). LMW
148 colloidal fractions could be compared to the low molecular weight exudates and HMW
149 colloidal fractions EPS extracted by various authors (Abdullahi et al., 2006, Hanlon et al.,
150 2006). The total amount of colloidal carbohydrate (neutral carbohydrates and uronic acids)
151 given in Table 1 ($146 \mu\text{g}\cdot\text{g}^{-1}$ sediment) fitted with common results described in literature: 50
152 to $5000 \mu\text{g}\cdot\text{g}^{-1}$ sediment. Similarly, the bound fractions were composed of carbohydrates (87
153 % LMW). The total amount of bound carbohydrates was closed to the concentrations of total
154 colloidal carbohydrates ($\pm 113 \mu\text{g}\cdot\text{g}^{-1}$). This result would indicate that, in general, the colloidal
155 and bound EPS are produced in close quantities in this benthic ecosystem. However, this
156 amount of bound carbohydrates is lower than other amounts measured for European mudflats
157 and suggests that our fractions were not contaminated by residual and internal carbohydrates.
158 Colloidal and bound fractions did not contain proteins, in contrast to many previous works (de
159 Brouwer et al., 2001; Underwood and Paterson, 2003; Hanlon et al., 2006, Hofmann et al.,
160 2009). This lack seems to confirm that our EPS fractions were not contaminated by residual and
161 internal storage polymers (Staats et al., 2000; Orvain et al., 2003).

162 Finally, the residual fractions were widely extracted compared to the EPS fractions (Table 2)
163 and presented a complex composition especially because of the presence of proteins (22 %)
164 and sulfated components (15 % of the total amount of carbohydrates) (Table 1). Our residual
165 polymers found could be compared to the complex cell wall-associated and the intracellular
166 polymers of diatoms, widely described in the literature (glucan, chrysolaminaran). Otherwise,

167 these residual fractions must also contain some refractory EPS that were not extracted with
168 the Dowex-resin.

169 3.2. Model of Underwood & Smith

170 The colloidal EPS quantities measured in the Marennes-Oléron mudflat were compared to the
171 predicted quantities given by the model of Underwood & Smith, which was used to determine
172 the amount of colloidal carbohydrates produced in European mudflats (Underwood and
173 Smith, 1998).

$$174 \quad \log(\text{coll. carbohydrates content} + 1) = 1.40 + 1.02 \times \log(\text{Chl } a \text{ content} + 1)$$

175 $\{r^2=64.6\%$

176 The model was applied to the concentrations of Chlorophyll *a* (in average, 21.5 µg Chl *a*/g dw
177 sediment) measured *in situ* during the sampling campaign. Considering the relationship and
178 the r^2 , the amount of colloidal carbohydrates extracted by the Dowex-resin was in accordance
179 with the model, suggesting that the Dowex method allowed extracting in full the colloidal
180 EPS.

181 3.3. Sugar characterization

182 GC-MS results indicated that the carbohydrate portions were formed of nine different types of
183 monomer units (Fig. 3), including seven neutral sugars and two uronic acids. The colloidal
184 EPS fractions had a high glucose content (>50 %), the bound EPS fractions were mainly
185 composed of rhamnose, xylose, glucose, galacturonic acid and the residual polymeric
186 fractions had a more varied composition in monosaccharides, including a greater unknown.

187 The monosaccharide distribution between LMW and HMW of the colloidal fractions was
188 quite similar, although there were a few amount of rhamnose in LMW fractions (Fig. 3, A1-
189 A2). Colloidal EPS fractions were mainly composed of glucose, which could explain why this
190 fraction is easily consumable by heterotrophic bacteria in the extracellular medium (van Duyl

191 et al., 1999; Bellinger et al., 2005; Hofmann et al., 2009). Our results were close to previous
192 works, which showed the predominance of glucose (50 %), galactose, xylose (15 %) or
193 galacturonic acid (15 %) in colloidal fractions extracted by the same way (Abdullahi et al.,
194 2006; Hanlon et al., 2006). In contrast to previous works where bound fractions were
195 extracted, glucose is not the main saccharide (less than 20 %) of bound EPS fractions (Fig. 3,
196 B1-B2). The content of specific sugars in these fractions can be better estimated and bound
197 EPS were mainly composed of rhamnose (28 %), xylose (22 %) and galacturonic acid (18 %).
198 The accurate composition of bound EPS, enriched in deoxy and specific sugars, is very
199 important for understanding the functional role of bound EPS. Deoxy sugars can promote
200 biostabilisation of sediments (Zhou et al., 1998; Giroldo et al., 2003) through their surface
201 active properties. Deoxy sugars can also influence the hydrophobic character of EPS, playing
202 a role on the adhesion of EPS to sediment or on the regulation of desiccation and salinity
203 (Spears et al., 2008). However, it was surprising that fucose has not been highlighted although
204 the GC-MS method allowed its detection. It is therefore possible that the lack of fucose was
205 linked to the environmental conditions or the physiological state and the quantity of benthic
206 diatoms forming the benthic biofilm during winter (Stal and Défarge, 2005; Bellinger et al.,
207 2009). The presence of inositol (*-myo*) is significant since no author has highlighted it.
208 Inositol is involved in the structural basis for a number of secondary messengers in eukaryotic
209 cells and is a major growth factor for many-organisms, especially for heterotrophic bacteria.
210 Residual fractions were mainly composed of polysaccharides rich in glucose (derived from β -
211 1,3-linked glucan or chrysolaminaran) and mannose, rhamnose, xylose (Fig. 3, C1-C2). It is
212 important to note that a portion of these sugars must come from refractory bound EPS.

213 On the whole, our results confirmed the relevance and the effectiveness of Takahashi's
214 method for *in situ* experiments. Bound fractions were biochemically different from the two
215 other fractions, thanks to the presence of large amounts of deoxy sugars and uronic acids. In

216 this way, it can be supposed that the levels of rhamnose, mannose or galacturonic acid played
217 a role during the development of the microphytobenthic biofilm by increasing binding forces
218 or enhancing the incorporation of water. The surprising lack of fucose has been correlated to
219 the sampling period. The presence of inositol was detected for a first time *in situ*. This sugar is
220 commonly used for GC/MS analysis (internal standard) and this could explain why it was not
221 identified as a component of EPS. Significant amounts of this growing factor for bacteria
222 were measured in the colloidal fraction, a fraction known as being a direct nutrient source for
223 the heterotrophic bacteria. Finally, it would be interesting to extract and characterize *in situ*
224 the same EPS fractions depending on environmental conditions.

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229 microbial biofilms in tidal flats” (contract ANR-06-BLAN-0393-01).

230 **References**

231 Abdullahi, A.S., Underwood, G.J.C., Gretz, M.R., 2006. Extracellular matrix assembly in
232 diatoms (Bacillariophyceae). V. Environmental effects on polysaccharide synthesis in the
233 model diatom, *Phaeodactylum tricornutum*. J. Phycol. 42, 363-378.

234 Azerado, J., Henriques, M., Sillankorva, S., Oliveira, R., 2003. Extraction of exopolymers
235 from biofilm: the protective effect of glutaraldehyde. Water Sci. Technol. 47, 175-179.

236 Bellinger, B.J., Abdullahi, A.S., Gretz, M.R., Underwood, G.J.C., 2005. Biofilm polymers:
237 relationship between carbohydrate biopolymers from estuarine mudflats and unialgal cultures
238 of benthic diatoms. Aquat. Microb. Ecol. 38, 169-180.

239 Bellinger, B.J., Underwood, G.J.C., Ziegler, S.E., Gretz, M.R., 2009. Significance of diatom-
240 derived polymers in carbon flow dynamics within estuarine biofilms determined through
241 isotopic enrichment. *Aquat. Microb. Ecol.* 55, 169-187.

242 Blumenkrantz, N., Asboe-Hansen, G., 1973. New method for quantitative determination of
243 uronic acids. *Anal. Biochem.* 54, 484-489.

244 de Brouwer, J.F.C., Stal, L.J., 2001. Short-term dynamics in microphytobenthos distribution
245 and associated extracellular carbohydrates in surface sediments of an intertidal mudflat. *Mar.*
246 *Ecol. Prog. Ser.* 218, 33-44.

247 de Brouwer, J.F.C., Stal, L.J., 2004. Does warm-water extraction of benthic diatoms yield
248 extracellular polymeric substances or does it extract intracellular chrysolaminaran? *Eur. J.*
249 *Phycol.* 39, 129-131.

250 Chiovitti, A., Molino, P., Crawford, S.A., Ten, R., Spurck, T., Wetherbee, R., 2004. The
251 glucans extracted with warm water from diatoms are mainly derived from intracellular
252 chrysolaminaran and not extracellular polysaccharides. *Eur. J. Phycol.* 39, 117-128.

253 Comte, S., Guibaud, G., Baudu, M., 2006. Relations between extraction protocols for
254 activated sludge extracellular polymeric substances (EPS) and EPS complexation properties
255 Part I. Comparison of the efficiency of eight EPS extraction methods. *Enz. Microb. Tech.* 38,
256 237-245.

257 Craigie, J.S., Wen, Z.C., van der Meer, J.P., 1984. Interspecific, intraspecific and nutritionally-
258 determined variations in the composition of agars from *Gracilaria spp.* *Bot. Mar.* 27, 55-61.

259 Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method
260 for determination of sugars and related substances. *Anal. Chem.* 28, 350-356.

261 van Duyl, F.C., de Winder, B., Kop, A.J., Wollenzien, U., 1999. Tidal coupling between
262 carbohydrate concentrations and bacterial activities in diatom-inhabited intertidal mudflats.
263 Mar. Ecol. Prog. Ser. 191, 19-32.

264 Filisetti-Cozzi, T.M., Carpita, N.C., 1991. Measurement of uronic acids without interference
265 from neutral sugars. Anal. Biochem. 197, 157-162.

266 Frølund, B., Palmgren, R., Keiding, K., Nielsen, P.H., 1996. Extraction of extracellular
267 polymers from activated sludge using a cation exchange resin. Water Res. 30, 1749-1758.

268 Giroldo, D., Vieira, A.A.H., Paulsen, B.S., 2003. Relative increase of deoxy sugars during
269 microbial degradation of an extracellular polysaccharide released by a tropical freshwater
270 *Thalassiosira* sp. (Bacillariophyceae). J. Phycol. 39, 1109-1115.

271 Hanlon, A.R.M., Bellinger, B., Haynes, K., Xiao, G., Hofmann, T.A., Gretz, M.R., Ball, A.S.,
272 Osborn, M., Underwood, G.J.C., 2006. Dynamics of extracellular polymeric substances (EPS)
273 production and loss in an estuarine, diatom-dominated, microbial biofilm over a tidal
274 emersion-immersion period. Limnol. Oceanogr. 51, 79-93.

275 Hofmann, T., Hanlon, A.R.M., Taylor, J.D., Ball, A.S., Osborn, A.M., Underwood, G.J.C.,
276 2009. Dynamics and compositional changes in extracellular carbohydrates in estuarine
277 sediments during degradation. Mar. Ecol. Prog. Ser. 379, 45-58.

278 Jaques, L.B., Ballieux, R.E., Dietrich, C.P., Kavanagh, L.W., 1968. A microelectrophoresis
279 method for heparin. Can. J. Physiol. Pharmacol. 46, 351-360.

280 Orvain, F., Galois, R., Barnard, C., Sylvestre, A., Blanchard, G., Sauriau, P.G., 2003.
281 Carbohydrate production in relation to microphytobenthic biofilm development: an integrated
282 approach in a tidal mesocosm. Microb. Ecol. 45, 237-251.

283 Perkins, R.G., Honeywill, C., Consalvey, M., Austin, H.A., Tolhurst, T.J., Paterson, D.M.,
284 2003. Changes in microphytobenthic chlorophyll *a* and EPS resulting from sediment
285 compaction due to de-watering: opposing patterns in concentration and content. Cont. Shelf
286 Res. 23, 575-586.

287 Perkins, R.G., Paterson, D.M., Sun, H., Watson, J., Player, M.A., 2004. Extracellular
288 polymeric substances: quantification and use in erosion experiments. Cont. Shelf Res. 24,
289 1623-1635.

290 Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D.,
291 Fujimoto, E.K., Goetze, N.M., Olson, B.J., Klenk, D.C., 1985. Measurement of protein using
292 bicinchoninic acid. Anal. Biochem. 150, 76-85.

293 Spears, B.M., Saunders, J.E., Davidson, I., Paterson, D.M., 2008. Microalgal sediment
294 biostabilisation along a salinity gradient in the Eden Estuary, Scotland: unraveling a paradox.
295 Mar. Freshwater Res. 59, 313-321.

296 Staats, N., de Winder, B., Stal, L.J., Mur, L.R., 1999. Isolation and characterization of
297 extracellular polysaccharides from the epipellic diatoms *Cylindrotheca clostrerium* and
298 *Navicula salinarum*. Eur. J. Phycol. 34, 161-169.

299 Staats, N., Stal, L.J., Mur, L.R., 2000. Exopolysaccharide production by the epipellic diatom
300 *Cylindrotheca clostrerium*: effects of nutrient conditions. J. Exp. Mar. Biol. Ecol. 249, 13-27.

301 Stal, L.J., 2003. Microphytobenthos, their extracellular polymeric substances, and the
302 morphogenesis of intertidal sediments. Geomicrobiol. J. 20, 463-478.

303 Stal, L.J., Défarge, C., 2005. Structure and dynamics of exopolymers in an intertidal diatom
304 biofilm. Geomicrobiol. J. 22, 341-352.

305 Stoodley, P., Sauer, K., Davies, D.G., Costerton, J.W., 2002. Biofilms as complex
306 differentiated communities. *Annu. Rev. Microbiol.* 56, 187-209.

307 Takahashi, E., Ledauphin, J., Goux, D., Orvain, F., 2009. Optimizing extraction of
308 extracellular polymeric substances (EPS) from benthic diatoms: comparison of the efficiency
309 of six EPS extraction methods. *Mar. Freshwater Res.* 60, 1201-1210.

310 Underwood, G.J.C., Smith, D.J., 1998. Predicting epipellic diatom exopolymer concentrations
311 in intertidal sediments from sediment chl. *a*. *Microb. Ecol.* 35, 116-125.

312 Underwood, G.J.C., Paterson, D.M., 2003. The importance of extracellular carbohydrate
313 production by marine epipellic diatoms. *Adv. Bot. Res.* 40, 184-240.

314 Witvrouw, M., De Clercq, E., 1997. Sulfated polysaccharides extracted from sea algae as
315 potential antiviral drugs. *Gen. Pharmac.* 29, 497-511.

316 Zhou, J., Mopper, K., Passow, U., 1998. The role of surface-active carbohydrates in the
317 formation of transparent exopolymer particles by bubble adsorption of seawater. *Limnol.*
318 *Oceanogr.* 43, 1860-1871.

319

320 **Table 1** Composition ($\mu\text{g/g}$ of dry sediment) of the different fractions extracted by the Dowex
 321 method from the Marennes-Oléron mudflat.

Fraction	Neutral carbohydrate content	Uronic acid content	Sulfate content	Protein content
LMW colloidal	59 ± 8	23 ± 9	0	0
HMW colloidal	57 ± 16	7 ± 2	0	0
LMW bound	76 ± 8	22 ± 10	0	0
HMW bound	11 ± 2	4 ± 2	0	0
LMW residual	413 ± 79	112 ± 38	0	180 ± 72
HMW residual	530 ± 155	304 ± 172	204 ± 66	203 ± 55

322 \pm : deviations were calculated from the heterogeneity of the different sampling squares and not from the true
 323 replicates of the biochemical analysis (<5%)

324

325 **Table 2** Distribution of carbohydrates in the three fractions of EPS.

% (w/w)	Fraction Ratio	Low Molecular Weight	High Molecular Weight
Colloidal carbohydrates	9	56	44
Bound carbohydrates	7	87	13
Residual carbohydrates	84	39	61
Total carbohydrates	100*	43	57

326 * (1618 μ g/g of dry sediment)

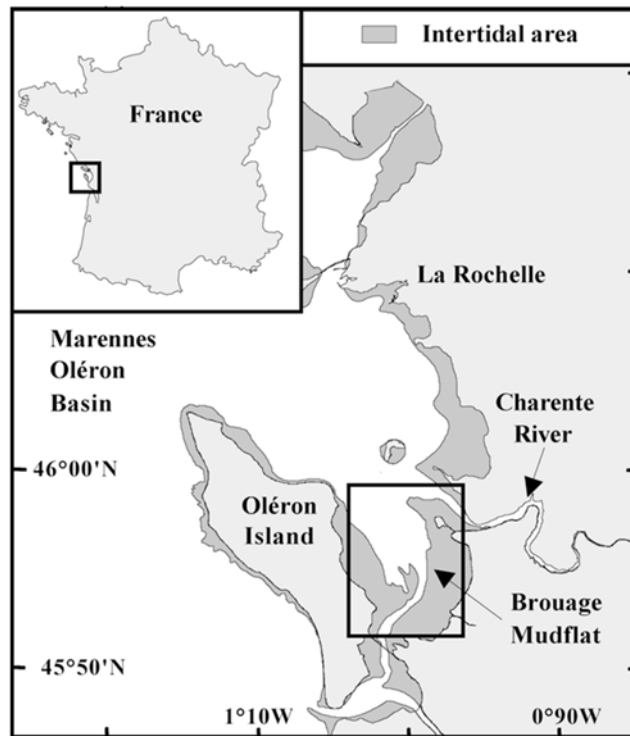
327

328 **Fig. 1.** Station where samples of surficial intertidal sediment were collected, two hours after
329 the beginning of emerged period.

330 **Fig. 2.** GC-MS chromatogram of carbohydrates detected in an HMW bound fraction.
331 Ionization: Electronic Impact (EI). Target ion: 40-650 m/z.

332 **Fig. 3.** Monosaccharide composition of the different EPS fractions collected on the Marennes-
333 Oléron mudflat (% of the carbohydrate content) after 2 hours of emersion. Ionization:
334 Electronic Impact (EI). Target ion: 40-650 m/z. The variability within true sample replicate
335 was less than 5%. (White): Unknown, undetermined on GC/MS.

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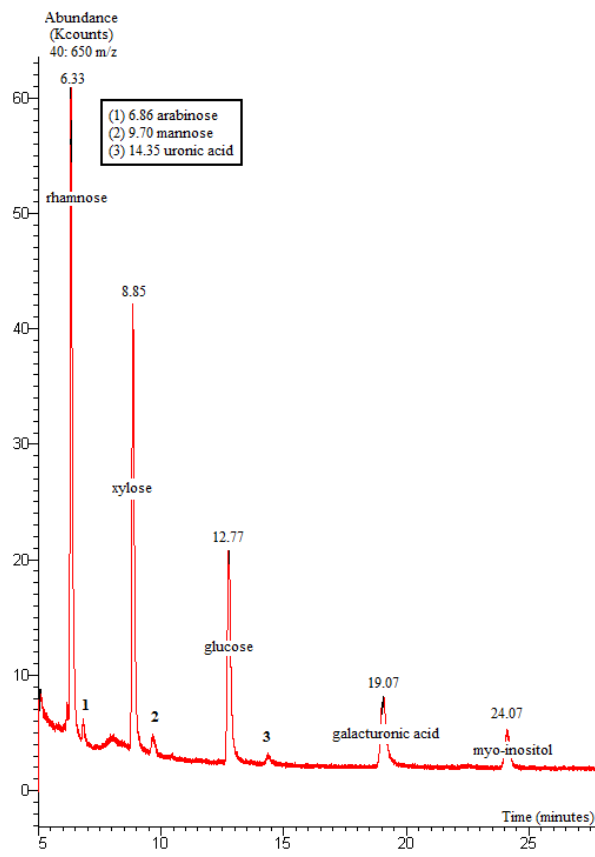
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FIGURE 1

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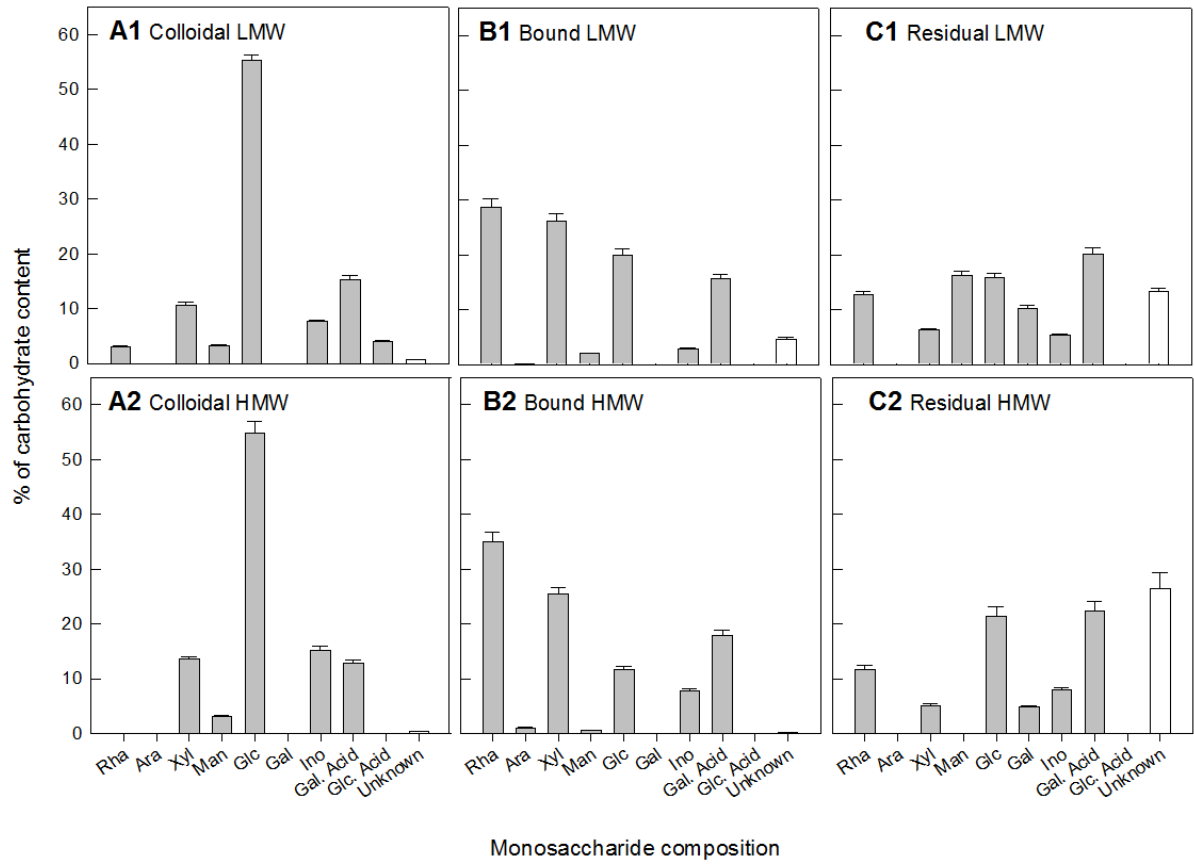
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FIGURE 2



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FIGURE 3