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Effects of Oil and Gas Well-Drilling Fluids on the Biomass and Community Structure of Microbiota that Colonize Sands in Running Seawater

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Abstract. Well-drilling fluid and a number of the known components (barite, clay, Aldacide®, Surflo®, and Dowicide®, were tested for effects on the biomass and community structure of the microbiota that colonize marine sands exposed for eight weeks to running ambient seawater. Shading the microbiota from light depressed the microflora without a significant effect on the biomass, while well-drilling fluids layered on the surface or mixed with the sand significantly increased a component of the bacteria and the microfauna as reflected in changes in the fatty acid composition. There were some shading effects from the surface layering of well-drilling fluids as reflected in the fatty acids from the microflora when compared to the sands mixed with well-drilling fluids. Barite had essentially no effect on the biomass or community structure while clays increased nearly all of the biomass indicators for the bacteria as well as the microfauna; the clay overlay mirrors the effect of the drilling fluids. Aldacide shifted the bacterial composition, depressing the proportions of microbes containing the cyclopropane fatty acids and the anaerobic pathways of desaturation. Concentrations of 1 and 15 μg/L increased the bacterial biomass as reflected in the total lipid (16:0) and extractable lipid phosphate coupled with a decrease in the total microeukaryotes. Surflo increased the biomass and shifted the bacterial community structure at concentrations between 4 and 800 μg/L. The lowest level also stimulated the microfauna. Dowicide at 100 μg/L increased the bacteria forming cis-vaccenic acid and the microfauna similar to low concentrations of Surflo.

Oil and gas well-drilling fluids are designed to bring up cuttings, maintain hydrostatic pressure, inhibit corrosion by sulfate reducing bacteria, and both cool and lubricate the drilling bits. These drilling fluids are basically aqueous clay suspensions containing barite with additives to prevent corrosion, seal, lubricate, emulsify, buffer, and increase density (Robichaux 1975). The possibility that tons of these fluids will be discharged and eventually deposited on the bottom of the sea (Shinn 1975; George 1975) prompted these studies on the impact of well-drilling fluids on the marine benthos.

Long term effects of well-drilling fluids on the biomass and community structure of the marine and estuarine benthos have been tested previously by monitoring the effects on the colonization of azoic sands by larvae in unfiltered seawater. Well-drilling fluids mixed with the sand depressed the numbers of individuals and species of annelids and coelenterates, and, when used as a thin covering layer, depressed the number of individuals and species of arthropods and molluscs when exposed to raw estuarine water for 13 weeks (Tagatz *et al.* 1979a). In the same test system, barite depressed numbers of molluscs and annelids (Tagatz and Tobia 1978) as well as nematodes and copepods (Cantelmo *et al.* 1979). Of the biocides added to well-drilling fluids (Bureau of Land Management 1978), Surflo® (chlorophenols) depressed the occurrence of chordates, annelids and especially molluscs (Tagatz *et al.* 1979b), and Dowicide® (pentachlorophenol) depressed the number of individuals and the number of species of molluscs (Tagatz *et al.* 1977, 1978). Aldacide® (paraformaldehyde) showed the least effect on the colonizing marine epibenthos (Tagatz *et al.* 1979b).

These experiments involved the colonization of azoic sands by the propagules in unfiltered seawater incubated on a platform 12 mi out in the Gulf of Mexico. In addition to studies of the benthic invertebrates, the study utilized the newly developed biochemical methodology (White *et al.* 1979a, 1979b; Bobbie and White 1980; Nickels *et al.* 1981a) for the analysis of the biomass and community structure of the marine benthic microbial community. The present tests examined impacts of oil and

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gas well-drilling fluids and specific components of these fluids to assess the consequences of releasing these fluids in the marine ecosystem.

Materials and Methods

The well-drilling fluid was collected from a barge moored next to an active drilling rig in Mobile Bay, AL. The fluids were transported and stored at 4°C and utilized within 10 days of collection. The composition of the fluids is currently being analyzed (Richards, unpublished data). The sands used for the substrate have been described (Nickels *et al.* 1981b). Barite, (94-96% barium sulfate) and clay (bentonite clay) were supplied by the Baroid Petroleum Service, NL Industries, Houston, TX. Aldacide®, which contains 91% paraformaldehyde (polymerized formaldehyde) and 9% inert materials, and Surflo B-33® which consists of 37.3% isopropyl alcohol, 17% sodium 2,2'methylene bis (4,6 dichlorophenol), 8.0% sodium salts of other chlorophenols and 37.5% inert ingredients, were supplied by the Baroid Petroleum Service, NL Industries Inc., Houston, TX. Dowicide G-ST® (Dow Chemical Company, Midland, MI.) contains 79% sodium pentachlorophenate, 11% sodium salts of other chlorophenols and 10% inert ingredients.

The experiments were performed on the United States Naval Stage I platform south of Panama City, FL. at 30° 7.5′ N, 85° 46.3′ W in the Gulf of Mexico between August 1, and September 27, 1978. The colonizing microbiota were exposed to well-drilling fluids in Plexiglas troughs 10 × 40 × 12 cm deep that were filled with sand to a depth of 5 cm. The sand had been dredged from 32 m just below the platform and dried in the sun for three weeks prior to the experiment. Unfiltered seawater was pumped from a depth of 26 m into a head box that maintained a flow rate of 200 ml/min over the sands at a depth of three cm. The experiments were allowed to run for eight weeks. The apparatus was originally designed by Hansen (1974) and modified as described by Hansen and Tagatz (1980) for experiments with oil and gas well-drilling fluids. The light intensity and spectral distribution were adjusted to match that on the bottom (32 m) with blue plastic sheeting used to cover the troughs. In experiments to test the effects of shading by the drilling fluid, light was screened out by covering the trays with opaque black plastic. Toxicants were added with constant delivery syringe pumps (Hansen and Tagatz 1980) and the concentration of Surflo and Dowicide in the seawater measured from the head boxes using gas-liquid chromatography as described (Tagatz *et al.* 1977; Hansen and Tagatz 1980). Aldacide was not measured. Clay, barite, and drilling fluids were added as 1 or 2 mm layers on the surface of the sands or mixed with the sand at ratios of 1 part to 10 parts of sand (w/w). The troughs were set up with 10 replicates of each treatment with 5 troughs of a given treatment placed side by side in a latin square arrangement. End troughs were not analyzed. At the end of the experiment sediment cores 2.54 cm in diameter were removed from the troughs and washed through 1 mm sieves to recover the macrofauna. The sands were allowed to stand for one minute and the seawater was decanted. The sands containing the meiofauna and microbes were quick-frozen at −70°C in plastic bags. The lipids were extracted from the sediment cores by the efficient single-phase chloroform-methanol-water solvent, derivatized by acid methanolysis, purified by thin layer chromatography, and the fatty acid methyl ester band recovered and assayed by glass capillary gas chromatography as previously described (Bobbie and White 1980; Nickels *et al.* 1981b). Lipid phosphate was determined after acid digestion (White *et al.* 1979b). The fatty acids are designated as the number of carbon atoms in the chain: the number of double bonds, and the position of the ultimate double bond (the double bond closest to the w end of the molecule) designated as w3, w6, etc. The prefixes a, i, and delta indicate anteiso, iso-branching and the cyclopropane ring.

Results

Oil and Gas Well-Drilling Fluids

The effects of oil and gas well-drilling fluids placed as a 1 or 2 mm layer on the top of sand or mixed in proportions of 1 part well-drilling fluid to 10 parts sand on the biomass and community structure of the microbiota colonizing the marine sands in eight weeks are given in Table 1. Screening out light with opaque plastic produced significant decreases in the polyenoic fatty acids of the alpha linolenic series 20:5w3, 22:6w3 and increased the ratio of eukaryotic microfauna to microflora (polyenoic w6/w3 ratio) (Table 1, column B). Screening decreased the algae with very little effect on the bacteria or the microfauna measured as 20:4w6. Drilling fluids, whether mixed with sand or layered on the sediment, showed considerably greater effects than merely screening out light (Table 1, columns C-E). There was a two-fold increase in the absolute amount of the 15:0, the 20:4w6 and the ratios 20:4w6/16:0 and total polyenoic fatty acids longer than 20 carbon atoms/16:0. The absolute amount of 24:0 increased in the presence of drilling fluid. Mixing well-drilling fluid with the sediment significantly increased the absolute amount of linoleic acid (18:2w6) and 22:6w3 in the microbiota when compared with overlaying the sand.

Barite Layers

Barite ($BaSO_4$) is a major component of oil and gas well-drilling fluids. Although a few significant changes were observed, layers of barite 1 to 2 mm thick on marine sands produced no consistent trends in the microbial biomass or community structure (Table 2).

Clay Layers

Clay layers significantly affected the bacterial and microeukaryotic biomass and community structure (Table 2). Bacterial community stimulation by a clay overlay was indicated in the absolute increases in the short and short-branched fatty acids (15:0, a + i 15:0) and in cis-vaccenic acid (18:1w7). Eukaryotic microfauna increased as seen in the higher palmitic acid (16:0), 20:4w6 and in the ratio of 20:4w6/16:0.

Table 1. Effects of darkness and oil and gas well-drilling fluids on the colonization of marine sands

	Control sand[a]		Dark sand[b]		1mm layer drill fluid[c]		2mm layer drill fluid[d]		1 to 10 mix drill fluid[e]	
Biomass	n moles per 40 g wet wt/ (31.5 g dry wt.)									
Lipid phosphate	249	(41)	211	(26)	221	(126)	220	(32)	284	(63)
a + i 15:0	7	(3)	7	(4)	13	(11)*	10	(4)	11	(3)*
15:0	8	(3)	7	(4)	16	(11)**	12	(5)**	13	(5)***
16:0	47	(16)	41	(23)	88	(47)***	63	(22)	67	(16)**
delta (17:0 + 19:0)	5	(5)	3.5	(2)	6	(3)	5	(1)	7	(1)
18:1w7	12	(5)	11	(6)	12	(7)	13	(4)	15	(3)
18:1w9	12	(8)	9	(6)	10	(3)	10	(3)	13	(4)
18:2w6	3	(2)	1.5	(1)	3	(3)	2	(1)	7	(8)***
20:4w6	4	(3)	4	(3)	10	(4)***	8	(4)**	9	(4)***
20:5w3	1.7	(1.7)	0.3	(0.3)*	2	(2)	2	(1)	1	(1)
22:5w3					0.4	(1)			0.2	(0.4)*
22:6w3	0.7	(0.5)	0.1	(0.1)*	1	(1)	1	(1)	4	(3)***
24:0	3	(2)	2	(1)	5	(3)*	5	(2)**	4	(2)***
Total polyenoics[f]	10	(4.4)	6	(4.2)	22	(7)***	13	(5)	31	(12)**
Lipid P/16:0	610	(290)	970	(1170)	405	(79)*	394	(154)*	319	(203)**
a + i 15:0/15:0	98	(6)	98	(6)	83	(16)*	83	(9)*	75	(14)***
a + i 15:0/16:0	15	(3)	15	(3)	16	(1)	16	(4)	13	(4)
Delta (17:0 + 19:0)/16:0	9.2	(2)	9	(2)	9	(2)	8	(1)	6	(1)**
18:1w7/16:0	26	(3)	30	(3)	23	(7)	21	(4)**	14	(0.7)***
18:1w7/18:1w9	130	(40)	130	(40)	123	(25)	127	(30)	86	(16)***
18:2w6/16:0	5.4	(2)	5	(2)	12	(14)*	3	(1)*	3	(1)*
20:5w3/16:0	4.5	(4)	5	(4)	2	(2)	3	(2)	2	(2)
20:4w6/16:0	5.4	(4)	5	(4)	14	(5)*	13	(3)**	14	(7)**
22:6w3/16:0	3.1	(0.8)	1	(0.8)	6	(6)***	1	(1)	0.7	(0.8)
Total polyenoic/16:0	14	(4)	12	(3)	23	(9)***	18	(6)*	38	(47)**
Polyenoic w6/w3	438	(668)	1210	(347)*	173	(37)	470	(321)	200	(276)

[a] Control sand, X (SD), n = 15
[b] Sand shaded from light, X (SD), n = 5
[c] Sand covered with 1 mm well-drilling fluid, X (SD), n = 5
[d] Sand covered with 2 mm well-drilling fluid, X (SD), n = 5
[e] Sand mixed 10 to 1 with well-drilling fluid, X (SD), n = 5
[f] Polyenoic fatty acids = Sum of 20:4w6, 20:5w3, 22:6w3
*,** and *** indicate statistical significance between the means by analysis of variance between control sand (column a) and the experimental treatments at the 0.1, 0.05 and 0.01 level

Aldacide

Aldacide changed the community structure of the colonizing microbiota at all concentrations tested (Table 3). There was a decreased proportion of the bacteria containing cis-vaccenic acid (18:1w7) compared to the total lipid (16:0). Bacteria and eukaryotes containing oleic acid (18:1w9) as well as the proportions of the bacteria containing the cyclopropane fatty acids delta 17:0 and delta 19:0 were also decreased compared to the total lipid. Significant changes in absolute amounts of fatty acids were not detected with 300 μg/L Aldacide but changes were noted at 15 μg/L and 1 μg/L concentrations in seawater. Increases in the total extractable lipid phosphate and decreases in the absolute amounts of 18:2w6, 24:0 and in the polyenoic fatty acids longer than 20 carbon atoms occurred with exposure to more dilute Aldacide.

Surflo

Surflo showed consistently higher membrane biomass as reflected in the extractable lipid phosphate (Table 3). There was a consistently higher ratio of cis-vaccenic acid (18:1w7) to oleic acid (18:1w9) with all of the three tested Surflo concentrations. The 4μg/L level of Surflo stimulated microbial growth significantly. The absolute amounts of bacterial fatty acids a + i 15:0, 15:0, and 18:1w7, and the microeukaryote indicators 20:4w6 and the total polyenoic fatty acids longer than 20 carbon atoms as well as the total lipids (16:0) were increased significantly when the colonizing microbiota was exposed to 4 μg/L Surflo.

Dowicide

Dowicide at a concentration of 100 μg/L affected

Table 2. Effects of barite and clay on the biomass and community structure of colonizing microbiota on marine sands

	Control sand[a]		1mm layer barite[b]		2mm layer barite[c]		1mm layer clay[d]		2mm layer clay[e]	
Biomass	n moles per 40 g wet wt/ (31.5 g dry wt.)									
Lipid phosphate	249	(41)	220	(289)	284	(22)	252	(32)	252	(63)
a + i 15:0	7	(3)	8	(3)	6	(4)	12	(4)**	12	(4)**
15:0	8	(3)	10	(4)	6	(4)	13	(4)***	13	(4)***
16:0	47	(16)	47	(20)	41	(22)	62	(15)*	67	(16)**
Delta (17:0 + 19:0)	5	(5)	5	(2)	4	(2)	4	(1)	6	(3)
18:1w7	12	(5)	9	(5)	12	(10)	16	(4)	20	(5)***
18:1w9	12	(8)	9	(5)	32	(34)**	13	(5)	12	(3)
18:2w6	3	(2)	1	(0.00)**	9	(17)	2	(1)	3	(2)
20:4w6	4	(3)	7	(6)	4	(3)	8	(3)**	8	(1)***
20:5w3	1.7	(1.7)	0.0	(0.0)	1	(1)	1	(2)	1	(2)
22:6w3	0.7	(0.5)	1	(1)	1	(1)	1	(0.0)	1	(1)
24:0	3	(2)	2	(1)	2	(1)	1	(1)	3	(1)
Total polyenoics[f]	10	(4.4)	9	(7)	14	(20)	10	(6)	13	(3)*
Lipid P/16:0	610	(290)	577	(230)	824	(447)	936	(122)	395	(120)
a + i 15:0/15:0	98	(6)	83	(6)**	98	(4)	87	(9)	96	(9)
a + i 15:0/16:0	15	(3)	17	(2)	15	(1)	18	(2)	18	(2)
Delta (17:0 + 19:0) 16:0	9.2	(2)	9.8	(2)	10	(2)	7	(0.9)	9	(2)
18:1w7/16:0	26	(3)	20	(2)***	24	(14)	26	(2)	30	(6)
18:1w7/18:1w9	130	(40)	108	(16)	94	(92)	135	(27)	170	(32)
18:2w6/16:0	5	(2)	3	(1)**	15	(24)*	3	(2)	5	(2)
20:5w3/16:0	4.5	(4)	1	(1)**	3	(2)	2	(3)	2	(1)
20:4w6/16:0	5.4	(4)	14	(9)**	7.4	(4)	14	(7)**	12	(3)*
22:6w3/16:0	3.1	(0.8)	1.4	(0.6)	1.6	(0.6)	0.9	(0.5)	1	(1)
Total polyenoic/16:0	14	(4)	17	(10)	13	(4)	18	(9)	6	(3)
Polyenoic w6/w3	438	(668)	570	(736)	200	(160)	316	(223)	330	(295)

[a] Control sand, X (SD), n = 15
[b] Sand covered with 1 mm Barite, X (SD), n = 5
[c] Sand covered with 2 mm Barite, X (SD), n = 5
[d] Sand covered with 1 mm Clay, X (SD), n = 5
[e] Sand covered with 2 mm Clay, X (SD), n = 5
[f] Polyenoic fatty acids = Sum of 20:4w6, 20:5w3, 22:6w3
*,** and *** indicate statistical significance between the means by analysis of variance between control sand (column a) and the experimental treatments at the 0.1, 0.05 and 0.01 level

the biomass and community structure of the microbiota colonizing marine sands (Table 3). Dowicide induced absolute increases in bacteria containing the anaerobic desaturation pathway which forms 18:1w7 and in the microfauna containing 20:4w6 and 16:0. The community structure shifted to a more bacteria-enriched assembly indicated by the increased proportions of 18:1w7/16:0 and decreased 20:5w3/16:0.

Discussion

In studies of estuarine detrital (Morrison *et al.* 1977; Bobbie and White 1980; King and White 1977; Fazio *et al.* 1979; Morrison and White 1980), marine microfouling (Nickels *et al.* 1981a) and marine sedimentary microbiota (White *et al.* 1979b; 1980b; Nickels *et al.* 1981b; Davis and White 1980), a suite of biochemical methods has been developed for the analysis of the biomass and community structure of the microbial assemblies. Validation of assignments of specific biochemical indicators to various components of these microbial assemblies have been made by comparing morphology by scanning electron microscopy to biochemical analysis (Morrison *et al.* 1977; White *et al.* 1980a), by manipulating communities with growth conditions and antibiotics (White *et al.* 1980a; Bobbie *et al.* 1981), by isolating monocultures from mixtures (White *et al.* 1980a), by mixing monocultures in various proportions followed by analysis (Bobbie and White 1980; White *et al.* 1980a) and by comparing the effects of specific predation by sand dollars to gut analysis (White *et al.* 1980b). With this information, it is possible to define the significance of the components analyzed in this study. Lipid phosphate measures the viable membrane biomass of all the microbes. The fatty acids a + i 15:0, 15:0, delta 17:0, delta 19:0, and 18:1w7 are measures of various components of the bacterial community. The 18:2w6 is found in the protozoa, fungi, algae and certain flexibacteria. Polyenoic fatty acids longer than 20 carbon atoms

Table 3. Effects of Aldacide, Surflo, and Dowicide on the biomass and community structure of colonizing microbiota on marine sands

	Control sand[a]	300 μg/L Aldacide[b]	15 μg/L Aldacide[c]	1 μg/L Aldacide[d]	800 μg/L Surflo[e]	40 μg/L Surflo[f]	4 μg/L Surflo[g]	100 μg/L Dowicide[h]
Biomass	n moles per 40 g wet wt/(31.5 g dry wt.)							
Lipid phosphate	249 (41)	252 (32)	284 (63)*	284 (63)*	284 (63)*	284 (32)**	284 (32)**	252 (32)
a + i 15:0	7 (3)	10 (3)	17 (19)	8 (4)	9 (4)	8 (5)	14 (15)*	8 (4)
15:0	8 (3)	8 (3)	9 (4)	9 (4)	9 (4)	9 (5)	15 (14)**	10 (5)
16:0	47 (16)	69 (21)**	51 (13)	51 (13)	50 (72)	50 (72)	80 (67)**	62 (17)*
Delta (17:0 + 19:0)	5 (5)	5 (2)	4 (1)	4 (1)	5 (2)	4 (1)	6 (5)	5 (1)
18:1w7	12 (5)	13 (4)	13 (3)	13 (3)	17 (6)*	12 (6)	19 (16)*	18 (3)**
18:1w9	12 (8)	15 (4)	10 (5)	10 (5)	8 (4)	7 (2)*	12 (10)	14 (7)
18:2w6	3 (2)	3 (2)	1 (1)*	1 (1)*	3 (1)	1 (1)*	2 (2)	1 (0.0)**
20:4w6	4 (3)	2 (2)	4 (2)	4 (2)	1 (0.0)	5 (1)*	8 (4)**	7 (2)*
20:5w3	1.7(1.7)	2 (1)	1 (1)	1 (1)	2 (2)	4 (1)**	2 (2)	1 (0.0)
22:6w3	0.7(0.5)	1 (0.0)	1 (1)	1 (1)	1 (1)	1 (1)	1 (2)	1 (2)
24:0	3 (2)	4 (4)	1 (1)**	1 (1)**	2 (3)	3 (3)	4 (4)	3 (1)
Total polyenoics[i]	10 (4.4)	7 (5)	7 (3)**	7 (4)*	7 (3)**	8 (3)	14 (8)*	11 (4)
Community structure	ratios (times 100)							
Lipid P/16:0	610 (290)	378 (110)	593 (220)*	412 (170)*	548 (135)	655 (295)	596 (502)	355 (81)*
a + i 15:0/15:0	98 (6)	124 (39)**	87 (14)	95 (12)	99 (16)	80 (14)**	90 (13)	82 (10)***
a + i 15:0/16:0	15 (3)	17 (7)	14 (4)	14 (5)	18 (4)	14 (5)	16 (4)	16 (4)
Delta (17:0 + 19:0)/16:0	9.2(2)	7 (2)*	8 (1)	7 (3)*	11 (3)*	10 (2)	8 (2)	9 (2)
18:1w7/16:0	26 (3)	22 (4)**	25 (1)	26 (2)	36 (6)***	25 (3)	24 (3)	30 (6)**
18:1w7/18:1w9	130 (40)	92 (51)	113 (55)*	92 (64)	183 (47)**	164 (30)*	165 (22)*	142 (57)
18:2w6/16:0	5 (2)	7 (2)*	3 (1)*	8 (4)	5 (2)	3 (2)**	3 (1)**	3 (0.4)**
20:5w3/16:0	4.5(4)	3 (3)	2 (1)	3 (4)	4 (3)	0.6 (0.6)*	2 (2)	1 (0.6)**
20:4w6/16:0	5.4(4)	3.8 (3)	4 (2)	4 (2)	1 (0.0)	5 (1)	8 (4)**	7 (2)**
22:6w3/16:0	3.1(0.8)	1 (0.8)	1 (0.4)	1 (0.9)	2 (2)	2 (1)	1.3 (0.0)	2 (4)
Total polyenoic/16:0	14 (4)	8 (9)	11 (3)*	14 (7)	9 (5)	14 (2)	16 (5)	16 (11)
Polyenoic w6/w3	438 (668)	74 (62)	592 (219)	220 (148)	240 (12)	190 (188)	96 (100)	356 (356)

[a] Control sand, $\bar{X}$ (SD), n = 15
[b] 300 μg/L Aldacide (90% paraformaldehyde), $\bar{X}$ (SD), n = 5
[c] 15 μg/L Aldacide (90% paraformaldehyde), $\bar{X}$ (SD), n = 5
[d] 1 μg/L Aldacide (90% paraformaldehyde), $\bar{X}$ (SD), n = 5
[e] 800 μg/L Surflo (25% dichlorophenol), $\bar{X}$ (SD), n = 5
[f] 40 μg/L Surflo (25% dichlorophenol), $\bar{X}$ (SD), n = 5
[g] 4 μg/L Surflo (25% dichlorophenol), $\bar{X}$ (SD), n = 5
[h] 100 μg/L Dowicide (79% sodium pentachlorophenate), $\bar{X}$ (SD), n = 5
[i] Polyenoic fatty acids = sum of 20:4w6, 20:5w3, 22:6w3
*,** and *** indicate statistical significance between the means by analysis of variance between control sand (column a) and the experimental treatments (columns b through h) at the 0.1, 0.05 and 0.01 level

and the long chain alkyl fatty acids (24:0) are found in the microeukaryotes. In polyenoic fatty acids, the w3 series are concentrated in the microflora and the w6 series in the microfauna. The total palmitic acid (16:0), a ubiquitous fatty acid, is a measure of the total lipid (both the neutral and phospholipids), so the ratio of lipid phosphate to 16:0 is a measure of the bacteria to higher microbiota, since neutral lipids tend to be low and phospholipids high in bacteria. Ratios of various fatty acids such as a + i 15:0/15:0 and a + i 15:0/16:0 represent the proportion of the short branched fatty acid-containing bacteria to the total bacteria and to the total community, respectively. The ratio of 18:1w7/16:0 and 18:1w7/18:1w9 represent the bacteria containing the anaerobic desaturation pathway to the total community and to the portion of the bacteria and microeukaryotes containing oleic acid. The ratio of polyenoic w6/w3 fatty acids is a rough ratio of the microfauna to the microflora; thus the effects of oil and gas drilling fluids and their components can be examined.

An overlay of well-drilling fluid on the marine sand has a significantly different effect than merely shading the microbiota from light. Shading decreases the microflora as reflected in the decrease in polyenoic fatty acids of the alpha linolenic series (w3). Well-drilling fluids, whether they are used as an overlay or mixed with the sand, increase a portion of the bacterial community as reflected in the two-fold increase in 15:0 and the microfauna as reflected in the increased polyenoic fatty acids 20:4w6, 24:0 and the total polyenoic fatty acids/16:0. There was a shading by the well-drilling fluid overlay as ween in the increase in 18:2w6 and 22:5w3 when the well-drilling fluid was mixed with the sand. The higher level of palmitic acid in the microbiota resulted in a significantly lower ratio of lipid phosphate to total palmitic acid and the high 15:0 significantly decreased the a + i 15:0/15:0 ratio.

The ratio of 20:4w6 and the total polyenoic fatty acids longer than 20 carbon atoms to 16:0 was significantly (two-fold) higher in the presence of the well-drilling fluids.

Layers of barium sulfate produced no consistent changes in microbial biomass or community structure in the colonization of the marine sediments. A clay overlay increased both the bacterial and microfaunal biomass as reflected in the short and short-branched fatty acids, the cis-vaccenic acid, the palmitic acid and the 20:4w6. The response of the colonizing microbiota to clay was much like that of the oil and gas well-drilling fluids. Clays have marked effects on the community structure of soil microbiota (Stotzky 1974; Hattori and Hattori 1976). Effects of antimicrobial compounds, often included in oil and gas well-drilling fluids, are detectable at the part per billion (μg/L) concentration (Table 3). Aldacide depressed the relative proportions of the bacterial components containing cis-vaccenic acid and the cyclopropane fatty acids. The 1 μg/L and 15 μg/L concentrations of Aldacide increased the total bacterial biomass and decreased the microeukaryotes as measured by the absolute amounts of 18:2w6, 24:0 and the polyenoic fatty acids longer than 20 carbon atoms. Surflo consistently stimulated microbial growth as reflected in the increased extractable lipid phosphate. The microbial community structure also shifted as reflected in the increased ratio of cis-vaccenic acid-forming to oleic acid-forming organisms (18:1w7/18:1w9). Surflo at a level of 4 μg/L stimulated bacteria with short and short branched fatty acids as well as those with cis-vaccenic acid. The microfauna as indicated by the 20:4w6 and the total polyenoic fatty acids longer than 20 carbon atoms were increased in the presence of 4 μg/L Surflo. When Dowicide was added to the seawater, the total microbial biomass measured as the extractable lipid phosphate and the total palmitic acid was unchanged, but there was a shift in the community structure. Dowicide induced an increase in the bacteria particularly those containing the cis-vaccenic acid pathway for unsaturation and a shift in the microeukaryotes as reflected in the different proportions of the w6 and w3 polyenoic fatty acids.

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