

Full Length Research Paper

Microbiological impacts of produce water discharges in nearshore shallow marine waters near Chevron's Escravos tank farm, Nigeria

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Microbiological and Physicochemical survey was undertaken in produced water and its receiving environment with the aim of verifying the likely impacts of produced water constituents on the immediate receiving marine near shore shallow environment. The sampling was carried out in two seasons, late wet season and late dry season. The results obtained indicate that the chemical constituents of the discharged produce water are capable of sustaining microbial growth and proliferation. Produce water from Escravos tank farm had relatively moderate concentrations of hydrocarbon utilizing microorganisms and sulphate reducing bacteria and the concentration of these organisms were much higher at the point of discharge of the produced water. Physicochemical analysis showed that Produce water had lower salinity and sulphate levels than the receiving marine water but the reverse was the case with the BOD, COD and Hydrocarbon constituents. The two seasons under investigation showed similar results. From the analytical data, it can be advanced that the impacts of produced water microbial flora on the receiving environment is limited to the vicinity of the discharge point of about 100 m in diameter and also to some extent up to a distance of 500 m upstream along the direction of flow of produced water discharges. It is expected that while the hydrocarbon degrading microorganisms plays a beneficial role of degrading and detoxifying abundant produced water hydrocarbons in the sediment and the surface water, Sulphate reducing bacteria might at the same time be playing a detrimental role of oxidizing certain organic compounds or hydrogen and reducing sulphate and other reduced sulphur compounds in the marine water and sediment to hydrogen sulphide which can be very toxic to bacteria, aquatic animals and man.

Key words: Produced water, sulphate reducing bacteria, hydrocarbon utilizing bacteria.

INTRODUCTION

Produced water is defined as the water (brine) brought up from the hydrocarbon bearing strata during the extraction of oil and/or Gas and this may include; Formation water, Injection water, small volumes of condensed water and trace amount of treatment chemicals (Ayes and Parker, 2001). Produced water is by far the largest volume by-product of waste stream associated with oil and gas production and its properties and volumes vary considerably depending on the geographical location of the field, the geological formation with which the produce water has been in contact for thousands of years and the type of hydrocarbon product being produced (Ayes and

Parker, 2001).

The biological impacts of the discharged produce water on the immediate environment will depend on whether the discharge is on a shallow near shore environment or in an open sea. In an open sea, the level of dilution and mixing is very high and as such the impact of the discharged produced water may not be significant but in a shallow near shore environment where the level of dilution and mixing is low, it is expected that the impact of the discharged produced water on the immediate environment will be significant.

The study is focused on the microbiological impacts of

produced water discharges on the immediate near shore environment and the target microorganisms are the hydrocarbon degrading organisms and sulphate reducing bacteria which seems to be indigenous to produced water. Population densities of microorganisms in produced waters are usually not very high, total bacterial counts can reach up to 10^5 - 10^6 ml by direct microscopic count (Maggot, 2005). These low population densities indicate that produced waters constitute a nutrient limiting environment. A major cause for concern is the presence of sulphate reducing bacteria (SRB) in produced water. A great variety of SRBs have been isolated from produced water in various oil fields around the world (Birkeland, 2005). SRB species such as *Archaeoglobus fulgidus*, *Desulfacinium infernum*, *Desulfobacter vibrioformis*, *Desulfomicrobium* sp, *Thermodesulfobacterium* have been isolated from the North sea oil fields (Birkeland, 2005), From the Russian oil fields, *Desulfotomaculum kuznetsorri* and *Desulfotomaculum nigrificans* have been isolated while *Desulfovibrio bastinii* and *Desulfovibrio gabonensis* have been isolated from Congo and Gabon offshore oil fields (Birkeland, 2005). In Nigeria, a significant concentration of unidentified sulphate reducing bacteria has been isolated from produced water effluents from Chevron's Escravos tank farm (Okoro, 1999). These discoveries indicate that oil fields may be the natural habitats of sulphate reducing bacteria.

Sulphate reducing bacteria are strict anaerobes that perform anaerobic respiration by oxidizing certain organic compounds or hydrogen and reducing sulphate and other reduced sulphur compounds to hydrogen sulphide, the hydrogen sulphide when released is very toxic to bacteria, aquatic animals and man (Atlas, 1984). Apart from liberation of hydrogen sulphide, sulphate reducing bacteria have been known to be responsible for corrosion of iron and steel in form of storage tanks, pipelines and pumps (Atlas, 1984).

A significant concentration of hydrocarbon utilizing microorganisms have also been isolated from produced water, and the studies conducted thereafter showed that produced water is easily biodegradable (Okoro, 1999; Okoro and Amund, 2002; Okoro, 2008), the presence of hydrocarbon degrading bacteria in produced water therefore can be of immense benefit to the receiving environment especially in the degradation of recalcitrant organic compounds in the receiving environment (Munn, 2004).

The aims and objectives of the present study therefore is to look at the Microbiological and Physicochemical aspects of produced water discharges and their potential impacts in near shore shallow marine waters.

MATERIALS AND METHODS

Study area

The study area is within the operational facility of Chevron's Escravos Tank farm in the Nigeria's oil rich Niger Delta. The GPS

bearings and coordinates of all the sampling points are indicated in all the tables.

Physicochemical analysis of water and sediment samples

The pH of the water and sediment samples were measured with a portable water proof pH meter (Jenway, 3150, USA), Temperature was measured using portable thermometer (Hanana, H1-93510, USA). Salinity was measured as Chloride using the Argentometric method as earlier described by Eaton et al. (1995). Per sulphate digestion method was used to estimate Phosphorus (Eaton et al., 1995).

The method used in the estimation of Barium, Sulphate and Nitrate was as described by CNL (1995). The appropriate powder pillows for Barium, Nitrate and Sulphate were added to 25 ml. Of the water sample and 1 g of the sediment sample as the case may be, the wave length of the colorimeter was adjusted to 500 nm using blank solution, the sample was introduced to the colorimeter and the concentration recorded accordingly.

Carbonate, Bicarbonate and COD were also determined as described by Eaton et al. (1995). Ammonia Nitrogen was determined by titrimetric method while TDS and TSS were determined by gravimetric method as described by Eaton et al. (1995).

Determination of biological oxygen demand

BOD bottles were filled with appropriate dilutions of the samples (50 ml) and the initial dissolved oxygen was measured. The BOD bottles with samples were sealed to exclude air followed by incubation at 20°C for 5 days after which the BOD was computed from the difference between the initial and the final dissolved oxygen (Eaton et al., 1995).

Detection of heavy metals

Heavy metals were detected using the Atomic absorption Spectrophotometer (Perkin Elmer 5100PC, England) after sample preparation and digestion as previously described (Eaton et al., 1995).

Detection of sulphate reducing bacteria

Sulphate reducing bacteria were estimated using sulphate reducing bacteria test kit (Rapid check 11) which was prepared and packaged by Strategic diagnostics Industries Inc. As described by CNL (1995). The SRB test kit contains a set of reagents which was mixed with 1 ml. The sample was incubated for 10 min at room temperature under anaerobic condition. The colour of the membrane of the test kit was compared with the standard colour in the colour card to determine the SRB concentration.

Enumeration of hydrocarbon utilizing microorganisms

Hydrocarbon utilizing microbial counts were obtained by plating out at low dilutions 10^{-1} - 10^{-3} of samples on mineral salt medium of Mills et al. (1978). The composition of the medium is as follows in (g/l): NaCl (10), $MgSO_4 \cdot 7H_2O$ (0.42), KCl (0.29), KH_2PO_4 (0.83), Na_2HPO_4 (1.25), $NaNO_3$ (0.42), Agar bacteriological (15), distilled water (1000 ml), and pH (7.2). The medium was autoclaved at 1.1 kg/cm² for 15 min. The inoculated mineral agar plates were then inverted over sterile membrane filters moistened with crude oil (Escravos light) and held in the lid of the petri dishes. The dishes

were wrapped round with a masking tape so as to increase the vapour pressure within the Petri dishes while the plates were incubated at 29°C for 6 days after which the growth of hydrocarbon degrading microorganisms were observed and counted. For fungal plates, 0.1 g of Penicillin was added to 250 ml mineral salt medium to inhibit bacterial growth.

Solvent extraction of residual oil

One gram of the sample or 25 ml as the case may be was introduced into a separating funnel containing 50 ml of Methylene chloride, this was followed by vigorous shaking for 10 min and filtration using Whatman no.1 filter paper as previously described by Eaton et al. (1995) and the filtrate was collected in a clean conical flask.

Gas chromatography of oils

Degraded oil were analyzed by Gas chromatography using Hewlett Packard 5890 series 11 Gas chromatograph equipped with single flame ionization detector (FID) fitted with Perkin Elmer Nelson analog digital converter (900 series) and a Compaq deskpro computer. A J and W scientific DB-1 capillary column of 15 m length and an internal diameter of 0.32 mm wide bore of 1 micron film thickness were used. A temperature program of 50 - 305°C increasing at 3.5°C/min for 27.15 min was employed. Hydrogen with a flow rate of 2 ml/min was used as a carrier gas while the flow rate of air was 400 ml/min. The detector temperature was 325°C while the injection port temperature was 305°C. 1 ml of the residual oil extract was dissolved in methylene chloride at the ratio of 1:1 and a sample volume of 0.2 µl was injected into the GC. Total hydrocarbon was measured as oil and grease in ppm.

Identification of hydrocarbon degrading microorganisms

The growth and morphology of bacterial isolates in minimal salts medium and on nutrient agar plates were noted with regards to the following characteristics; Form, Pigmentation, Texture, Color and Elevation. Fungal cultures were stained with Methylene blue and observed under a microscope (x40) and each fungal culture was identified based on its morphological characteristics as described in Munn (2004). Bacterial cultures were stained using Gram staining procedure and proper identification was done using a computerized BBL Enterotube identification test kits, manufactured by Becton Dickson Microbiology systems Inc. USA.

RESULTS

Physico-Chemical Properties of Produced water and the associated receiving marine environment

The Physicochemical properties of produced water and the receiving marine environment during the late dry and rainy season's survey are shown in Tables 1 and 2 respectively. Both the produced water and the receiving marine environment have a moderate alkaline pH during the late dry season but the late rainy season's survey showed a slightly neutral pH for produced water and a slightly acidic pH for the receiving marine environment. Considerable concentrations of oil and grease were present in the discharged produced water and also within

the area of about 100 m circumference where produced water is being discharged. Among the heavy metals analyzed, only lead and chromium showed considerable presence in both the produced water and the receiving marine environment. The temperature of the produced water and the point of discharge are relatively higher than that of the marine environment.

Relative population densities of microorganisms found in the produced water and the receiving marine environment

The relative population densities of microorganisms found in the produced water and the receiving marine environment during the two seasons under investigation are shown in Tables 3 and 4 respectively. The total heterotrophic and hydrocarbon utilizing microbial counts were highest in the bottom sediment samples at the point of discharge of produced water. Upstream surface water and bottom sediment samples also showed higher microbial counts than the downstream counterparts. Considerable concentration of SRB were present in produced water, surface water and bottom sediment at the point of discharge but the concentrations were more in the bottom sediment samples. Upstream bottom sediment samples also showed considerable concentrations of sulfate reducing bacteria while those of downstream did not.

Hydrocarbon utilizing microorganisms isolated from produce water and the receiving marine environment

Various groups of hydrocarbon utilizing microorganisms, especially Bacteria and Fungi which were isolated from the produced water and the receiving marine environment during the two seasons under investigation are shown in Table 5. It was observed that some of the microorganisms found in the produced water were also present in the receiving marine environment. The bacterial flora of produce water was dominated by *Pseudomonas* sp. during the two seasons under investigation; the *Aspergillus* sp. dominated the fungal flora.

DISCUSSION

A wide variety of microbial species has been isolated from produced water and there is a strong indication that produced water harbour original and specific indigenous microorganisms (Maggot, 2005). Although population densities of microorganisms in produced water is usually not very high, total bacterial counts can reach up to 10^5 - 10^6 cfu/ml, these rather low population densities according to Maggot (2005), indicate that oil field waters constitute a nutrient limited environment. In the present

Table 1. Chemical composition of produced water and the receiving environment at Escravos (late dry season).

	Treated produced water	Receiving water at discharge point	Bottom sediment at discharge point	500 M Upstream from discharge point (SW)	500M upstream from discharge point (BS)	500M downstream from discharge point (SW)	500M downstream from discharge point (BS)
pH	8.34	8	8.40	7.30	7.80	7.40	7.60
Temp. (°C)	65	48	42	38	36	32	32
Co ₃ (mg/l)	14.16	0.00	64.40	0.00	23.60	0.00	14.40
HCO ₃ (mg/l)	1894	405	2640	65.70	1480.50	69	1325
COD (mg/l)	1110	240	1820	200	1480	300	1250
BOD ₅ (mg/l)	640	230	1260	60	820	70	540
DO (mg/l)	1	3	0.56	4	0.65	3	1.50
Mg (mg/l)	0.37	451	625	590	0.68	27	0.34
SO ₄ (mg/l)	12	450	550	1300	220	1425	140
Ba (mg/l)	16	8	16	46	0	0	0.30
NO ₃ (mg/l)	3.98	0.33	1.68	35	0.50	0.00	0.48
NH ₃ (mg/l)	3.25	1.84	2.40	1.96	1.60	1.96	3.20
Salinity (Cl)mg/l	5105	11012	9650	10612	8560	10211	9480
TSS (mg/l)	58	585	28	172	32	178	28
TDS (mg/l)	11492	16484	25230	17860	13200	16260	9200
Oil and grease (mg/l)	44	22	36	0	14	0	16
Ca (mg/l)	68	250	330	279	0	268	0.046
P (mg/l)	1.14	0.20	0.62	0	1.56	0	1.26
Cd (mg/l)	0.06	0.03	1.20	0.04	0	0.04	0
Cr (mg/l)	0.10	0.04	0.82	0.12	0.48	0.12	0
Cu (mg/l)	0	0.03	0.08	0	0.16	0	0.28
Pb (mg/l)	0.15	0.19	0.93	0.12	0.82	0.12	0
K (mg/l)	54	17.6	23.40	298	96.40	246	48.40
Zn (mg/l)	0	0	0.55	0.02	0	0.01	0.056

SW = Surface water, BS = Bottom sediment. TDS = Total dissolved solids, TSS = Total suspended solids. GPS bearings: Discharge point: 04° 22' 12" E, 06° 09' 15" N, 500 M Upstream: 04° 47' 30" E, 06° 23' 00" N, 500 M Downstream: 04° 39' 30" E, 06° 23' 10" N.

study, Produce water from Escravos tank farm has bacterial counts of 23 - 28 × 10⁵ Cfu/ml and only about 3.92-5.20% have the capability to degrade petroleum hydrocarbons. The results of the physicochemical analysis showed that nutrients (N and P) in produced water are not

really limiting. Head et al. (2003) have noted that Nitrogen is unlikely to be limiting in petroleum reservoirs and produce water because of abundant ammonium ions buffered by reservoir minerals which should be the ideal nitrogen source for *in situ* microbial activity. Okoro and

Amund (2002) have also advanced that produced water from Escravos tank farm is readily biodegradable and it contains sufficient nutrients and carbon sources that can support microbial growth and proliferation.

In the present study, the two microbial groups

Table 2. Chemical composition of produce water and the receiving environment at Escravos (late wet season).

	Treated produced water	Receiving water at discharge point	Bottom sediment at discharge point	500 M upstream from discharge point (SW)	500 M upstream from discharge point (BS)	500M downstream from discharge point (SW)	500 M downstream from discharge point (BS)
pH	7.9	7.3	7.8	6.8	7.2	6.1	6.6
Temp. (°C)	58	42	38	32	30	29	28
Co ₃ (mg/l)	0	0	0.18	0	0.56	0	1.28
HCO ₃ (mg/l)	2660	7.73	1650	51	2840	26	3640
COD (mg/l)	4610	1387	5210	248	4280	682	3840
BOD ₅ (mg/l)	900	140	1280	60	1200	10	680
DO (mg/l)	1	2	0.50	4	0.85	4	1.30
Mg (mg/l)	113.3	787	326	149	233	1080	246
SO ₄ (mg/l)	57	60	164	425	525	305	310
Ba (mg/l)	27	22	14	3	23	7	32
NO ₃ (mg/l)	4.3	0.5	6.40	0.20	0.56	6.30	460
NH ₃ (mg/l)	3	1.24	3.26	0.68	3.42	1.88	0.38
Salinity (Cl)mg/l	11516	7033	9506	7607	8508	6358	7610
TSS (mg/l)	63	250	23	182	14	230	08
TDS (mg/l)	17348	19633	14880	15322	16840	14232	13406
Oil and grease (mg/l)	66	12	26.40	0	14.20	0	9.60
Ca (mg/l)	91	110	138	178	48	226	148
P (mg/l)	62	18	340	410	432	430	480
Cd (mg/l)	0.03	0.06	0.08	0.01	0.068	0.03	0.56
Cr (mg/l)	0.20	0.12	0	0	0.43	0.03	0
Cu (mg/l)	0.50	0	0.16	0.28	0	0	0.038
Pb (mg/l)	0.08	0.06	0.43	0.02	0.008	0.08	0
K (mg/l)	114	128	326	0	142	0	0.38
Zn (mg/l)	0.11	0.12	0.21	0	0.53	0	0.15

SW = Surface water, BS = Bottom sediment. TDS = Total dissolved solids, TSS = Total suspended solids. GPS bearings: Discharge point: 04° 22' 12" E, 06° 09' 15" N., 500 M: Upstream: 04° 47' 30" E, 06° 23' 00" N., 500 M: Downstream: 04° 39' 30" E, 06° 23' 10" N.

of particular interest are the hydrocarbon utilizing bacteria (HUB) and the sulphate reducing bacteria (SRB). A wide variety of hydrocarbon utilizing bacteria have been isolated from produced water from Escravos tank farm (Okoro and Amund, 2002; Okoro, 2008), Sulphate reducing bacteria have also been detected at considerable high

levels in produced water (Okoro, 1999). The aim of the present study therefore is to identify possible impacts, these two groups of microorganisms are likely to cause in the receiving shallow and near shore marine environment where the dilution and mixing rates of the produced water constituents with the receiving marine environ-

ment is low.

The analytical data from the present study showed that produced water chemical constituents is biodegradable, the study also revealed that produce water had considerable concentrations of hydrocarbon utilizing bacteria (HUB) during the two seasons under investigation

Table 3. Relative population densities of microorganisms found in produce water and the receiving water during the late dry Season sampling period.

	Total heterotrophic bacterial counts	Total hydrocarbon utilizing bacterial counts	% Hydrocarbon utilizing bacteria	Total heterotrophic fungal and yeast counts	Hydrocarbon utilizing fungal and yeast counts	% Hydrocarbon utilizing fungi and yeasts	SRB counts
Treated produced water (cfu/ml × 10 ⁵)	23	1.20	5.20	0.28	0.096	34.20	1.0
Receiving water at discharge point (cfu/ml × 10 ⁵) SW	30	5.60	18.60	0.52	0.015	2.88	1.20
Bottom sediment at discharge point (cfu/g × 10 ⁵) BS	126	14	11.11	0.98	0.055	5.60	10
500 m upstream from discharge point (cfu/ml × 10 ⁵) SW	14	0.16	1.14	0.85	0.008	0.94	0
500 m upstream from discharge point (cfu/g × 10 ⁵) BS	48	1.38	2.87	1.46	0.032	2.19	1.0
500 m downstream from discharge point (cfu/ml × 10 ⁵) SW	13	0.024	0.18	0.65	0.001	0.15	0
500 m downstream from discharge point (cfu/g × 10 ⁵) BS	44	0.86	0.51	1.36	0.0012	0.088	0

SW = Surface water, BS = Bottom sediment. GPS bearings: Discharge point: 04° 22' 12" E, 06° 09' 15" N., 500 M: Upstream: 04° 47' 30" E, 06° 23' 00" N., 500 M: Downstream: 04° 39' 30" E, 06° 23' 10" N.

Table 4. Relative population densities of microorganisms found in produce water and the receiving water during the late wet Season sampling period.

	Total heterotrophic bacterial counts	Total hydrocarbon utilizing bacterial counts	% Hydrocarbon utilizing bacteria	Total heterotrophic fungal and yeast counts	Hydrocarbon utilizing fungal and yeast counts	% Hydrocarbon utilizing fungi and yeasts	SRB counts
Treated produced water (cfu/ml × 10 ⁵)	28	1.10	3.92	0.12	0.0032	2.66	0.10
Receiving water at discharge point (cfu/ml × 10 ⁵) SW	96	3.20	3.33	1.48	0.068	4.59	0.20
Bottom sediment at discharge point (cfu/g × 10 ⁵) BS	148	4.50	3.04	2.65	0.042	1.58	2.50
500 m upstream from discharge point (cfu/ml × 10 ⁵) SW	26	0.068	0.26	1.50	0.0012	0.08	0
500 m upstream from discharge point (cfu/g × 10 ⁵) BS	32	0.62	1.93	0.18	0.005	2.77	0.10
500 m downstream from discharge point (cfu/ml × 10 ⁵) SW	22	0.015	0.068	0.32	0.0011	0.61	0
500 m downstream from discharge point (cfu/g × 10 ⁵) BS	28	0.068	0.24	1.80	0.021	1.16	0

SW = Surface water, BS = Bottom sediment. GPS bearings: Discharge point: 04° 22' 12" E, 06° 09' 15" N., 500 M: Upstream: 04° 47' 30" E, 06° 23' 00" N., 500 M: Downstream: 04° 39' 30" E, 06° 23' 10" N.

(1.10 - 1.20 × 10⁵ Cfu/ml) same is applicable to Sulphate reducing bacteria (SRB) (0.10 - 1 × 10⁵ Cfu/ml). Bottom sediment section of produced

Table 5. Hydrocarbon utilizing microorganisms isolated from produce water and the receiving water during the two seasonal periods under investigation.

	Late dry season period		Late wet season period	
	Bacteria	Fungi/yeasts	Bacteria	Fungi/yeasts
Treated produce water	<i>Corynebacterium</i> sp. <i>Pseudomonas</i> sp. <i>Pseudomonas mallei</i>	<i>Aspergillus niger</i> <i>Penicillium crysogenum</i>	<i>Pseudomonas</i> sp., <i>Bacillus</i> sp., <i>Pseudomonas pseudomallei</i> , <i>Flavobacterium</i> sp.	<i>Aspergillus niger</i> ,
Receiving water at the discharge point. SW	<i>Vibrio</i> sp., <i>Pseudomonas</i> sp., <i>Acinetobacter lwoffii</i> . <i>Alkaligenes</i> sp. <i>Pseudomonas mallei</i>	<i>Penicillium crysogenum</i> <i>Aspergillus niger</i> <i>Aspergillus tamarrii</i>	<i>Acinetobacter lwoffii</i> , <i>Pseudomonas</i> sp., <i>Enterobacter</i> sp., <i>Alkaligenes</i> sp., <i>Flavobacterium</i> sp.	<i>Aspergillus niger</i> <i>Aspergillus fumigatus</i> <i>Penicillium crysogenum</i>
Bottom sediment at the discharge point. BS	<i>Flavobacterium</i> sp. <i>Micrococcus</i> sp., <i>Alkaligenes</i> sp. <i>Vibrio</i> sp., <i>Bacillus</i> sp. <i>Corynebacterium</i> sp.	<i>Aspergillus flavus</i> <i>Aspergillus niger</i> <i>Rhodotorula</i> sp. <i>Candida</i> sp	<i>Alkaligenes</i> sp., <i>Bacillus</i> sp., <i>Corynebacterium</i> sp., <i>Pseudomonas</i> sp.	<i>Aspergillus niger</i> , <i>Candida</i> sp., <i>Penicillium</i> sp., <i>Rhizopus</i> sp.
500 m upstream from discharge point. SW	<i>Vibrio</i> sp., <i>Pseudomonas mallei</i> <i>Corynebacterium</i> sp., <i>Pseudomonas</i> sp.	<i>Aspergillus flavus</i> <i>Penicillium pinophyllum</i>	<i>Micrococcus</i> sp., <i>Pseudomonas</i> sp., <i>Enterobacter</i> sp., <i>Corynebacterium</i> sp., <i>Achromobacter</i> sp., <i>Pseudomonas mallei</i> .	<i>Aspergillus flavus</i> , <i>Candida</i> sp., <i>Penicillium pinophyllum</i>
500 m upstream from discharge point. BS	<i>Vibrio</i> sp., <i>Bacillus</i> sp., <i>Flavobacterium</i> sp.	<i>Rhizopus</i> sp. <i>Aspergillus niger</i> <i>Penicillium crusogenum</i>	<i>Corynebacterium</i> sp., <i>Vibrio</i> sp., <i>Bacillus</i> sp., <i>Micrococcus</i> sp	<i>Penicillium</i> sp., <i>Aspergillus niger</i> , <i>Aspergillus flavus</i>
500 m downstream from discharge point. SW	<i>Enterobacterium</i> sp., <i>Bacillus</i> sp.	<i>Aspergillus niger</i> <i>Penicillium</i> sp.	<i>Alkaligenes</i> sp., <i>Aeromonas</i> sp., <i>Pseudomonas mallei</i> , <i>Pseudomonas pseudomallei</i>	<i>Penicillium</i> sp., <i>Penicillium crysogenium</i> , <i>Aspergillus niger</i>
500 m downstream from discharge point. BS	<i>Vibrio</i> sp., <i>Alkaligenes</i> sp., <i>Micrococcus</i> sp.	<i>Penicillium pinophyllum</i> , <i>Rhizopus</i> sp	<i>Pseudomonas</i> sp., <i>Flavobacterium</i> sp	<i>Aspergillus niger</i> , <i>Rhizopus</i> sp.

SW = Surface water, BS = Bottom sediment. GPS bearings: Discharge point: 04° 22' 12" E, 06° 09' 15" N., 500 M Upstream: 04° 47' 30" E, 06° 23' 00 N., 500 M: Downstream: 04° 39' 30" E, 06° 23' 10" N.

water discharge point showed relatively high concentration of HUB ($4.50 - 14 \times 10^5$ Cfug) and SRB ($2.50 - 10 \times 10^5$ Cfug) during the two seasons under investigation. Samples taken up to a distance of 500m upstream which corresponded with the directional flow of the discharged produced water also showed relatively high concentrations of HUB and SRB counts. On the contrary, samples taken from opposite direction (500 m downstream) showed very low concentrations of HUB and there was no indication of the

presence of SRB in both sediment and water samples.

This is a strong indication that Produced water is the source of the accumulated HUB and SRB in the bottom sediment and surface water samples of the discharge area. Birkeland (2005) have advanced that oil fields are the natural habitat of SRBs, the first indication of this was in 1926 when SRB was first detected in produced water samples from a number of oil fields in Illinois, USA by Bastin and associates. Since then, a great

variety of SRBs have been isolated from oil fields around the world (Birkeland, 2005).

The environmental implications of produced water being discharged in a shallow near shore environment is focused on the fact that the level of mixing and dilution of produced water constituents with the receiving marine environment is likely to be low which may lead to the accumulation of most of the constituents especially the hydrocarbon in the sediment. The accumulation of hydrocarbon degrading microorganisms from

produced water in the sediment is desirable and will lead to a positive impact because the accumulated hydrocarbon utilizing microorganisms will be responsible for the degradation and detoxification of toxic organic compounds. On the contrary, the accumulation of sulphate reducing bacteria in the sediment will lead to a negative impact because of the following reasons. Sulphate reducing bacteria in the sediment reduces sulphate to sulphide while oxidizing degradable organic electron donors, the presence of sulphide in the environment poses some health and safety risks because of the toxicity, corrosive and souring of hydrogen sulphide generated (Hubert and Voordouw, 2007), except in some cases where the competition for available nutrients, carbon sources and organic electron donors have led to other microorganisms out competing SRBs and rendering them less potent as demonstrated by Hubert and Voordouw (2007), the presence of SRB in any environment is not desirable. The heavy metal constituents of produce water are relatively low and as such are not likely to have any significant impact in the immediate receiving environment.

Conclusion

The present study have demonstrated that produce water discharges in near shore shallow environment can lead to the accumulation of hydrocarbon and the indigenous microbial flora of the produced water both in the surface water and the sediment at the discharge point up to a distance of 500 m upstream along the directional flow of the produced water. Substantial concentrations of these microorganisms are being accumulated over a long period of time, while the HUBs are beneficial to the environment in terms of its hydrocarbon degradation potentials; the SRBs are detrimental because of the production of hydrogen sulphide which is toxic to humans and marine animals. The study also showed that there was no accumulation of heavy metals at the bottom sediment of the discharge point.

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