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Penetration of Sulfate Reducers through a Porous North Sea Oil Reservoir

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The presence of mesophilic benzoate-degrading sulfate-reducing bacteria in the water systems of three Norwegian oil platforms was investigated. Strain 4502 was isolated from the injection water system, and specific antibodies were produced against this isolate. It was present in the injection water system during a period of 3 years, but not in the in situ reservoir water. Later it was found in water samples collected from the oil field production system. This showed that strain 4502 had penetrated the reservoir together with the injection water and eventually reached the production well.

During oil production, water is often injected into the reservoir to maintain reservoir pressure. The Norwegian oil fields are located offshore, and seawater is therefore used as injection water. This injection water is transported through the reservoir and eventually reaches the production wells together with oil and gas. Sulfate reducers have been reported from water samples from both the injection water systems (25) and production systems (3, 4, 6, 20, 21, 23) of Norwegian oil fields. The injection of sulfate-rich seawater might stimulate the growth of sulfate-reducing bacteria with subsequent production of H_2S (20, 26). This biogenic H_2S production causes several problems during oil production, e.g., corrosion of iron and steel alloys in the oil wells, reduction of the oil quality by souring oil and gas (8), reduction of the permeability of the oil formation (2, 8, 9, 11–13, 17), and finally, a health hazard to the platform personnel (18). Biogenic sulfate reduction may also increase the H_2S content of the export gas to a level where H_2S removal is required, a costly process in offshore gas production. The quality of the injection water is crucial for the growth of sulfate-reducing bacteria in the injection system (26). Sulfate-reducing bacteria are able to grow either directly (1, 24) or indirectly (13) on oil components and water injection additives (25). Model experiments have shown that bacteria are able to penetrate the reservoir pores together with the injection water (5, 15, 16, 19, 22, 27), and it has been assumed that microbes could also penetrate the oil reservoir. However, until now, no field observations have shown reservoir penetration by one particular microorganism. In order to demonstrate penetration, a suitable indicator microorganism is required. This paper reports the isolation of one such indicator bacterium from injection water followed by isolation of the same bacterium from water from the oil field production system under conditions which implied that the microbe had penetrated the reservoir.

The water systems at the three Gullfaks oil platforms (GFA, GFB, and GFC) have been monitored regularly for the distribution and activity of microorganisms. Several techniques have been used, including immunological techniques and different kinds of enrichment cultures (3, 4, 20). Sulfate-reducing bacteria belonging to different genera are able to degrade benzoate (10), and benzoate was used as a substrate in enrichment cultures on a regular basis during monitoring.

A benzoate-degrading sulfate-reducing bacterium, strain 4502, was first isolated from the injection water at GFA. Specific polyclonal antibodies were produced against strain 4502 and used to investigate the presence of this serotype in the water and biofilm samples. The bacterium was found to be present in the injection water systems at GFA and GFB. Later the strain was also identified in water samples collected from the oil field production systems of these platforms.

Water samples and biofilms were collected from the injection water systems of three Norwegian oil platforms, GFA, GFB, and GFC. The reservoir conditions were as described previously (20). The permeability of the Gullfaks reservoir ranges from 1,000 to 4,000 millidarcy, with a mean pore size of 20 μm. The reservoir pressure was 28 MPa. The distances between the injection wells and the production wells on this oil field were 1 to 4 km. The transport time for the injection water to reach the production well was at least 5 months, determined by increases in the concentrations of sulfate and magnesium in the oil field production system water at seawater breakthrough. From the production system, only water samples were collected. On each sampling date, anoxic water samples were collected without exposure to oxygen from three different locations in the injection water system. Biofilms were grown in situ on biocoupons (mild steel; Oilfield Microbiology Services, Ltd., Aberdeen, Scotland) located in the pipeline under anoxic conditions and elevated pressure. At GFA, the biocoupons were subjected to a pressure of 1 MPa, whereas the biocoupons at GFB and GFC were subjected to a pressure of 24 MPa. The biocoupons with biofilm were removed from the injection water system after in situ incubation of 2 to 12 months and replaced with new biocoupons. The biocoupons were transported anaerobically onshore, immobilized in specially designated containers, and immersed in anoxic injection water. Oil field production system water was sampled anaerobically as described previously (20).

An oxyalkyl aromatic surfactant was added to the injection water at GFA and GFB during the period from 1988 to 1990. The aromatic surfactant was never added to the injection water at GFC.

Water samples (5 ml) were transferred anaerobically into sterile serum bottles (50 ml) containing 45 ml of anoxic medium (28) with sulfate as the electron acceptor and benzoate as the carbon and electron donor. Biocoupons were transferred into sterile, anoxic 13-ml serum bottles containing 2 ml of sterile filtered anoxic injection water. The biofilm was suspended by sonication for 1 to 2 min, and 8 ml of sterile filtered

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TABLE 1. Enrichment cultures from GFA

Yr	Mo	Enrichment[a] with the following sample:		
		Injection water system		Oil field production system water[b]
		Water	Biocoupon	
1990	October	+	+	
1991	February	−	+	
	September	−	NS	
	October	−	−	
	December	−	NS	
1992	February	−	+	
	March	−	NS	
	July	+	NS	
	October	+	+	
	November	−	NS	
1993	February	−	NS	
	March	+	+	
	April	−	NS	
	May	−	NS	
	September	−	+	
	October	−	NS	−
1994	February	−	−	+
	May	−	NS	+
	June	−	NS	NS
	October	−	−	−

[a] +, enrichment of benzoate-degrading sulfate-reducing bacteria; −, no enrichment of benzoate-degrading sulfate-reducing bacteria; NS, not sampled.
[b] First sample obtained in October 1993.

anoxic injection water was added. One milliliter of this biofilm suspension was used as inoculum and transferred into a 50-ml bottle as described above. The enrichment cultures were incubated at 30°C. Growth and sulfide production were recorded after 1, 2, and 3 months of incubation. Hydrogen sulfide production was detected with copper sulfate (7). Pure cultures were isolated by dilution series as described previously (6). Survival of strain 4502 was tested by incubation at 45 and 60°C for 1 month, with subsequent incubation at 30°C.

Polyclonal antiserum (anti-4502) was produced against strain 4502 as described previously (6). Antigens were characterized and compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western immunoblotting as described previously (3).

Identification of strain 4502 in the enrichment cultures was carried out by fluorescent antibody (FA) and 4′,6-diamidino-2-phenylindole (DAPI) double staining (14, 20).

Immuno-cross-reactivity tests were carried out to verify the specificity of the antiserum. The sulfate reducers included in the tests were *Desulfobacter* B 54 (DSM 8776), *Desulfotomaculum thermocisternum* ST 90 (DSM 10259), *Thermodesulforhabdus norvegicus* A8444 (DSM 9990), *Archaeoglobus fulgidus* 7324 (DSM 8774), *Desulfovibrio desulfuricans* 4303, and the mesophilic benzoate-degrading strain 4701, all isolated from North Sea oil field water (20).

Strain 4502 was isolated from a biocoupon in the injection water flow line at GFA retrieved in September 1993. The isolate consisted of short rods and showed a strong tendency to form biofilm on surfaces during growth. Growth was observed at 30°C with benzoate as the substrate. Growth was not observed at 45°C or higher temperatures, but the isolate survived incubation at 45 and 60°C for 1 month. Benzoate-degrading sulfate reducers were detected in enrichment cultures with water samples from the injection water systems of GFA (Table 1) and GFB (Table 2) collected from October 1990 to September 1993 (GFA) and April 1994 (GFB). Oil field production system water was sampled in October 1993, but no benzoate-degrading sulfate-reducing bacteria were detected in the first samples. However, in 1994, benzoate-degrading sulfate-reducing bacteria were detected in several oil field production system water samples from GFA and GFB. The microbe could not be detected in in situ reservoir water sampled from individual production wells. Enrichment cultures of benzoate-degrading sulfate-reducing bacteria were not obtained from the injection water system or oil field production system water from platform GFC (data not shown). The polyclonal antiserum (anti-4502) did not cross-react with any of the sulfate reducers included in the test. The enriched benzoate-degrading sulfate-reducing bacteria obtained from different water and biofilm samples were compared with strain 4502 by SDS-PAGE, immunoblotting, and FA staining. By immunoblotting, they showed similar immunological patterns, with common bands of 26, 36, 50, and 73 kDa (Fig. 1). The protein profiles obtained by SDS-PAGE were similar to that of strain 4502, and the FA staining results also showed that they were all immunologically similar to strain 4502 (data not shown).

Strain 4502 was present in samples from both water and biofilm in the injection water system, and later also in the oil field production system water. Thus, strain 4502 served as an indicator microorganism and made it possible to show that sulfate-reducing bacteria can be transported by injected seawater through the oil reservoir. The question then arises whether strain 4502 is capable of growth under reservoir conditions. Strain 4502 is a mesophilic sulfate-reducing bacterium, and the initial temperature in this reservoir is approximately 74°C (20). The injection of seawater cools down parts of the reservoir close to the injection well, and mesophilic sulfate-reducing bacteria might therefore be able to inhabit this part of the reservoir after some period of flooding. The temperature in the reservoir was too high for growth of strain 4502, but not high enough to kill this bacterium during the time required to penetrate the reservoir. Strain 4502 does not grow at 45°C or higher temperatures, and endospore formation has not been observed, and it is therefore not likely that it had inhabited the reservoir prior to the flooding. The fact that it could not be detected in in situ reservoir water supports this theory. If strain 4502 had been present in the reservoir since the oil was formed, it must have survived for millions of years under con-

TABLE 2. Enrichment cultures from GFB

Yr	Mo	Enrichment[a] with the following sample:		
		Injection water system		Oil field production system water[b]
		Water	Biocoupon	
1990	October		+	
1991	December	−	−	
1992	March	−	NS	
	April	−	−	
	July	+	NS	
	December	−	NS	
1993	February	−	NS	
	March	−	−	
	May	−	NS	
	October	−	−	−
1994	April	−	+	−
	June	−	NS	+
	September	−	−	+
	November	−	−	+

[a] −, no enrichment of benzoate-degrading sulfate-reducing bacteria; +, enrichment of benzoate-degrading sulfate-reducing bacteria; NS, not sampled.
[b] First sample obtained in October 1993.

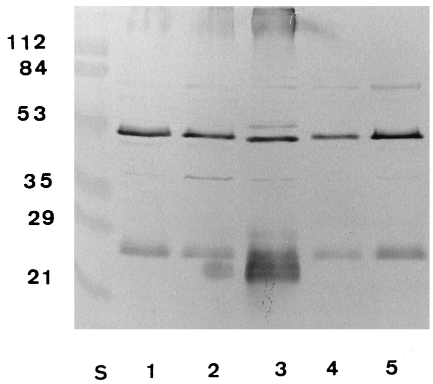

FIG. 1. Immunoblotting with anti-4502. Lanes 1, 2, 4, and 5, benzoate-degrading sulfate-reducing bacteria from oil field production system water; lane 3, strain 4502; lane s, molecular mass standards, with values (in kilodaltons) indicated to the left of the gel.

ditions where it cannot grow. The carbon sources for benzoate-degrading sulfate-reducing bacteria in oil field water systems and in the reservoir are most probably degradation products from crude oil and other aromatic compounds. One possible substrate could be an aromatic surfactant added to the injection water on GFA and GFB in the period from 1988 to 1990. From 1990 through 1993, enrichment cultures of benzoate-degrading sulfate-reducing bacteria were obtained in both water samples and biofilm samples from the injection system. Benzoate-degrading sulfate-reducing bacteria disappeared from the injection water at GFA and GFB in April 1993 and December 1992, respectively, but were still detected in the biofilm. The aromatic surfactant was not added to the injection water on GFC, and benzoate-degrading sulfate-reducing bacteria were never found at this platform, neither in the injection water system nor in the oil field production system water.

High-permeability zones would facilitate the transportation of strain 4502 through the reservoir. The elevated pressure in the oil reservoir (28 MPa) seems not to hinder growth of strain 4502, since it was isolated from a biofilm grown at a pressure of 24 MPa. Strain 4502 also survived the large decrease in pressure from 24 to 0.1 MPa during sampling.

Apparently, strain 4502 was established in the injection water systems at GFA and GFB during the period when the aromatic surfactant was added to the injection water on these two platforms, and it is likely that strain 4502 was growing in the injection water system with this surfactant as the substrate. The strain then appeared in the oil field production system water after surviving transport through the oil reservoir with the injected seawater.

These findings imply that microorganisms present in the injection water will eventually contaminate the water-flooded part of the oil reservoir. Under conditions favorable for growth, these microbes will be active. The injection of sulfate-reducing bacteria able to grow under reservoir conditions could accordingly contribute significantly to H_2S production in the reservoir.

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