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Laboratory Study on Adaptability of Anaerobic Bacteria to Different Salinities for the Purpose of Microbial Enhanced Oil Recovery at the Nord Sea

Uchendu Udochukwuka Ifeanyi*, Onyeizu Rowland Uchenna, Stephen Austin Chigozie
Department of Environmental Management and Toxicology, Michael Okpara University of Agriculture Umudike, Abia State
*Corresponding Author: uchendu.udochukwuka@mouau.edu.ng

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Abstract

This research is based on the study of microbial enhanced oil recovery (MEOR) and the possibilities of its use in the Danish section of the Nord Sea. The study applies experimental procedure to investigate if anaerobic bacteria can survive under reservoir conditions and produce products important in oil recovery. A laboratory experiment was set up to simulate the salinity conditions at the Nord Sea. Result showed that it was possible to increase the adaptability limit of the considered bacteria growth to salinities up to 140 g/l. Salinity of 20 g/l and 140 g/l has the highest and lowest total gas production of 3970 ml and 110 ml respectively. A decline in pH with time was observed across all salinity. The highest pH was measured at 140 g/l also pH showed a direct correlation with salinity. Electrical conductivity increases as pH increases, it also increased with increasing salinity. The highest gas production was after 24 hours and gas production across all salinities decreased with increasing time. The highest gas production was between the pH of 5 to 7. Gases produced during this experiment includes, carbon dioxide, methane, nitrogen, traces of hydrogen sulphide, and two other unknown gases which we were unable to detect. A linear model was used to plot the relationship between various measured parameters.

1. Introduction

Microbial enhanced oil recovery (MEOR) involves the use of reservoir microorganisms or specifically selected natural bacteria to produce metabolic events that leads to enhanced oil recovery. The processes that facilitate oil production are complex and may involve multiple biochemical processes [1, 2]. Microbial biomass or biopolymers may plug high permeability zones and lead to increased mobilization of residual oil, it may increase gas pressure by the production of carbon dioxide, it may also reduce the oil viscosity due to digestion of large molecules [3, 4].
During oil production, primary oil recovery can account for between 30-40 % oil production, while additional 15-25% can be recovered by secondary methods such as water injection leaving behind about 35-55 % of oil as residual oil in the reservoirs [5]. This residual oil is usually the target of many enhanced oil recovery technologies and it amounts to about 2-4 trillion barrels or about 67 % of the total oil reserves [6]. The recent oil decline in the Danish sector of the North Sea and the need to identify the most plausible technology to enhance oil recovery makes this research relevant [7,8]. Most of the reservoir rocks in the Danish sector are made up of carbonate rocks and MEOR has been postulated to be more effective in carbonate reservoirs [9]. In Danish sector of the North Sea, oilfields were estimated to have recoverable hydrocarbon reserves of 240 million m^3 of oil and 120 billion m^3 of gas at January 2007 [10, 11].

MEOR processes are somewhat akin to in situ bioremediation process. Injected nutrients, together with indigenous or added microbes, promote in situ microbial growth and generation of products that mobilize additional oil and move it to producing well through reservoir repressurization, interfacial tension/oil viscosity reduction and selective plugging of the most permeable zones [12]. Alternatively, the oil-mobilizing microbial products may be produced by fermentation and injection into the reservoir. Bacteria are considered promising candidates for microbial enhanced oil recovery [13]. Many petroleum reservoirs have high concentration of sodium chloride and require the use of bacteria that can tolerate these conditions. Bacteria producing biosurfactants and biopolymers can grow at sodium concentration up to eight percent and selectively plug sandstone to create a biowall to recover additional oil [14]. The aim of this research is to study the adaptability of anaerobic bacteria to different salinities and check the effect of the microbes on permeability of the Danish Nord Sea Chalk.

2. Methodology

2.1.Sample Collection and Analysis

The anaerobic bacteria used for this experiment was collected from CHP-biogas plant at Ribe in Denmark. It was stored at a temperature of 53°C which is the optimum temperature for anaerobic bacteria. In order to check the adaptability of the considered bacteria and see how effective MEOR method could prove for the Danish field, a laboratory-based experiment was carried out. Sodium chloride (NaCl) was first added to seven flasks according to the salinity desired 20 g/l, 40 g/l, 60 g/l, 80 g/l, 100 g/l, 120 g/l, 140 g/l except for the control (0 g/l) which had no NaCl. The fermentation bottles and media were initially purged of air by passing pure nitrogen for about 5 min according to [1]. This was to create anaerobic conditions in the bottles. For each salinity there was a replicate. 700 ml of water and 50 ml of molasses was then added to each flask. Flasks were then placed in the water bath and heated until a temperature of 53^{o}C was attained. 50 ml of the anaerobic bacteria was then added to each flask. All flasks were covered and mixed properly to attain a homogenous content within each flask and placed back in the water bath. The initial pH and conductivity of all flasks were measured and recorded. The set up was completed with water displacement apparatus for gas collection and measurement of volume produced. The whole process was maintained in water bath at 53^{o}C. Cumulative gas production was calculated as total sum of gas produce as a result of water displacement during the period of 120 h. Liquid samples were carefully taken out from the fermentation bottles every 24 h for pH measurement using a pH meter (Model: PH 2000 Radiometer Analytical). Determination of gas composition from the experiment was carried out using Clarus 500 GC Perkin Elmer. Statgraphic plus (Version 4) was used for the statistical analysis of the result obtained.

Table 1: Initial pH and Electrical Conductivity (E C) reading

SALINITY (g/l)	INITIAL pH	INITIAL E C (mS/cm)
0	7.83	10.36
20	7.57	35.17
40	7.48	55.4
60	7.39	75.7
80	7	99.2
100	6.97	113.4
120	7.02	128
140	7.01	139.6

This experiment was conducted for 120 hours (5 days) and during this period no nutrient (molasses) was added, this was to estimate the frequency of consumption of nutrient by these bacteria and to study the quantity of gases and acids they can produced with a specified amount of nutrient within the stipulated 120 hours. At the end of the experiment, the gas obtained was analysed using Gas

Chromatography. Hydrogen Sulphide Test Strips (Lead acetate test strips) was used for this experiment.

3. Results and Discussion

Table 2 shows the pH measured for each salinity, as well as electrical conductivity, temperature, volume of gas produced.

Table 2: pH, Electrical Conductivity, Temperature, Volume of Gas Produced at Various Salinity.

SALINITY (g/l)	pH	E C (mS/cm)	TEMP (°C)	GAS VOLUME (ml)	HOURS
	5.7	12.54	43	1300	24
	5.55	13.31	36.8	0	48
0	5.15	12.81	36.9	0	72
	5.08	13.25	39.5	40	96
	5.1	13.07	39.2	0	120
	6.12	34.9	41.8	2000	24
	5.66	34.7	39.5	1100	48
20	5.76	36.5	38.8	850	72
	5.1	35.1	39.9	20	96
	5.16	37.4	42.2	0	120
	6.46	56	39.5	0	24
	5.85	57.2	40.7	0	48
40	5.52	57.2	38.6	0	72
	4.93	57.1	41.4	250	96
	5.08	58	42.4	10	120
	6.5	77.1	41.9	0	24
	6.42	75.9	39.5	0	48
60	6.44	76.6	38.2	0	72
	6.16	76.6	42.1	130	96
	6.04	76.9	43.9	50	120
	6.74	94.4	38.2	60	24
	6.74	93.3	39.7	40	48
80	6.4	93.2	39.6	70	72
	6.88	95.6	40.2	20	96
	6.59	95.4	40.2	5	120
	6.87	109.4	41.5	230	24
	6.94	112.4	42.6	0	48
100	6.9	107.7	41.4	60	72
	6.5	114.4	42.3	20	96
	6.43	111.7	43.6	10	120
	7	123.5	42.5	40	24
	7.11	122.7	40.9	50	48
120	7.02	124.6	44.2	40	72
	7.07	121.9	43.7	20	96
	6.91	118.4	43	5	120
	7.96	139.2	42.2	30	24
	7.27	138.8	43.4	50	48
140	7.24	139	43.6	20	72
	7.37	138.8	45.3	10	96
	7.39	138.7	45.4	0	120

From Table 2, it can be seen that at 20 g/l salinity, has the highest total gas production (3970 ml), followed by 0 g/l salinity with 1340 ml, the least gas production was at salinity of 140 g/l, this is due high salt content which affects the activities of the anaerobic bacteria. The table also shows a decline in pH with time across all salinity. The highest pH was measured at 140 g /l. It can then be concluded that pH has a direct correlation with salinity, the higher the salinity the higher the pH. Electrical conductivity increases as pH increases. It also increases with increasing salinity. The highest gas production was after 24 hours, and gas production across all salinities decreased with increasing time. The table also shows a relationship between pH and gas production, the highest gas production was between the pH of 5 to 7. Figures 1-12 show various relationships between the measured parameters and plot of fitted model.

3.1 Surface Plot for Various Combinations of Parameter

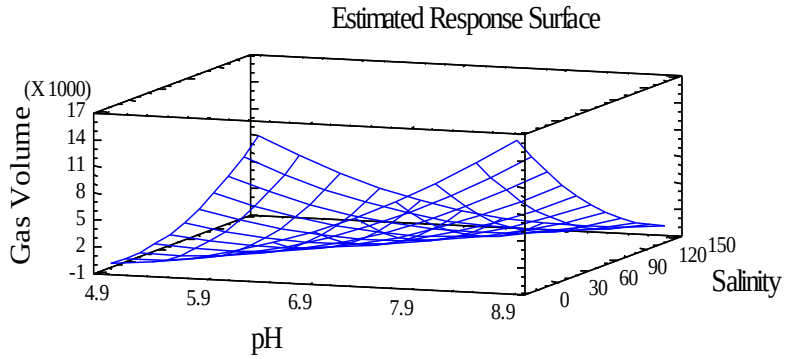

Figure 1: Gas volume vs. pH vs. salinity

Figure 1 shows the estimated gas volume as a function of pH and salinity. The height of the surface represents the value of gas volume. It is observed that an increase in gas volume corresponds with an increase in pH and corresponding decrease in salinity.

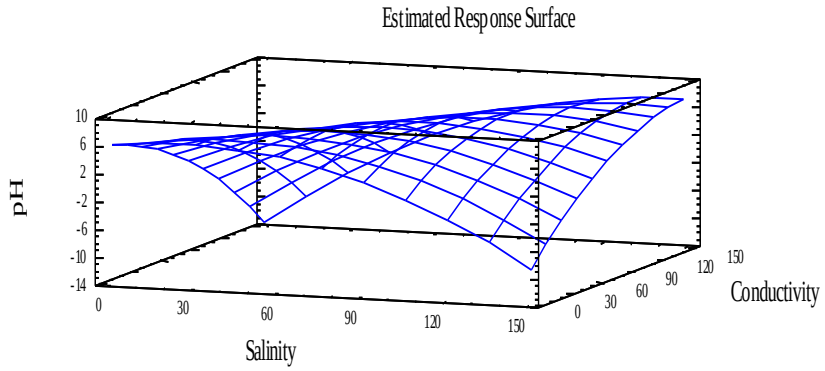

Figure 2: pH vs. salinity vs. conductivity

Figure 2 shows the estimated pH as a function of salinity and conductivity. The height of the surface represents the value of pH. It is observed that a decrease in pH corresponds with a decrease in salinity and corresponding increase in conductivity.

3.2 Scatter Plot for Various Combinations of Parameter

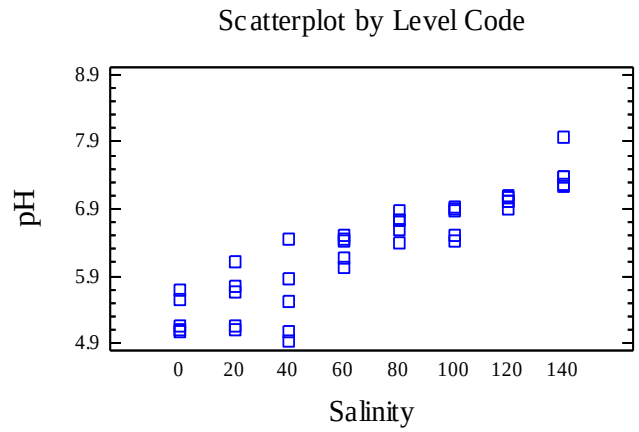

Figure 3: Scatterplot of pH vs. salinity

Figure 3 shows a positive trend of increase in salinity as pH increases, at salinity of 40 g/l, the pH measurement across the 120 hours varies considerably.

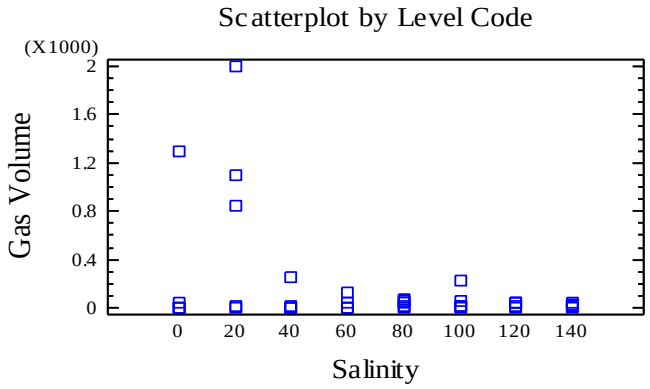

Figure 4: Scatterplot of gas volume vs. salinity

Figure 4 shows the distribution of gas production at different salinities. It shows that at salinity of 20g/l the gas production was highest, and has the highest statistical range.

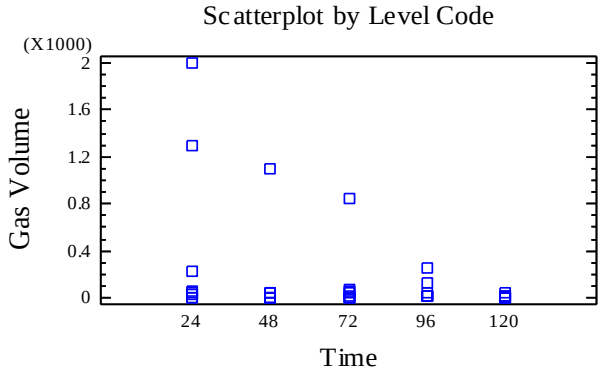

Figure 5: Scatterplot of gas volume vs. time

Figure 5 shows that gas volume was highest after just 24 hours and decreases with increasing time. This can be attributed to the fact that no nutrient was added during the experimental period, therefore less metabolic process.

3.3 Plots of Fitted Model

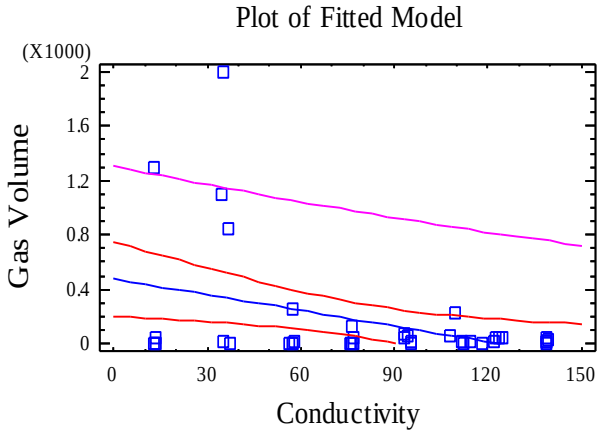

Figure 6: Plot of fitted model of gas volume vs. conductivity

Figure 6 shows the results of fitting a linear model to describe the relationship between gas volume and conductivity. The equation of the fitted model is shown in Equation (1).

$$\text{Gas volume} = 473.969 - 3.82979*\text{conductivity} \quad (1)$$

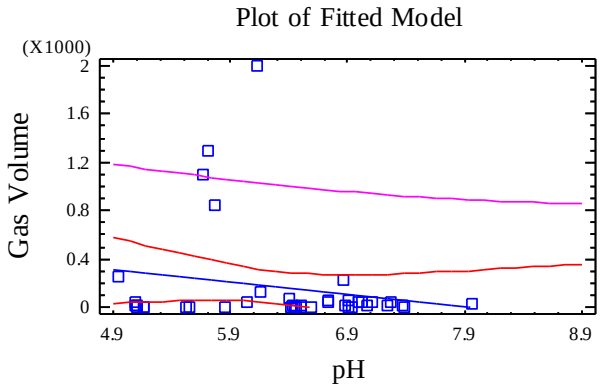

Figure 7: Plot of fitted model of gas volume vs. pH

Figure 7 shows the results of fitting a linear model to describe the relationship between gas volume and pH. The equation of the fitted model is shown in Equation (2).

$$\text{Gas volume} = 799.606 - 100.566*\text{pH} \quad (2)$$

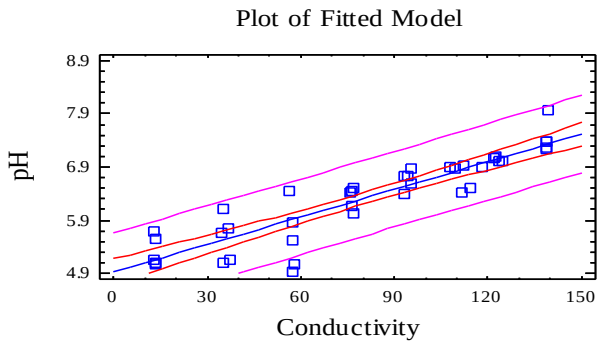

Figure 8: Plot of fitted model of pH vs. conductivity

Figure 8 shows the results of fitting a linear model to describe the relationship between pH and conductivity. The equation of the fitted model is shown in Equation (3).

$$pH = 4.92943 + 0.0172351*conductivity \quad (3)$$

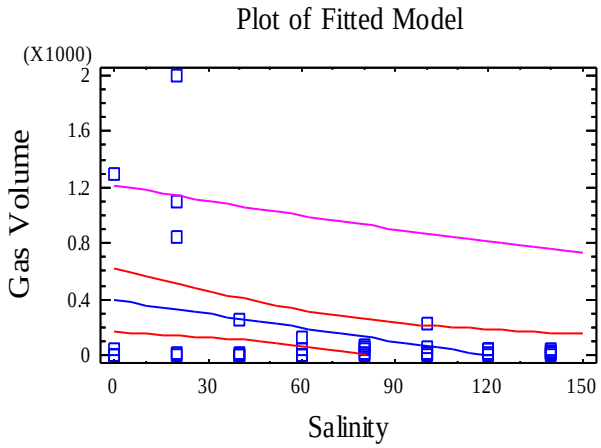

Figure 9: Plot of fitted model of gas volume vs. salinity

Figure 9 shows the results of fitting a linear model to describe the relationship between gas volume and salinity. The equation of the fitted model is shown in Equation (4).

$$Gas\ volume = 392.333 - 3.27262*salinity \quad (4)$$

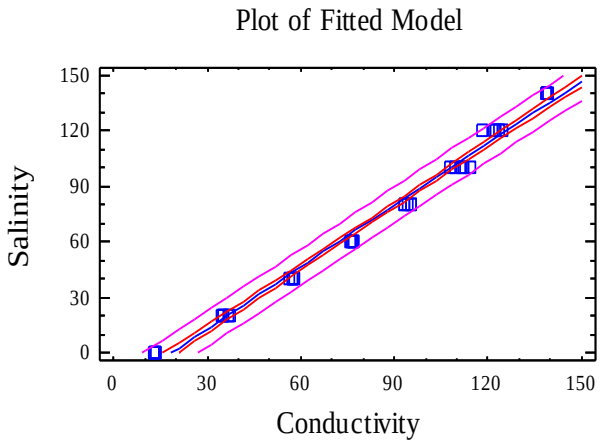

Figure 10: Plot of fitted model of salinity vs. conductivity

Figure 10 shows the results of fitting a linear model to describe the relationship between salinity and conductivity. The equation of the fitted model is shown in Equation (5).

$$Salinity = -20.33 + 1.11337*conductivity \quad (5)$$

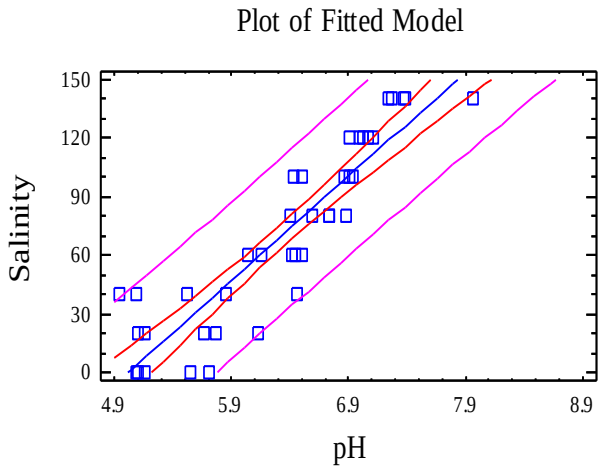

Figure 11: Plot of fitted model of salinity vs. pH

Figure 11 shows the results of fitting a linear model to describe the relationship between salinity and pH. The equation of the fitted model is shown in Equation (6).

$$\text{Salinity} = -266.638 + 53.2002*\text{pH} \qquad (6)$$

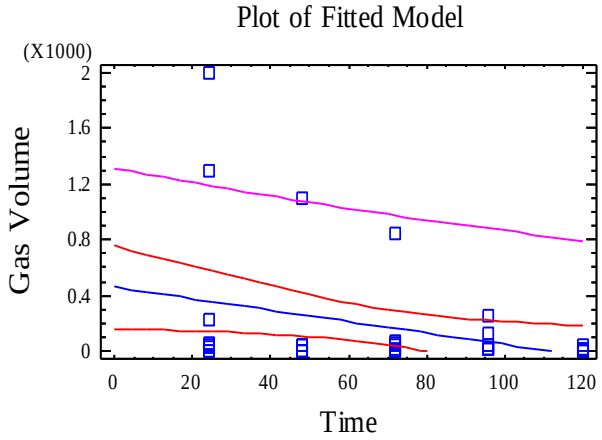

Figure 12: Plot of fitted model of gas volume vs. time

Figure 12 shows the results of fitting a linear model to describe the relationship between gas volume and time. The equation of the fitted model is shown in Equation (7).

$$\text{Gas Volume} = 459.125 - 4.10938*\text{time} \qquad (7)$$

3.4 Gas Composition Analysis

From Table 3, it is observed that Nitrogen concentration measured during the gas analysis increases with increase in salinity; therefore, we can deduce that there is a direct correlation between the amount of Nitrogen produced and salinity, this is supported by earlier study of [15]. Carbon dioxide on the other hand was random across the different salinity although the highest value was observed in 0 g/l, this shows that the microbes produce more carbon dioxide at low or zero salinity. The analysis also shows that methane gas was produced, but it was only detected at 20 g/l, we can recall that 20 g/l has the highest gas production over the 120 hours; therefore it is possible that the amount of gas produced has an influence on the presence of methane gas as stated by [16]. There were three unknown gases whose name were not detected by the GC, however, past literature [17,18] suggest

that hydrogen sulphide is likely to be one of these three gases. Generally, production of carbon dioxide for all salinity was between the range of 67 – 99 % of the total gas production, this is however very desirable as it will help to break down rock formation and aid oil recovery [19].

Table 3: Gas Composition at Various Salinity

Gas Composition (%)						
Salinity (g/l)	**CO_2**	**CH_4**	**N_2**	**Unknown 1**	**Unknown 2**	**Unknown 3**
0	99.027	-	0.930	0.020	0.020	-
20	97.238	2.590	0.170	0.003	0.003	-
40	97.929	-	1.960	0.050	0.050	0.006
60	67.531	-	31.450	0.340	0.510	0.178
80	78.972	-	20.088	0.940	-	-
100	87.594	-	12.406	-	-	-
120	69.802	-	29.382	0.447	0.369	-
140	-	-	95.488	2.622	1.890	-

The result for hydrogen sulphide test using strips (Sterile filter paper strips impregnated with lead acetate) was negative, this means no hydrogen sulphide was detected, however, literature from other past works [17,20] indicates the presence of hydrogen sulphide in the gas produced during fermentation of anaerobic bacteria. The reasons why hydrogen sulphide was not detected could be that the temperature recommended for the test strip was too low for the anaerobic bacteria (35°C was recommended for the test, however the anaerobic bacteria has an optimum temperature of 53°C) therefore, probably growth did not occur and as such, H_2S was not produced [1].
Results obtained from the experiment shows that gas production peaked at 20 g/l salinity and the least gas production was observed at 140 g/l as shown in Figure 4, therefore it was observed that increase in salinity corresponds with a decrease in gas production, this shows that the anaerobic bacteria produces more metabolite at lower salinity as supported by [21]. There was a steady decline in volume of gas produced after 24 hours as shown in Figure 5; this was because there was no additional nutrient added during the 120 hours experimental period, therefore the microbes gradually used up all the available nutrients, this decline in nutrient correlates with decline in production of metabolite [22]. It was also observed that gas production was highest between the pH of 5 -7 as shown in Figure 7.
From Figures 3, 8, 10 and 11, salinity, conductivity and pH show a direct correlation with each other, as increase in salinity corresponds with increase in conductivity and also an increase in pH and vice versa, also increase in pH corresponds with increase in conductivity and vice versa [23].

4. Conclusion

The result of this experiment shows that production of gases at high salinity was possible although the highest gas production was observed at salinity of 20 g/l. Gas production decreased with time and this can be attributed to the face that no nutrient was added during the 120 hours of the experiment. From the statistical analysis, the following were deduced: gas volume decreases with time, gas volume increases as salinity decreases, gas volume decreases with increasing pH, pH increases with increasing salinity, conductivity increases with increasing pH. The gas analysis shows that more than 60 % of the gas produced was carbon dioxide. This study recommends that further experiment should involve adding molasses when the volume of the gas produced becomes low, this increases the gas volume produced as growth and propagation of microbes start as this is very important for further oil recovery. During the fermentation days the amount of carbon dioxide gas produced is limited due to the fact that it was carried out with a limited amount of nutrient.

Therefore, more investigation should be carried out on mass balance of this process to have a better understanding of the production of metabolites.

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