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Jordi Urmeneta
Antoni Navarrete

Department of Microbiology, University of Barcelona, Barcelona, Spain

Mineralogical composition and biomass studies of the microbial mats sediments from the Ebro Delta, Spain

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Correspondence to:
Jordi Urmeneta. Department of Microbiology.
University of Barcelona.
Av. Diagonal, 645. 08028 Barcelona. Spain.
Tel.: +34–934034628.
Fax: +34–934034629.
E-mail: jordi@bio.ub.es

Summary The mineral composition of the microbial mats at La Banya spit was studied. The spit is formed by a narrow sand bar and a peninsula and is located south of the main body of the Ebro Delta (Tarragona, Spain). Although quartz was the predominant mineral component in all sampling sites, clay, feldspars, calcite, aragonite, halite, dolomite and gypsum were also found. An increase in both the fine material (clay) and the halite content was observed in the sites influenced by nearby salterns. The amount of each mineral did not differ significantly along a 55 cm deep profile, except for halite and aragonite, which reached a maximum in the surface and decreased with depth. Dolomite, which ranged from 0.5 to 5% (w/w), is a possible indicator of sulfate-reducing bacteria activity in the past. Organic carbon and total nitrogen were quantified for biomass assessment. Total nitrogen ranged from 0.1 to 0.56% in the uppermost layer, where the microbial mat is active, but was undetectable at deeper layers. Organic carbon ranged from 1 to 5.5% in the active microbial mat layers and decreased to 0.3% at deeper layers. During the summer, both organic carbon and total nitrogen contents (biomass) of the microbial mat samples from some sites increase, whereas other sites show constant concentrations throughout the year, and others have a fluctuant biomass content.

Key words Microbial mats · Organic carbon · Total nitrogen · Mineralogical

Introduction

The development of the Ebro Delta, the third largest delta in the Mediterranean (320 km^2), after those of the Nile and Rhone rivers, probably began after the last glaciation, expanding seaward from the Holocene period until present. The data of historical evolution of the Ebro Delta plain (the first reliable maps date back to the 15th Century) show how the main channel shifted its position several times, giving rise to different prograding delta lobes. During a storm in 1937, a new channel mouth opened towards the north, and the eastern delta lobe was progressively eroded and abandoned. This provided a surge of sediment that was transported both northward and southward along the coast by littoral drift. Since then, only the edges of the two spits of the Ebro Delta, El Fangar in the north, and La Banya in the south, have been actively prograding [23]. Besides, the tendency of the coastline has been generally regressive in the last few decades. This is a result of the decrease of sediment input from the drainage basin, due to the construction of large water reservoirs (mainly during the 1960s), which retain much of the coarser material before it reaches the delta [23].

Microbial mats are the oldest of all known ecosystems. They fringed all continents and shallow waters of Earth as early as 3,500 million years ago. Their lithified remains originated the geological structures known as stromatolites [1, 12]. A microbial mat is a community consisting primarily of different populations of bacteria, which form thin horizontal layers. These layers grow actively and can be several millimeters to a few centimeters thick. The upper layer is formed mostly by cyanobacteria (mainly of the genera *Microcoleus*, *Lyngbya*, *Spirulina* and *Phormidium*) and diatoms. Below, there are usually different populations of anaerobic phototrophic bacteria: purple sulfur bacteria (Chromatiaceae), green sulfur bacteria (Chlorobiaceae) and green non-sulfur bacteria (*Chloroflexus* sp.) [11, 16]. In the bottom layer, there is a population of sulfate-reducing bacteria (SRB). The black color of this layer is due to the SRB activity (hydrogen sulfide production and metallic sulfides formation, mainly pyrite, FeS_2). We also observed a population of heterotrophic and fermentative microorganisms, as well as sulfur oxidizers that do not form stable layers and do not have a defined position at the mat. Methanogenic bacteria have also been detected in some microbial mats [21]. Some eukaryotic organisms, such as the amoebamastigote *Paratetramitus jugosus* and the ciliate *Pseudocohnilembus pusillus* [19], are associated to microbial mats.

Nowadays, microbial mats can be found in restricted locations around the world, mainly in coastal zones and extreme environments. The continuous elastic covering found on the sand

flat and drainage areas in La Banya spit of the Ebro Delta is considered to be among the largest in Europe (about 3 km^2) [23].

Numerous researchers have demonstrated that bacteria can induce the precipitation of calcium carbonate (calcite and aragonite) [2, 3, 8, 14, 17, 20] at the same time that they favor the lithification of the mats into stromatolites, as long as environmental conditions are suitable [4, 9, 13]. The aim of this study is to describe the mineralogical characteristics of the sediment as a physical support for the microbial mats, to discuss the possible modifications of the structure and mineralogical composition of these sediments as a consequence of the bacterial activity, and to assess both the biomass distribution in the mats and its evolution throughout the year.

Materials and methods

Mineralogical assays First, samples were taken at ten different locations at La Banya spit of the Ebro Delta. These locations are the A, B, C, D, D2, E, F, G, H and K sites, which have been previously described [11, 22]. Each sample was obtained by using a 14.5 cm^2 steel cylindrical corer. After being transported to the laboratory in Petri dishes at 4°C, samples were frozen at –80°C in the laboratory and then freeze-dried at –55°C and 200 mTorr (in a Virtis apparatus), and then powdered with a steel ball mill (15 min for each sample). Powdered samples were analyzed in an X-ray diffractometer of Bragg–Brentano (θ, 2θ) geometry (model Siemens D-500), equipped with a graphite secondary monochromator. Copper radiation of 40 kV and 30 mA ($K\alpha$, λ=1.5418 Å), a 0.05° reception window and a 1° divergence window were used. We compared the diffractograms obtained from the samples with others obtained from pure minerals. Commercial software Diffrac-at, equipped with the data of Joint Committee of Powder Diffraction Standards, was used to identify the components of each sample. We used the Chung method [5, 6] for the semiquantitative analysis.

Nitrogen and carbon analyses Organic carbon was analyzed to quantify total microbial biomass. Total carbon and total nitrogen were also measured. Each sample was obtained by using the corer described, and was then put in liquid nitrogen. The in situ frozen samples were freeze-dried at –55°C and 200 mTorr (in a Virtis apparatus) in the laboratory. The next step was to pulverize them in a Spex Mixer/Mill (Spex Industries, Inc.). To measure the total carbon and total nitrogen contents, 3 mg (±0.5 μg) of each sample was weighed in a ultramicrobalance (Mettler UM3) using tin sumps. An equal volume of vanadium pentoxide was added to each sample to facilitate the combustion. To measure organic carbon, 5 mg of each sample was weighed, and, to eliminate the inorganic carbon (carbonates), 25 μl of HCl (2 N) was added to each sample. All samples were burned at 1500°C in a Nitrogen Analyzer 1500 (Carlo Erba Strumentazione). We also used an acetanilide pattern for the analysis. Two replicates were taken from each site.

Results and Discussion

Mineralogical assays Figure 1 shows the diffractogram obtained in samples from site B with the names of the minerals responsible for each peak. The X-ray diffraction mineralogical assay is basically a qualitative analysis. However, the signal intensity in counts per second (cps), which depends on the substance analyzed, is proportional to the amount of this substance in the sample. For this reason, it is possible to perform a semiquantitative analysis to obtain the proportions of the different minerals in the samples. The results are shown in Table 1.

Quartz was the predominant mineral component in all samples, followed by calcite and clay. The quartz/clay rate ranged from 2.3 to 6.8 in all samples, except for sites F and H, where the rate was approximately 1. Those sites were influenced by the activity of the nearby salterns, which produced a higher input of fine material (clay). The effect of the salterns is also obvious on the halite content. Water conductivity in sites F and H was normally three times higher than seawater (95,500 μS/cm).

We then made mineralogical analyses of samples from sites B and G along a 55 cm deep profile. Significant differences were observed only in the halite and aragonite content. The amount of both compounds was higher at the surface and decreased with depth. Halite is the precipitated form of the sodium chloride contained in seawater. Therefore, the higher the rate of evaporation on the surface, the higher the halite contents there (11 to 22.1%

Table 1 Mineralogical composition (expressed in percentage w/w) of sediment from nine sampling sites of microbial mats of the Ebro Delta

Site	Quartz	Calcite	Clay	Halite	Aragonite	Dolomite	Felspars	Gypsum
A	49.4	18.1	14.8	6.8	6.9	2.3	0.7	0.9
B	54.9	21.4	10.2	5.7	3.3	1.6	2.0	0.9
C	45.8	19.2	13.9	8.1	8.0	2.1	2.3	0.4
D	36.4	16.0	15.3	9.7	16.5	2.0	2.7	1.5
D2	35.5	21.7	14.0	9.2	13.5	3.0	1.3	1.9
E	49.6	24.4	16.6	4.0	2.0	1.1	0.8	1.6
F	23.4	16.0	23.2	13.9	19.1	3.3	0.0	1.1
G	53.5	23.4	7.8	7.2	2.1	1.7	3.3	0.9
H	28.4	21.2	23.0	14.2	6.1	5.8	0.9	0.5
Average	41.88	20.16	15.42	8.76	8.61	2.54	1.56	1.08

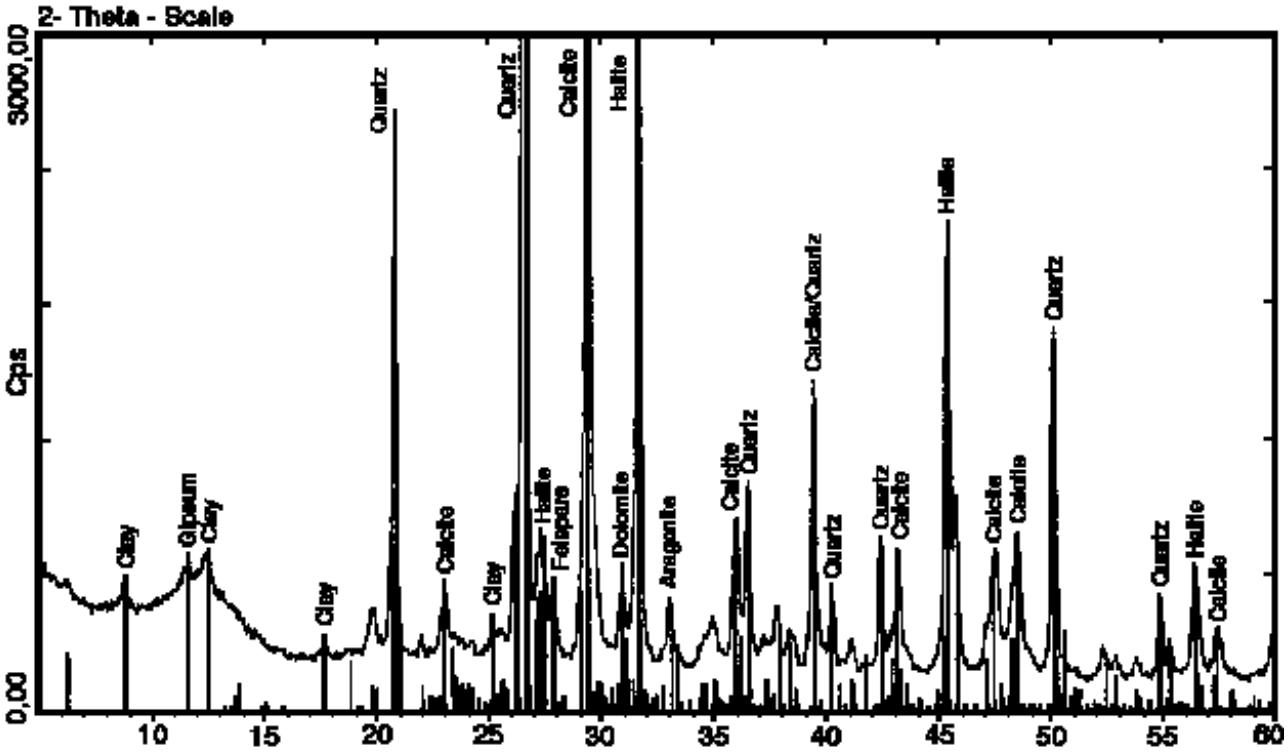

Fig. 1 X-ray diffractogram of site B sample of microbial mat sediment of the Ebro Delta. X-axis shows the 2θ diffraction angle and Y-axis, the signal intensity, expressed in counts per second (cps)

w/w). Aragonite is a particular form of calcium carbonate from biological origin, and it loses its crystallographic structure with time. The surface layer is exposed to the action of organisms that produce aragonite (shells of mollusks, carbonate precipitated by the cyanobacterial photosynthesis, etc.). Microbial mats with a strong precipitation of calcium carbonates by photosynthetic activity (sites D, D2 and F, see Table 1) showed the highest content of aragonite (ranged from 13.5 to 19.1% w/w), which under several favorable conditions can lithify into stromatolites. Dolomite (calcium and magnesium carbonate) concentration ranged from 1.1 to 5.8% (w/w). The activity of sulfate reducing bacteria as a possible origin of the dolomite has been suggested in previous studies [25].

Nitrogen and carbon analyses Total carbon, total nitrogen and organic carbon content were analyzed in the cross sections from 55 cm depth of sites B and G (Fig. 2). Both organic carbon and total nitrogen contents hit maximum levels at the surface, where the microbial mat is active. The two plots are significantly different because the degree of development of the mat at these sampling sites is also different. Microbial mat samples from site G have higher biomass content (48.7 to 75.1 μg of chlorophyll *a* per cm^2) than samples from site B (42.1 μg of chlorophyll *a* per cm^2) [11].

Table 2 shows the results obtained in the different sites at different times of the year. Total carbon ranged from 37.2 to 92.8 mg/cm^2, total nitrogen ranged from 0.3 to 10.8 mg/cm^2 and organic carbon ranged from 6.8 to 52.4 mg/cm^2. In most

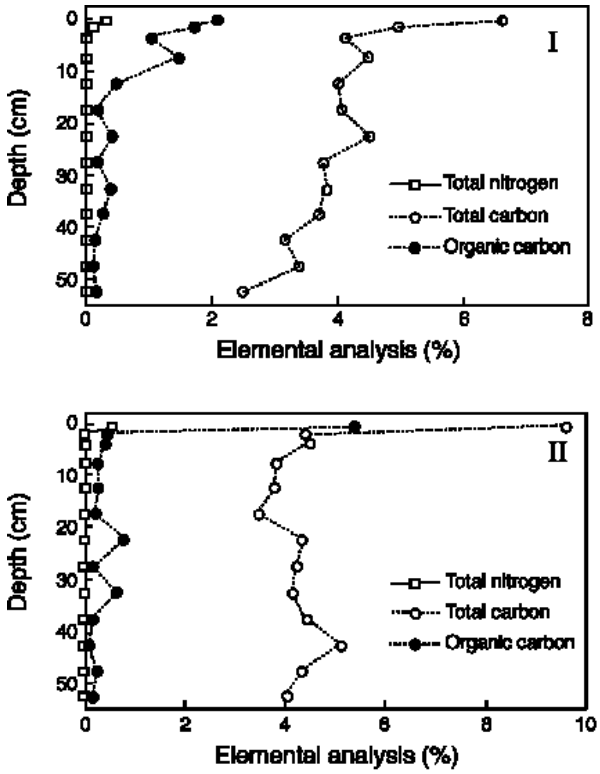

Fig. 2 Total carbon, organic carbon and total nitrogen profiles (expressed in percentage w/w) of a cross section of 55 cm depth in site B (I) and in site G (II) of microbial mats of the Ebro Delta

of the microbial mats studied, both organic carbon and total nitrogen contents (biomass) increased in summer (sites A, B, E and F), whereas at other sites, they were almost constant throughout the year (sites D, D2 and G) and others had a fluctuant biomass content (C, H and K). These changes could be due to the physicochemical environmental conditions, highly variable in some sites and very uniform in others.

Table 2 Total carbon, organic carbon and total nitrogen content in the microbial mats of the Ebro Delta at different times of the year: March (I), May (II), July (III) and December (IV)

I

Site	mg total C / cm^2	mg total N / cm^2	mg org C / cm^2	org C / t N
A	50.05	2.86	30.79	10.77
B	48.40	2.37	19.96	8.43
C	62.16	4.00	32.91	8.23
D	51.34	2.52	38.41	15.26
D2	49.35	5.90	39.05	6.62
E	42.47	0.35	6.83	19.73
F	49.11	7.56	42.32	5.60
G	47.25	2.59	22.23	8.59
H	52.48	4.49	21.68	4.83
K	39.47	4.40	18.85	4.28

II

Site	mg total C / cm^2	mg total N / cm^2	mg org C / cm^2	org C / t N
A	52.70	2.99	27.23	9.12
B	66.90	2.97	29.92	10.07
C	66.06	5.53	44.78	8.10
D	63.58	5.43	31.62	5.82
D2	76.18	6.14	47.99	7.82
E	40.79	1.57	12.03	7.65
F	72.68	5.48	51.53	9.41
G	37.20	7.14	25.50	3.57
H	49.95	6.15	32.26	5.25
K	49.04	6.67	35.24	5.28

III

Site	mg total C / cm^2	mg total N / cm^2	mg org C / cm^2	org C / t N
A	66.02	4.80	43.24	9.00
B	92.82	3.82	39.28	10.28
C	44.20	7.77	31.49	4.05
D	47.53	10.78	33.82	3.14
D2	86.72	7.64	46.15	6.04
E	45.58	3.82	20.44	5.35
F	74.96	6.85	52.43	7.65
G	37.26	10.53	26.47	2.51
H	45.70	5.34	26.16	4.90
K	40.15	10.47	27.00	2.58

IV

Site	mg total C / cm^2	mg total N / cm^2	mg org C / cm^2	org C / t N
A	53.33	3.86	36.48	9.45
B	55.77	2.67	29.86	11.18
C	ND*	ND	ND	ND
D	ND	ND	ND	ND
D2	ND	ND	ND	ND
E	ND	ND	ND	ND
F	53.69	4.70	36.71	7.81
G	53.29	5.14	32.05	6.24
H	49.33	4.19	33.88	8.09
K	73.02	4.80	49.76	10.37

*ND, not determined.

In sand samples from other areas at the Ebro Delta having no microbial mats, nitrogen cannot hardly be detected (less than 1 $\mu g/cm^2$), and the amounts of organic carbon are very low (less than 4 mg/cm^2). Therefore, according to these last observations, we can assume that all nitrogen measured at the microbial mat samples is of organic origin. For this reason, both total nitrogen and organic carbon can be good indicators of microbial biomass. The organic carbon/total nitrogen ratio is almost constant at each site. High differences in this ratio can indicate significant changes in the microbial populations and/or in the physicochemical conditions of the analyzed site. Biomass contents of the microbial mats depend on the degree of development of the community and are directly related to the capacity of sediment stabilization [10].

The influence of benthic microbiota on the erosion of the upper layers of marine sediments has been reported [23, 24]. The microbial mat coverage protects the sediment against erosion produced mainly by storm currents and wind. Bacterial decay, desiccation, burrowing activity, competition from floating algal masses and growth of halophilic vegetation are responsible for mat discontinuities [7, 22]. In these cases, the strongest and most persistent currents can break the mat into large flakes, and the underlying sediment can be eroded and transported away.

In situ studies of all sites in the field, such as the community structure and physiological status of microbial mats [15, 18], as well as the cultivation of the most abundant cyanobacterial genera found at each site in laboratory conditions, would lead to a better understanding of the interactions between marine sediments and benthic microbiota.

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References

1. Awramik SM (1984) Ancient stromatolites and microbial mats. In: Cohen Y, Castenholz RW, Halvorson HO (eds) Microbial Mats Stromatolites. Alan R. Liss. New York, pp 1–22
2. Boquet E, Boronat A, Ramos-Cormenzana A (1973) Production of calcite (calcium carbonate) crystals by soil bacteria is a general phenomenon. Nature 246:527–529
3. Chafetz HS (1986) Marine peloids: a product of bacterially induced precipitation of calcite. J Sedim Petrol 56:812–817
4. Chafetz HS (1994) Bacterially induced precipitates of calcium carbonate and lithification of microbial mats. In: Krumbein WE, Paterson DM, Stal LJ (eds) Biostabilization of sediments. Bibliotheks und Informationsystem der Universität, Oldenburg, pp 149–163
5. Chung FH (1974) Quantitative interpretation of X-ray diffraction pattern of mixtures. I. Matrix-flushing method for quantitative multicomponent analysis. J Appl Crystal 7:519–525

6. Chung FH (1975) Quantitative interpretation of X-ray diffraction pattern of mixtures. III. Simultaneous determination of a set of reference intensities. J Appl Crystal 8:17–19
7. Cornée A, Dickman M, Busson G (1992) Laminated cyanobacterial mats in sediments of solar salt works: some sedimentological implications. Sedimentology 39:599–612
8. Freytet P, Verrecchia EP (1998) Freshwater organisms that build stromatolites: a synopsis of biocrystallization by prokaryotic and eukaryotic algae. Sedimentology 45:535–563
9. Gerdes G, Krumbein WE, Reineck H-E (1994) Microbial mats as architects of sedimentary surface structures. In: Krumbein WE, Paterson DM, Stal LJ (eds) Biostabilization of sediments. Bibliotheks und Informationsystem der Universität, Oldenburg, pp 165–182
10. Grant J, Gust G (1987) Prediction of coastal sediment stability from photopigment content of mats of purple sulfur bacteria. Nature 330:244–246
11. Guerrero R, Urmeneta J, Rampone G (1993) Distribution of types of microbial mats at the Ebro Delta, Spain. BioSystems 31:135–144
12. Knoll AH (1989) The paleomicrobiological information in proterozoic rocks. In: Cohen Y, Rosenberg E (eds), Microbial mats: Physiological ecology of benthic microbial communities. American Society for Microbiology, Washington DC, pp 469–484
13. Krumbein WE, Cohen Y, Shilo M (1977) Solar Lake (Sinai) 4. Stromatolitic cyanobacterial mats. Limnol Oceanogr 22:635–656
14. Lalou C (1957) Studies on bacterial precipitation of carbonates in sea water. J Sedim Petrol 27:190–195
15. Lopez-Cortes A (1999) Paleobiological significance of hydrophobicity and adhesion of phototrophic bacteria from microbial mats. Precambrian Res 96:25–39
16. Mir J, Martínez-Alonso M, Esteve I, Guerrero R (1991) Vertical stratification and microbial assemblage of a microbial mat in the Ebro Delta (Spain). FEMS Microbiol Ecol 86:59–68
17. Morita RY (1980) Calcite precipitation by marine bacteria. Geomicrobiol J 2:63–82
18. Navarrete A, Peacock A, Macnaughton SJ, Urmeneta J, Mas-Castellà J, White DC, Guerrero R (2000) Physiological status and community composition of microbial mats of the Ebro Delta (Spain) by signature lipid biomarkers. Microb Ecol 39:92–99
19. Olendzenski L, Urmeneta J (1993) Selected organisms from the microbial mats of Baja California (Mexico) and Ebro Delta (Spain). In: Guerrero R, Pedrós-Alió C (eds) Trends in Microbial Ecology, Spanish Society for Microbiology, Barcelona, pp 543–546
20. Oppenheimer CH (1961) Note on the formation of spherical aragonite bodies in the presence of bacteria from the Bahama Bank. Geochim Cosmochim Acta 23:295–296
21. Oremland RS, King GM (1989) Methanogenesis in hypersaline environments. In: Cohen Y, Rosenberg E (eds) Microbial mats: Physiological ecology of benthic microbial communities. American Society for Microbiology, Washington DC, pp 180–190
22. Park RK (1977) The preservation potential of some recent stromatolites. Sedimentology 24:485–506
23. Rampone G, Urmeneta J, Puigdefàbregas C, Guerrero R (1993) Geographical distribution of the microbial mats in the Ebro Delta: Structural diversity and role in the stabilization of the sediments. Verh Internat Verein Limnol 25:1014–1019
24. Stal LJ (1994) Microbial mats: Ecophysiological interactions related to biogenic sediment stabilization. In: Krumbein WE, Paterson DM, Stal LJ (eds) Biostabilization of sediments. Bibliotheks und Informationsystem der Universität, Oldenburg, pp 149–163
25. Vasconcelos C, McKenzie JA, Bernasconi S, Grujic D, Tien AJ (1995) Microbial mediation as a possible mechanisms for natural dolomite formation at low temperatures. Nature 377:220–222