Microbiological Impacts of Produce Water Discharges in Nearshore Shallow Marine Waters Near Chevron's Escravos Tank Farm, Nigeria

Dr. Chuma C. Okoro

Department of Biological Sciences and Biotechnology
Caleb University, Lagos Nigeria
Tel: 08033072754, 01-7430285
e-mail: chuma2k2001@yahoo.com
P. O. Box 146, University of Lagos Post Office, Lagos, Nigeria

Abstract: A microbiological survey was undertaken in produced water and it's receiving environment with the aim of verifying the likely impacts of produced water microbial flora especially the hydrocarbon utilizing types and the sulphate reducing bacteria 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 produced water from Escravos tank farm had relatively moderate concentrations of hydrocarbon utilizing microorganisms and sulphate reducing bacteria and the concentration of these organisms are much higher at the point of discharge of the produced water including the surface water and the bottom sediment. Bottom sediment samples up to a distance of 500m upstream also showed relatively moderate concentration of hydrocarbon utilizing microorganisms and sulphate reducing bacteria. A distance of 500m downstream showed relatively low concentrations of hydrocarbon utilizing bacteria without any presence of sulphate reducing bacteria. The two seasons under investigation showed similar results. The results obtained indicate that the impacts of produced water microbial flora on the receiving environment is limited to the vicinity of the discharge point of about 100 meters in diameter and also to some extent up to a distance of 500m upstream along the direction of flow of produced water discharges. This assertion is supported by the experimental data which showed considerable accumulation of produced water hydrocarbons in the sediment at the discharge point up to 500m upstream with relatively high concentration of hydrocarbon degrading microorganisms and sulphate reducing bacteria. 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, Sulfate 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 sediment to hydrogen sulphide, the hydrogen sulphide when released can be very toxic to bacteria, aquatic animals and man. [Journal of American Science 2010;6(3):93-101]. (ISSN: 1545-1003).

Keywords: Produced water, Sulphate reducing bacteria, Hydrocarbon utilizing bacteria

1. 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 bi-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 produced water has been in contact for thousands of years and the type of hydrocarbon product being produced (Ayes and Parker, 2001).

The characteristics features of produced water such as microbial load, salinity, density, trace metals and organic content can vary widely between fields and even within the same field, same goes with the receiving environment and the biological impacts of the discharge 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 millilitres 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, significant concentrations of unidentified sulphate reducing bacteria have 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., and 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 isolate the indigenous microbial flora of produced water and the receiving shallow near shore environment where the produced water is being discharged with more emphasis on the hydrocarbon utilizing bacteria and sulphate reducing bacteria and also to further investigate the relationship between the indigenous microbial flora of the discharged produced water and that of the receiving environment up to a distance of 500m downstream and upstream from the discharge point with the aim of knowing how much the indigenous microbial flora of the produced water have impacted on the receiving environment.

2.0 Materials and Methods:

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

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

Determination of Biological Oxygen Demand

BOD bottles were filled with appropriate dilutions of the samples (50ml) and the initial dissolved oxygen was measured. The BOD bottles with samples were sealed to exclude air followed by incubation at $20^{O}C$ 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 using sulphate reducing bacteria test kit (Rapid check 11) which was prepared and packaged by Strategic diagnostics Industries Inc. As described in CNL, 1995. The SRB test kit contains a set of reagents which was mixed with 1ml. Of the sample and incubated for 10mins 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), $MgSO_4.7H_20$ (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/cm^2 for 15 mins. 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^{0}C for 6 days after which the growth of hydrocarbon degrading microorganisms were observed and counted. For fungal plates, 0.1g of Penicillin was added to 250ml mineral salt medium to inhibit bacterial growth.

Solvent extraction of Residual Oil

One gram of the sample or 25mls as the case may be was introduced into a separating funnel containing 50mls of Methylene chloride, this was followed by vigorous shaking for 10mins and filtration using Watman no.1 filter paper as described previously described *(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 1micron film thickness were used. A temperature program of 50-305^{0}C increasing at 3.5^{0}C per minute for 27.15min was employed. Hydrogen with a flow rate of 2ml per min was used as a carrier gas while the flow rate of air was 400ml per min. The detector temperature was 325^{0}C while the injection port temperature was 305^{0}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. Bacterial cultures were stained using grams staining procedure and proper identification was done using a computerized BBL Enterotube identification test kits, manufactured by Becton Dickson Microbiology systems Inc. USA.

3.0 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 100m 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 is 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.

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	500M 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.(^{O}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

1. **SW = Surface water, BS= Bottom sediment. TDS=Total dissolved solids, TSS= Total suspended solids .**
2. **GPS BEARINGS: Discharge point: 04 22 12 E, 06 09 15 N, 500M UPSTREAM: 04 47 30 E, 06 23 00 N, 500M DOWNSTREAM: 04 39 30 E, 06 23 10 N.**

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.

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	500M Upstream from Discharge Point (SW)	500M Upstream from discharge point (BS)	500M Downstream from discharge point (SW)	500M Downstream From discharge point (BS)
pH	7.9	7.3	7.8	6.8	7.2	6.1	6.6
Temp.(^{O}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., 500M UPSTREAM: 04 47 30 E, 06 23 00 N., 500M DOWNSTREAM: 04 39 30 E, 06 23 10 N.

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	Hydrocar-bon Utilizing Fungal and Yeast Counts	% Hydrocarbon Utilizing Fungi and Yeasts	SRB COUNTS
Treated Produced Water (cfu/mlx10^5)	23	1.20	5.20	0.28	0.096	34.20	1.0
Receiving Water at discharge point (cfu/mlx10^5) SW	30	5.60	18.60	0.52	0.015	2.88	1.20
Bottom sediment at discharge point(cfu/gx10^5) BS	126	14	11.11	0.98	0.055	5.60	10
500m upstream from discharge point(cfu/mlx10^5) SW	14	0.16	1.14	0.85	0.008	0.94	0
500m upstream from discharge point(cfu/gx10^5) BS	48	1.38	2.87	1.46	0.032	2.19	1.0
500m downstream from discharge point(cfu/mlx10^5) SW	13	0.024	0.18	0.65	0.001	0.15	0
500m downstream from discharge point(cfu/gx10^5) 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., 500M UPSTREAM: 04 47 30 E, 06 23 00 N., 500M 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/mlx10^5)	28	1.10	3.92	0.12	0.0032	2.66	0.10
Receiving Water at discharge point (cfu/mlx10^5) SW	96	3.20	3.33	1.48	0.068	4.59	0.20
Bottom sediment at discharge point(cfu/gx10^5) BS	148	4.50	3.04	2.65	0.042	1.58	2.50
500m upstream from discharge point(cfu/mlx10^5) SW	26	0.068	0.26	1.50	0.0012	0.08	0
500m upstream from discharge point(cfu/gx10^5) BS	32	0.62	1.93	0.18	0.005	2.77	0.10
500m downstream from discharge point(cfu/mlx10^5) SW	22	0.015	0.068	0.32	0.0011	0.61	0
500m downstream from discharge point(cfu/gx10^5) 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., 500M UPSTREAM: 04 47 30 E, 06 23 00 N., 500M DOWNSTREAM: 04 39 30 E, 06 23 10 N.

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**
1. Treated Produce Water	*Corynebacterium sp. Pseudomonas sp. Pseudomonas mallei*	*Aspergillus niger Penicillium crysogenum*	*Pseudomonas sp., Bacillus sp., Pseudomonas pseudomallei, Flavobacterium sp.*	*Aspergillus niger,*
2. Receiving Water at the Discharge Point. SW	*Vibrio sp., Pseudomonas sp., Acinetobacter lwoffii. Alkaligenes sp. Pseudomonas mallei*	*Penicillium crysogenum Aspergillus niger Aspergillus tamarrii*	*Acinetobacter lwoffii, Pseudomonas sp., Enterobacter sp., Alkaligenes sp., Flavobacterium sp.*	*Aspergillus niger Aspergillus fumigatus Penicillium crysogenum*
3. Bottom Sediment at the Discharge point. BS	*Flavobacterium sp. Micrococcus sp., Alkalegenes sp. Vibrio sp., Bacillus sp. Corynebacterium sp.*	*Aspergillus flavus Aspergillus niger Rhodotorula sp. Candida sp*	Alkaligenes sp., Bacillus sp., Corynebacterium sp., Pseudomonas sp.	*Aspergillus niger, Candida sp., Penicillium sp., Rhizopus sp.*
4. 500m upstream from discharge point. SW	*Vibrio sp., Pseudomonas mallei Corynebacterium sp., Pseudomonas sp.*	*Aspergillus flavus Penicillium pinophylum*	*Micrococcus sp., Pseudomonas sp., Enterobacter sp., Corynebacterium sp., Achromobacter sp., Pseudomonas mallei.*	*Aspergillus flavus, Candida sp., Penicillium pinophylum*
5. 500m upstream from discharge point. BS	*Vibrio sp., Bacillus sp., Flavobacterium sp.*	*Rhizopus sp. Aspergillus niger Penicillium crusogenum*	*Corynebacterium sp., Vibrio sp., Bacillus sp., Micrococcus sp*	*Penicillium sp., Aspergillus niger, Aspergillus flavus*
6. 500m downstream from discharge point. SW	*Enterobacterium sp., Bacillus sp.*	*Aspergillus niger Penicillium sp.*	*Alkalegenes sp., Aeromonas sp., Pseudomonas mallei, Pseudomonas pseudomallei*	*Penicillium sp., Penicillium crysogenium, Aspergillus niger*
7. 500m downstream from discharge point. BS	*Vibrio sp., Alkaligenes sp., Micrococcus sp.*	Penicillium pinophylum, Rhizopus sp	*Pseudomonas sp., Flavobacterium sp*	*Aspergillus niger, Rhizopus sp.*

SW = Surface water, BS= Bottom sediment. GPS BEARINGS: Discharge point: 04 22 12 E, 06 09 15 N., 500M UPSTREAM: 04 47 30 E, 06 23 00 N., 500M DOWNSTREAM: 04 39 30 E, 06 23 10 N.

4.0 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 study, Produce water from Escravos tank farm have a bacterial counts of 23-28 x 10^5 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 insitu 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 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), Sulfate 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 environment is low.

The analytical data from the present study showed that produced water had considerable concentrations of hydrocarbon utilizing bacteria (HUB) during the two seasons under investigation ($1.10 – 1.20 \times 10^5$ Cfu/ml) same is applicable to Sulphate reducing bacteria(SRB) ($0.10– 1 \times 10^5$ Cfu/ml). Bottom sediment section of produced water discharge point showed relatively high concentration of HUB ($4.50 – 14 \times 10^5$ Cfu/g) and SRB ($2.50 – 10 \times 10^5$ Cfu/g) 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 (500m 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 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 outcompeting SRBs and rendering them less potent as demonstrated by Hubert and Voordouw,(2007), the presence of SRB in any environment is not desirable.

5.0 Conclusion:

The present study have demonstrated that Produced water discharges in near shore shallow environment leads to the accumulation of 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 500m 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.

Acknowledgement:

Chevron Nigeria Limited is gratefully acknowledged For Providing Logistics and Laboratory Facilities for this Research.

CORRESPONDING AUTHOR:
Dr. Chuma C Okoro
Department of Biological Sciences and Biotechnology
Caleb University, Lagos Nigeria
Tel: 08033072754, 01-7430285
e-mail: chuma2k2001@yahoo.com
P. O. BOX 146, UNIVERSITY OF LAGOS POST OFFICE, LAGOS, NIGERIA

References:.

1. Atlas RM. Petroleum Microbiology. Mac-millian Publishing Company 886 Third Ave. Newyork, USA.2004;960
2. Ayes RC,Parker M . Offshore Produced Water waste management: Technical Report to Canadian Association of Petroleum Producers (CAPP).2001; 19.
3. Bastin ES Anderson B, Creer CA, Mouton, G. The problem of natural reduction of sulphates.

Bull. Am. Assoc. Petrol. Geol. 1926:10: 1270-1299.
4. Birkeland N K. Sulfate reducing bacteria and Archaea. In Petroleum Microbiology; Oliver, B and Magot, M (ed.) ASM Press, Washington DC.2005; 35-54.
5. Chevron Nigeria Limited. Monthly Effluent Water reports (With Analytical Methods). July, 1995.
6. Eaton AD, Clesceri L S, Greenberg A E,. Standard Methods for the examination of water and waste water (19th edition). United Books press Inc. Batimore, Maryland, U.S.A 1995;1126
7. Head IM, Jones DM, Larter, S R. Biological activity in the deep subsurface and the origin of heavy oil. Nature 2003 ;426: 344-352.
8. Hubert C, Voordouw G. Oil field Souring control by Nitrate reducing Sulfurospirillum spp. That out compete Sulfate -reducing bacteria for organic electron donors. Appl. Environ. Microbiol. 2007;73(8):2644-2652.
9. Jonathan-Wills M A . A survey of offshore oil field drilling wastes and Disposal techniques to reduce the ecological impact of sea dumping. Sakhalin Environment Watch., 2000;22:12-16.
10. Magot M. Indigenous Microbial Communities in Oil fields., In Petroleum Microbiology. Oliver, B and Magot, M (ed.) ASM Press, Washington DC.2005; 35-54.
11. Middledich BS. Ecological Effect of Produced Water discharges from Offshore Oil and Gas Production Platforms. Final Report on API Project . 1984; 248. API Washington D.C.
12. Mills A L, Breuil C, Colwell RR. Enumeration of Petroleum degrading Marine and Estuarine Microorganisms by Most Probable Number Method. Can. J. Microbiol. 1978;24:552-557.
13. Munn CB. Marine Microbiology . C.B.Munn (ed.) Bios. Scientific. Publishers, Newyork. 2004;281..
14. Neff J M. The Potential Impacts of Drilling Fluids and other effluents from exploratory drilling in the living resources. Bachus R H.(ed.) Cambridge Massachussets 1985;(In Press).
15. Okoro CC. Microbial Degradation of Hydrocarbons In Produced Water from Crude Oil Production Operations In Escravos Tank Farm. Ph.D. Thesis, University of Lagos. 1999;269.
16. Okoro CC, Amund OO. Biodegradation of Hydrocarbons in Untreated Produced Water Using Pure and Mixed Microbial Cultures. J. Sci. Tech. And Environ.2002;2 (2): 14-23.
17. Okoro C C. Biodegradation of Hydrocarbons In Untreated Produced Water using Pure Fungal Cultures. Afric. J. Microbiol. Res.2008; 2:217-223.

DATE OF SUBMISSION: NOVEMBER 24, 2009

Dr. Chuma C Okoro is presently a senior lecturer at the Department of Biological Sciences and Biotechnology Caleb University, Lagos, Nigeria.