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Molecular analysis of microbial community structures in Nigerian oil production and processing facilities in order to access souring corrosion and methanogenesis

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ABSTRACT

Microbial communities associated with corrosion in samples from Nigeria facilities were examined. The SRB, heterotrophic nitrate reducing bacteria activities and SRB and acid producing bacteria counts were higher in Escravos than those in Meren. The corrosion rates were also higher in the Escravos samples possessing higher capacity in methane production. Pyrosequencing surveys indicate that Escravos samples had high fractions of potential hydrocarbon degrading bacteria and methanogenic archaea (mainly *Methanosaeta* and *Methanococcus*). Microbial activities at Meren were dominated by bacteria (*Marinobacterium*, *Halomonas*, *Pseudomonas* and *Kosmotoga* spp.). The research was the first to analyze microbial community structures in the oil facilities investigated.

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1. Introduction

Nigeria is Africa's largest oil producer and the 6th largest oil producing country in the world with a production capacity of about 2.53 million barrels per day and holds the 10th world's largest oil reserves of about 25 billion barrels. Its crude is classified mostly as "light" and "sweet" as the oil is largely free of sulfur. This sweet oil is similar in composition to the petroleum extracted from the North Sea.

The constant demand for oil has driven more interest in the study of petroleum reservoir microbial diversity as microbial activity in an oil reservoir can have significant implications on oil quality and recovery [1]. For example, microbial activity can have costly negative effects such as hydrocarbon degradation, clogging, souring and corrosion of pipelines [2–5] or beneficial effects on the rate or extent of oil recovery and productivity [6,7]. In the recent years, an assessment of microbial diversity and habitat conditions within a petroleum reservoir is being increasingly recognized as an important component of reservoir management [8]. Therefore a comprehensive assessment of the diversity, metabolic processes and habitat conditions for petroleum microorganisms is of practical importance for assessing the economic potentials of oil fields. It will also help in the understanding of how *in-situ* biotic factors may affect oil production operations [9]. A study by van der Kraan et al. [10] have also indicated how the presence of specific microbial communities could present information on some characteristics of oil reservoir such as temperature, acidity, salinity, redox-potential etc.

The main goal of the present investigation therefore is to characterize microbial communities associated with corrosion and souring in Escravos oil processing facility and its associated temporary waste storage facility (Skimmer pit) and Meren offshore oil production facility. Escravos processing facility is located nearshore in the Niger Delta, about 100 km south east of Lagos, Nigeria. It is composed of a tank farm where Escravos crude oil is stored and loaded, a processing facility for raw crude coming from Abiteye, Okan and Olero creek (with a capacity of about 70,000 barrels of crude per day) and a gas to liquid facility (EGTL) that processes about 325 million cubic feet per day of natural gas. Within the Escravos facility is an oily waste storage pit (skimmer pit) where oily waste that drains from the facility is stored temporarily before

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treatment and disposal. Skimmer pit is a short term storage facility for liquid oily wastes arising from oil production activities. The pits are dug on the ground and the base and walls are lined with heavy oil impermeable material such as polythene or oil resistant rubber sheeting to prevent groundwater contamination. As a result of frequent movement of hot liquids used for flushing and cleaning of the facility into the skimmer pit, its temperature is usually elevated ranging between 45 °C and 60 °C [11]. In addition to this, some residual chemicals and salts used for production purposes can also find its way into the skimmer pit. When the skimmer pit is filled to the brim, it is usually treated with biocides and part of the liquid waste can sieve into the nearby sea water while the rest are treated thermally.

Meren field on the other hand is located south east of Lagos, Nigeria with an estimated oil reserves of 1.8 billion barrels, out of which about 750,000 barrels has been produced as at 2002 [12]. The current production capacity is about 85,000 barrels per day. Sea water picked up to 25 ft below sea level is treated with continuous injection of sodium hypochlorite with residual chlorine maintained at 1.5 mg/L. Apart from continuous chlorination, two other biocides (amine and quaternary ammonium compound and a gluteraldehyde) have been applied alternatively for 6 h weekly at a concentration of 200 ppm. Even with the biocide application, it has been reported that total elimination of sulfate reducing bacteria (SRB) from Meren field is difficult [12,13].

In the present study, samples were collected from Meren and Escravos facility and its associated skimmer pit and analyzed both chemically and microbiologically. In the characterization of microbial communities associated with the oil fields under investigation, we adopted culture independent 16S rRNA gene pyrosequencing approach which has been proven to be very efficient and reliable as opposed to the culture dependent methods which greatly underestimates the microbial diversity associated with an environment [8,9,14–19]. Culture independent 16S rRNA gene based surveys are extremely valuable in providing an overall view of the community composition in a specific ecosystem regardless of the metabolic activities of the community members [7,20].

2. Materials and methods

2.1. Sample description

Water (S1–S4), oil sludge (S5) and oil (S6) and sediment (S7) samples were collected from Escravos and Meren oil production facilities (Fig. 1) from April 15–20, 2013 in sterile Nalgene sample bottles and filled to the brim to exclude air. Sample 1 (S1) was collected at a produced water discharge point at upstream of the Escravos River while 3 other samples were collected from the skimmer pit (S2), oily sludge (S5) and skimmer pit sediment (S7), respectively at the Escravos facility as shown in Fig. 1A. At Meren offshore field (Fig. 1B), samples were collected from injection (S3), produced water (S4), production wellhead (S6), respectively. Solid deposit samples from corroded pipelines (S8) were collected the same period in sterile 500 mL zip lock nylon bags. Samples were analyzed for chemical compositions (Section 2.2) and also transferred to a CO_2 anaerobic hood with an atmosphere of 90% N_2 and 10% CO_2 (v/v) for microbiological analyses as described in Section 2.3. All the remaining samples were shipped to the University of Calgary at room temperature and processed within two weeks of collection.

2.2. Chemical analysis

The pH of the samples was measured using an Orion pH meter. Aqueous sulfide was analyzed using the diamine method [21] and NH_4^+ with the indophenol method [22]. Sulfate, NO_3^-, NO_2^- and the volatile fatty acids (VFA) acetate, propionate and butyrate were analyzed by high-performance liquid chromatography (HPLC), as described by Grigoryan et al. [23]. Measurement of dissolved oxygen concentration in samples was determined using the method of Eaton et al. [24]. For analysis of inorganic anions 100 μL of sample was diluted with 400 μL of HPLC anion buffer, while 300 μL of the sample was acidified with 20 μL of 1 M phosphoric acid for VFA analysis.

2.3. Measurement of microbial activities and most probable numbers

The activities of SRB, as well as of heterotrophic nitrate reducing (hNRB) and of sulfide-oxidizing nitrate-reducing bacteria (soNRB) were measured in Coleville synthetic brine (CSB-K) medium [3]. CSB contained, per liter: 1.5 g NaCl, 0.54 g $MgCl_2$ $5H_2O$, 0.21 g $CaCl_2$ $2H_2O$, 0.3 g NH_4Cl, 0.05 g KH_2PO_4, 0.10 g KCl, 2–3 drops of 1% resazurin. After autoclaving, 1 mL of selenite tungstate, 1 mL of 1 M Na_2S, 30 mL of 1 M $NaHCO_3$, 1 mL of trace element solution and pH was adjusted to 7.4 using HCl. The trace element solution contained, per liter: 0.5 mL concentrated H_2SO_4, 2.28 g $MnSO_4$ H_2O, 0.5 g $ZnSO_4$ $7H_2O$, 0.5 g H_3BO_3, 0.025 g Na_2MoO_4 $2H_2O$, 0.045 g $CoCl_2$ $6H_2O$, and 0.58 g $FeCl_3$. Medium was anaerobically dispensed in 70 mL aliquots into 125 mL serum bottles with a gas phase of 90% N_2 and 10% CO_2 and closed with sterile butyl rubber stoppers. Medium was amended with 40 mM lactate (or 3 mM VFA) and 20 mM sulfate for measurement of SRB activity, with 3 mM VFA and 10 mM nitrate for measurement of hNRB activity and with 5 mM sulfide and 10 mM nitrate for measurement of soNRB activity. The medium bottles were inoculated by injection of 3.5 mL of sample and were incubated anaerobically at 37 °C with shaking. Using a sterile syringe needle, 1 mL was taken periodically from medium bottles to determine the inorganic anions concentrations. Microbial activities were calculated as $100/t_{1/2}$ units/day, where $t_{1/2}$ is the time (days) needed to reduce half of the sulfate (SRB activity) or nitrate concentration (hNRB and soNRB activities), or oxidize half of the sulfide concentration (soNRB). The most probable number (MPN) of lactate-utilizing SRB was determined, using vials with 9 mL of anaerobic API RP-38 broth. These were inoculated with 1 mL of sample and with 10-fold dilutions derived thereof. Formation of a black iron sulfide precipitate was used to score the presence of SRB. The vials were incubated at 37 °C for 30 days after which the MPN was determined [25]. For acid-producing bacteria (APB) ZPRA-5 phenol red-dextrose medium with a salinity of 5000 ppm was used [26]. Change in color from orange to yellow showed presence of acid producers.

2.4. Carbon steel coupon corrosion testing

Carbon steel ASTM A366 (ASTM international designation A 1008/A) coupons (2 × 1 × 0.1 cm) containing 0.08% (w/w) carbon were cleaned according to a standard protocol [27] in which the coupons were abraded with 400 grit silicon carbide paper and then placed in a dibutyl-thiourea–HCl solution for 2 min. The coupons were then neutralized in a saturated bicarbonate solution for 2 min, rinsed with deionized water (dH_2O) and then with acetone and finally dried in a stream of air. The coupons were weighed 3 times and the average weight was recorded as the starting weight. Duplicate coupons in small plastic holders to prevent them from contacting the glass wall or each other were placed in 120 mL serum bottles containing 70 mL of sample under anaerobic conditions (headspace of 90% N_2 and 10% CO_2 (v/v)). One experimental set contained unfiltered sample, whereas another set contained sample filtered through 0.2 μm acrodisc syringe filters. Serum bottles with 70 mL of filtered Milli-Q water were used as a control.

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