RESEARCH ARTICLE

THE EFFECTS OF SUBSTRATE CHARACTERISTICS MEDIUM ONIMPROVEMENT OF (MEOR) IN NIGER DELTA AREA OF NIGERIA

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ABSTRACT

In this paper investigation was conducted to examine the effect of substrate characteristics medium (heavier hydrocarbon mixture in a reservoir) on the improvement of Microbial Entrained Oil Recovery (MEOR) in Niger Delta Area of Nigeria. The effect of substrate concentrate (heavier hydrocarbon) was demonstrated upon the action of the following microorganism, *pseudomonas sp streptococcus sp, escheerichia coli, chromobacterium, bacillus sp. flavobacterium* and *micrococcus*. Results obtained illustrates high growth rate in order of magnitude *pseudomonas > bacillus sp. > eschevichia coli > flavobacterium > streptococcus > micrococcus > chromobiacterium*. The rate of gas, water lighter hydrocarbon, biomass production from the degradation of the heavier hydrocarbon can be attributed to the effectiveness of the microbial activity in each reactor as well as the physicochemical properties of the substance favour the process. The experimental results obtained showed that the increase in gas, water, lighter hydrocarbon and biomass concentration with increase in period of exposure. The mathematical model developed in this paper was used in the simulation of the microbial growth and substrate kinetics. As the rate of production of products increases with microbial the rate of substrate concentration (heavier hydrocarbon) decreases. The research demonstrate the useful of various microorganism in improving microbial entrained oil Recovery (MEOR) in Niger Delta area of Nigeria using a favourable environment and physicochemical conditions.

INTRODUCTION

Microbial enhanced oil recovery refers to the use of micro-organisms to retrieve additional oil from dead/existing wells, thereby enhancing the petroleum production of an oil reservoir. In this method, microorganism are introduced into oil wells to produce harmless byproducts such as slippery natural substances or gases, all of which help propel oil out of the well. This is because these processes help to mobilize the oil and facilitate oil flow; they allow a greater amount to be recovered from the well. The use of micro-organisms and their metabolic products to enhance oil production involves the injection of selected microorganisms into the reservoir and the subsequent stimulation and transportation of their in-situ growth product in order that their presence will aid in further reduction of residual oil left in the reservoir after secondary recovery is exhausted. The MEOR is unlikely to replace conventional EOR (Enhance Oil Recovering) methods, because MEOR itself has certain constraints. This unique process seems superior in many respects, however, because self-duplicating units, namely the bacteria cells, are injected into the reservoir and by their in-situ multiplication they magnify their beneficial effects. The aim of the study is to model the rate of substrate/microbial characteristics of oil well

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upon the influence of microbial entrained oil recovery (MEOR). Also to recognize the biochemical influence that specific microorganism that is injected into a well would have on the oil (crude) that could result to pressure building in a well. This study correlates the physiochemical changes in the reservoir upon the action of the micro-organisms. Further study was carried out in terms of Monod and Michaelis Mention equation in relationship to the MEOR as well as developing the residence time of the microorganisms in terms of radium (rm), depth (h), porosity q, and Kinetic of biochemical process taking place in the reserviour. The work is centered on the effect of microbial characteristics of oil upon the influence of microbial entrained oil recovery.

The paramount importance of MEOR is to improve the recovery of oil entrapped in porous media while increasing economic profits MEOR is a tertiary oil extraction technology allowing the partial recovery of the commonly residual two-third of oil, thus increasing the life of mature oil reservoirs (Reinhard and Drehfahl, 1999; Monlgomery, 2000; NCDC, 2001, Keller, Sirivithayapakom and Mingjie, 2001 & Rogerson and Berger 1981). Increment in Oil Production: This is done by modifying the interfacial properties of the system oil-water minerals, with the aim of facilitating oil movement through porous media. In such a system, microbial activity affects fluidity (viscosity) reduction, miscible flooding;

displacement efficiency (decrease of interfacial tension, increase of permeability: sweep-efficiency mobility control, selective plugging) and driving force reservoir pressure). Upgrading: In this case, microbial activity acts may promote the degradation of heavy oils into lighter ones. Alternatively, it can promote desulphurization due to denitrification as well as the removal of heavy metals (Briggs and Haldane, 2001; Ukpaka, 2006, 2007, 2009, 2010, 2011; Sarkar, Goursaud, Sharma and Georgious, 1989; & Schiumberger Article, 2011; 2011a). Several research and successful applications support the claims of MEOR as a mature technology. Regardless of those facts; there are still uncertainties about the technology. Successful stories are specific for each MEOR field application and published information regarding supportive economical advantages is however inexistent. Despite this, there is consensus considering MEOR one of the cheapest existing methods. However, obscurity exists on predicting whether or not the deployment of MEOR will be successful. MEOR is, therefore, one of the future research are with great priority as identified by the "Oil and Gas in the 21st century task force. This is probably because MEOR is a complementary technology that may help recover the 377 billion barrels of oil that are unrecoverable by conventional technologies bias (Sheehy, 2008; Adamczew, S.K., Siepak and Gramowsk, 2000; Thomas and Li, 2000; Kowalezyk, Swietlik and Dojlido, 2002 & Ying and Weber, 1979).

The technology started in 1926 when Beckam proposd the utilization of micro organism as agents for recovering the remnant oil entrapped in porous media. Since recovering that time numerous investigations have been developed and are extensively reviewed. The first field best was carried out in the Lisbon field is Arkansas, USA. During the time, Kuznetsov discovered the microbial gas production from oil. The main type of field experiments developed in those countries consisted in injecting exogenous microbes in 1958, selective plugging with microbial produced biomass was proposed by Heinnigen and colleagues. The oil crisis of 1970 triggered a great interest in active MEOR research in more than 15 countries. From 1970 to 2000, basic MEOR research focused on microbial ecology and characterization of oil reservoirs (Sitnikov, Eremin, and Ibattulin, 1994; Zobell and Frank, 2004; and Sea, 2006). In 1983, Ivanov and colleagues developed the strata microbial activation technology. By 1990, MEOR achieved an inter disciplinary technology status. In 1995, a survey of MEOR projects 322 in the USA showed that 91% of the projects successfully increased oil production, and there was not a single case of reduced oil production. Today, MEOR is gaining attention owing to the high prices of oil and the imminent ending of this resource. As a result, several countries are willing to use MEOR in one third of their oil recovery programs by 2010 (Calude, 1946; Sea, 2006 and Cloude and frank, 2000).

MEOR is classified as surface MEOR and underground MEOR based on the place where microorganism works. For surface MEOR, biosurfactant, biopolymer and enzyme are product in the surface facilities. These biological products are injected into the target place in the reservoirs as chemical FOR methods. While for underground MEOR, micro-organisms nutrients and/or addictives are injected into the reservoir and let them sustain, grow, metabolize and ferment underground. Underground MEOR is sorted as:

Cyclic microbial recovery (Huff and Puff, single well stimulation), Wax removal and Paraffin inhibition (well bore clean-up), Microbial flooding recovery, Selective plugging recovery, and Acidizing, fracturing. A solution of micro-organisms and nutrients is introduced into an oil reservoir during injection. The injector is then shut in for an incubation period allowing the micro-organisms to product carbon dioxide gas and surfactants that help to mobilize the oil. The well is then open and oil and products resulting from the treatments are produce (O'brien and Dixon, 1976; Mihecic and Luthy, 1988 and Sea, 2006). Recovery by this method utilizes the effect of microbial solution on a reservoir. The reservoir usually conditioned by water preflush, then a solution of microorganisms and nutrients is injected. As this solution is pushed through the reservoir by drive water, it forms gases and surfactants that help to mobilize the oil. The resulting oil and product solution is then pimped out through production wells (Lazar, Petrisor and Yen 2007; Ollivier and Magot, 2005 and Sea, 2006). The selective plugging recovery of bacterial suspensions followed by nutrients to produce biopolymer and microbial itself, which may plug the high permeability zone in the reservoir. The reduction of permeability would change the inject profile and achieve conformance control. Microorganisms for MEOR; Characteristics of Bacteria: Dynamic ecology with many species; Growth determined by environment, Nutrients, cell material and energy, Physical, Chemical, and Biological.

The Microorganisms for MEOR should have the following properties: small size, resistant against high pressure, capability of withstanding brine and seawater, anaerobic using of nutrients, unfastidious nutritional requirements, appropriate biochemical construction for production suitable amounts of MEOR chemicals and lack of any undesirable characteristics. There are various advantages and disadvantages for MEOR; thus for advantages of MEOR we have; infected microbes and nutrients are cheap; easy to handle in the field and independent of oil prices, economically attractive for mature oil field before abandonment, increases oil production, existing facilities require slight modification, easy application, less expensive set up, low energy input requirement for microbes to produce MEOR agents and cellular products are biodegradable and therefore can be considered environmentally friendly. Similarly, for the disadvantages of MEOR we have, the oxygen deployed in aerobic MEOR can act as corrosive agent on non-resistant topside equipment and down-hole piping, anaerobic MEOR requires large amount of sugar limiting its applicability in offshore platforms due to logistical problems, exogenous microbes require facilities for their cultivation, and indigenous microbes need a standardized framework for evaluating microbial activity, e.g. specialized coring and sampling techniques.

Environmental Constraints

Several factors happening at the same time affect microbial growth and activity. In oil reservoirs, such environmental constraints permit to establish criteria as to asses and compare the suitability of microorganism. Those constraints may not be as harsh as other environments or earth. Some environmental constraints creating selective pressures on cellular systems that may also affect microbial communities in oil reservoirs are; which includes:

Temperature: Enzymes are biological catalysts whose functions is affected by a variety of factors including temperature, which at different ranges may improve or hamper enzymatic mediated reactions. This will have an effect over the optimal cellular growth or metabolism. Such dependency permits to classify microbes according to the range of temperature at which they can grow. (Van, lame, Singh and Ward, 2006; Flyiwara, 2004 and Ukpaka, 2010).

Pressure: direct effects; the effects of pressure on microbial growth under deep ocean conditions were investigated by Zobel and Johnson in 1949. They called barophilic to those microbes whose growth was enhanced by increasing pressure. Other classification of microorganisms is based on whereas microbial growth is inhibited at standard conditions or above 40Mpa. From a molecular point of view, the review of Daniel shows that at high pressures the DNA double helix becomes dencer, and therefore both gene expression and protein synthesis are affected (Swietlik, Kowalczyk and Dojlido, 2002).

In Direct Effect: Increasing pressure increase gas solubility and this may affect the redox potential of gases participating as electron acceptors and donors, such as hydrogen or CO_2 (Kerr and Capone, 1988). *Pore*

Size/Geometry: One study has concluded that substantial bacterial activity is achieved when there are interconnections of pores having at least 0.2pi diameter. It is expected that pore size and geometry may affect chemo-taxis. However this has not been proven at oil reservoir conditions (Thomas and Li, 2002). *pH:* The acidity of alkalinity has an impact over several aspects in living and non-living systems. For instance (Swietlik, Kowakzyk and Dojlido, 2002).

Surface Charge: Changes in cellular surface and membrane thickness may be promoted by pH due to its ionization power of cellular membrane embedded proteins. The modified ionic regions may interact with mineral particles and affect the motion of cells through the porous media (Sea, 2006).

Enzymatic Activity: embedded cell proteins play a fundamental roll in the transport of chemicals across the cellular membrane. Their function is strongly dependent on their state of ionization, which is in turn strongly affected by pH.

Oxidation Potential: The oxidation potential is, as in any reaction system, the thermodynamic driving force of anaerobic respiration, which takes place on oxygen depleted environments. Prokarytoes are among the cells that have anaerobic respiration as metabolic strategy for survival. The electron transport takes place along and across the cellular membrane. Electrons are transferred from an electron donor (molecule to be oxidized anaerobically) to an electron acceptor (NO_3, SO_4, MnO_4, etc.). The net Eh between a given electron donor and acceptor; hydrogen ions and other species in place will determine which reaction will first take place. For instance, nitrification hierarchically more favoured than sulphate reduction. This allows for enhanced oil recovery by disfavouring biologically produced H_2S, which derives from reduced So4. In this process, the effects of nitrate reduction on wettability, interfacial tension, viscosity, permeability, biomass and biopolymer production remain unknown.

Electrolyte Composition: Electrolytes concentration and other dissolved species may affect cellular physiology. Dissolving electrolytes reduces thermodynamic activity, vapour pressure and antroprotolysis of water. Besides, electrolytes promote an ionic strength gradient across cellular membrane and therefore provides a powerful driving force allowing the diffusion of water into or out to cells. In natural environments, most bacteria are incapable of living at aw below 0.95. However, some microbes from hypersaline environment such as pseudomonas species and halococcus thrive at lower aw and are therefore interesting for MEOR research.

Biological Factors: Although it is widely accepted that predation, parasitism, syntrophism and other relationships also occur in the microbial world, little is known in this relationships on MEOR and they have been disregarded in MEOR experiments.

In other cases, some micro-organisms can thrive in nutrient deficient environments (oligatrophy) such as deep granitic and basaltic aquifers. Other microbes, living in sediments, may utilize available organic compounds (heterotrophy). Organic matter and metabolic products between geological formations can diffuse and support microbial growth in distant environments.

MATERIALS AND METHOD

Collection of Samples

Crude oil samples were collected from different oil wells in Niger Delta area of Nigeria, using plastic container and then transported to Department of Microbiology for isolation, identification and characterization of microorganisms present in the different crude oil samples. Some part of the samples was taken to the Department of Microbiology and the remaining where transferred to the Department of Chemical/Petrochemical Engineering; both departments are located in Rivers State university of Science and Technology, Port Harcourt. The some of the physicochemical properties of the crude oil mixture were measured before setting-up the experiment.

Microbial Sample

Total microbial counts were measured by a standard plate count technique using difeoplate count techniques using dico plate count agar (APHA, 1992).

Microbial Culture

Crude oil samples collected from Niger Delta area of Nigeria was used for the investigation. The microbes from the Petroleum were isolated, identified and characterized as *pseudomonas sp, streptococcus sp, Escherichia coli, chromobacterium, bacillus sp, flaro bacterium* and *micrococcus* according to the methods of Buchanan and Gibbons, (1974).

Analysis for Some the Physicochemical Parameter

The analysis for each of the parameters was carried out according to the international standard as specified in Table 1.

Table 1: Standard for measuring the physiochemical properties of some parameters

Parameters	Analytical Method
pH	APHA 4500H'8
Turbidity (NTU)	APHA 21308
Total Dissolved solids (mg/g)	APHA 25108
Conductivity (μS/cm)	APHA 25110A
Biochemical Oxygen Demand (mg/l)	APHA 5210D
Chemical oxygen Demand (mg/l)	APHA 5220D
Dissolved Oxygen (mg/l)	APHA 5230D
Chloride (mg/l)	APHA 4500Cr8
Total Alkalinity (mg/l0	ASTM D10678
Nitrate (mg/l)	EPA 352.1
Phosphate (mg/l)	APHA 4500-PD
Sulphate (mg/l)	APHA 4500 SO/"E
Total Iron (mg/l)	APHA 31118

Experimental Procedure

Procedure: Forty-two experimental set-up were prepared for this investigation with each sub-experimental for this investigation with each sub-experimental unit of six samples, thus, reactor A-*pseudomonas sp*, reactor B-*streptococcus s*p., reactor C-*escherichia coli*, reactor D-*chromobacerium*, reactor E-*bacillus sp*., reactor F-*flavobacterium* and reactor G-*micrococcus*. The experiments were set-up on groups, thus, for group A reactors we have A_1, A_2, A_3, A_4, A_5 and A_6; for group B reactors we have B_1, B_2, B_3, B_4, B_5 and B_6; for group C reactors - C_1, C_2, C_3, C_4, C_5 and C_6; for group D reactors - E_1, E_2, E_3, E_4, E_5 and E_6; for group F reactors - F_1, F_2, F_3, F_4, F_5 and F_6; and finally, for group G reactor we have G_1, G_2, G_3, G_4, G_5 and G_6. Equal volume of the crude oil (heavier hydrocarbon) and microbes were introduced into the different reactors set-up. The various microorganisms introduced into reactors were allowed to feed on the heavier hydrocarbon and each of the experimental unit set up was sampled at the intervals of two weeks for a period of 3 months.

The final mass of the bioreactors were measured to ensure that equal mass was introduced into each reactor and the reactors were then close to avoid escape of gases produced due to the activities of the microorganisms and the pressure build up was determined experimentally by connecting a tube into the pressure measuring instrument known as single leg manometer. After this process each of the unit was sampled as mentioned above, that is the experimental unit will be opened to allow the escape of gases produced as a result of microbial action for each of the bioreactor as well as the water produced also removed. And the heavier hydrocarbon left and the population were measured to ascertain the heavier hydrocarbon concentration as well as the microbial population. The investigation was conducted for the various experimental unit set-up using various species of microorganisms isolated and identified from the crude oil samples. The experiment of the impact of microbial characteristics on recovery was set-up in the Department of chemical/Petrochemical Engineering laboratory of Rivers State University of Science and Technology, Nkpolu, Port Harcourt.

The Model

Conceptualization Model of MEOR

Development of detailed mathematical models for MEOR is a uniquely challenging task, not only as a result of the inherent complexity of the microbes, but also because of the variety of physical and chemical variable that control their behavior in subsurface porous media. The most important point made by the authors is that the chemical reaction engineering of the microbial process imposes quite severe constants. These are expressed by the relation between the residence time of the bacteria in a cylindrical reaction region of radius r and depth h and porosity q, which is

$$\tau_{res} = r^2 \ mh\phi \left(1 - S_{OR}\right)/Q \qquad (1)$$

where, Q is the volumetric flow rate, and S_{OR} is the residual oil saturation, and the time τ_{rxn}, required for the microbial reaction to produce a desired concentration Greg of some metabolite C_{req} from nutrient N, according to the stoichiometric relationship.

$$N \rightarrow v_N C \qquad (2)$$

To estimate the reaction time, the authors assumed isothermal plug flow through the reactor, that consumption of *N* is first order and irreversible, and that it is injected at initial concentration no. The rate equation is

$$\frac{dc}{dt} = -v_N \frac{dn}{dt} = k_1 v_N^n \qquad (3)$$

where the stoichiometric coefficient v_N defines the conversion efficiency of nutrient into product. When integrated subject to the initial condition n (0) = n_0.

$$n = n_o e^{-k_1 t} \qquad \Rightarrow \frac{dn}{dt} = k_1 n_0 e^{-k_1 t} \qquad (4)$$

The kinetic equation for c is therefore

$$\frac{dc}{dt} = v_N k_1 n_0 e^{-k_1 t} \qquad (5)$$

which, when integrated subject to the initial condition c(0) = 0, gives:

$$C = v_N k_1 n_0 \left[\frac{e^{-k_1 t}}{k_1}\right]_0^1 = v_N n_0 \left[1 - e^{-k_1 t}\right] \qquad (6)$$

The limiting state implied by this equation is complete consumption of the nutrient, and from this result, the reaction time needed to establish the desired concentration C_{req} is

$$C_{req} = v_N n_0 \left[1 - e^{-k_1 t} rxn\right] \Rightarrow \tau_{rsn} = \frac{1}{k_1} in\left[1 \frac{C_{req}}{v_N n_0}\right] \qquad (7)$$

Logistic Growth of Bacteria

Assuming that the consumption of nutrient is proportional to the product of the microorganisms and nutrient concentrations (which is the law of mass action), and that the growth rate of the bacteria is also proportional to product of nut rent and

microorganisms concentrations, it is possible to solve the coupled differentia equations for both concentrations. In particular, it is possible to show that the sum of these concentrations is constant. Thus, if M and N are the respective concentrations of microorganisms and nutrients, k is the growth rate of microorganisms per unit concentration of nutrient, and the initial concentrations arc $M_o M(O)$ and N_o N(O), the law of mass action leads to the differential equations:

$$\frac{dM}{dt} = KMN, \quad \frac{dN}{dt} = KMN \qquad (8)$$

From which it is obvious that

$$\frac{dM}{dt} + \frac{dN}{dt} = 0 \qquad (9)$$

Or $M + N = M_o + N_o = C$ (10)

This last relation can be used to express N in terms, of M, leaving the single differential equation.

$$\frac{dM}{dt} = KM(C - M) \qquad (11)$$

$$\int_{M_o}^{M} \frac{dM}{M(C - M)} = kt \qquad (12)$$

and since

$$\int_{M_o}^{M} \frac{dM}{M(C-M)} \, dM = \frac{1}{C} \int_{M_o}^{M} \left[\frac{1}{M} + \frac{1}{C-M} \right] dM = \frac{1}{C} \, In \, \frac{M}{C-M} - In \frac{M_o}{C-M_o} \qquad (13)$$

Simple algebra leads to the solution

$$M = C \frac{1}{1 + (N_0 / M_0) e^{-KCt}} \quad N = C \frac{(N_o / M_o) e^{-kCt}}{1 + (N_o / M_o) e^{-kCt}} \qquad (14)$$

Thus, the bacterial concentration is limited •by the nutrient concentration, which is the essence of the logistic growth law. This solution can be verified by differentiation:

Monod Equation

Simulators of MEOR nearly always rely on the Monod equation, which expresses the rate of bacterial growth in terms of the substrate or nutrient concentration. In terms of the above notation, this is:

$$\frac{dM}{dt} = \left(\frac{dM}{dt} \right)_{max} \frac{N}{K + N} \qquad (15)$$

where K is a constant, which is essentially the same as the Michaelis Menten equation for enzyme kinetics. In order to get an expression for either the nutrient or the bacteria concentration as a function of time, it is necessary to impose the condition that the sum of the bacteria and nutrient concentrations is constant. But the above derivation shows that this result is an immediate consequence of the law of mass action. One practical limitation of the Monod equation is that the usual procedure for the estimation of the maximum growth rate and the constant K relies on estimation of the reaction rate, which must be obtained from the reaction extent (derived by chemical analysis) by some form of numerical differentiation. A further limitation is that its integrated form is an implicit, rather than explicit expression for the reaction extent. This is best demonstrated by the classical derivation by Brigs and Haldane (1925) of the Michaelis-Menten equation for the kinetics of the conversion of substrate S to product P catalyzed by enzyme E. Denoting the respective

concentrations of these species by S, P and B, the forward and reverse rate constants for the enzyme- substrate combination.

$$E + S \frac{K_1}{K_{-1}} ES \xrightarrow{k_2} P \qquad (16)$$
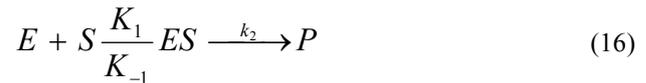
as k_1 and k_{-1} respectively, and the rate constant for the irreversible decomposition of the adduct ES as k_2, and the concentration of this adduct as A application of the law of mass action gives

$$\frac{dP}{dt} k_2 A, \quad \frac{dA}{dt} = k_1, \, ES - (k_{-1} + k_2) A \qquad (17)$$

If the steady state hypothesis is valid, the derivative of A can be set equal to zero, resulting in

$$A = \frac{K_1}{K_{-1} + K_2} \quad ES \cong \frac{1}{K_m} ES \qquad (18)$$

and since the total concentration of enzyme in the system is the sum of A and E,

$$E_0 = E + \frac{1}{K_m} ES \rightarrow E = E_0 \bigg/ \left(1 + \frac{S}{K_m}\right) = E_0 \frac{K_m}{K_m + S} \qquad (19)$$

The rate of production of P is therefore, and since the equation for the overall process is S → F, this is equivalent to the differential equation

$$\frac{dS}{dt} = -\frac{k_2 E_0 S}{K_m + S'} = \frac{k_2' S}{K_m + S} \qquad (20)$$

This is clearly of the same form as the Monod equation (19), but the above derivation shows that this analytical form results from mechanistic considerations that might not necessarily apply to bacterial growth. Now when this equation is integrated from the initial condition S(0) S_0, the result is the transcendental equation:

$$\int_{S_0}^{S} \left(1 + \frac{K_m}{S}\right) dS = S - S_0 + K_m \, In \, \frac{S}{S_0} = -k_2' t \qquad (21)$$

Although this equation cannot be solved analytically, it numerical solution present difficulties. It can be visualized as the point of intersection of the graph of the logarithmic function km In (S_o/S) (which crosses the S axis at S - S_o), with the family of parallel lines $S - S_o + k_2't$ (which also passes through the point (S_o, S) for t 0). For higher values of t, the intersection point becomes closer to the vertical axis S = 0, which corresponds to complete transformation of the substrate at t = ∞ .

In the most general case, one can expect both types of kinetic laws to be important. On one hand, application of the law of mass action to microbial populations results in the linear logistic equation, and on the other, application of the Jaw of mass action (and the crucial steady-state approximation) to an enzyme- catalyzed process results in the Michaelis-Menten (Monod) equation. The relation between these two kinetic models becomes important when considering the production of metabolites - such as biosurfactants - by bacteria. If one assumes that the steady-state hypothesis applies to the rate-limiting step in the enzymatic biosynthesis of the surfactant, the kinetics can be expected to follow the Michaelis-Menten equation. But since the bacteria can be expected to multiply at the same time, this means that the total enzyme concentration E_0, which was regarded as con stunt in the above derivation will vary with time. If the nutrient is growth- limiting and the generation of the byproduct is fast compared with the

multiplication rate of the bacteria, the rate of the former process can be expected to increase as the total concentration of enzyme in the system increases. In the light of these considerations, it is clear that the design of an MEOR process relying on in situ biosurfactant production will require carefully controlled experimentation to determine the specific growth rate as well as the Michaelis-Menten parameters of the rate-limiting enzyme reaction.

Substrate Kinetics

Considering the concentration of substrate varies with time as cell growth continues, consequently a material balance on molar concentration of a given component can be presented as:

$$\frac{d(V_c S)}{dt} = V_c R_s \qquad (22)$$

where V_c = the cultured volume
S = substrate concentration

If there is no change in volume due to additive or gas stripping, then V_c is constant. Hence equation (22) reduces to:

$$\frac{dS}{dt} = R_S \qquad (23)$$

Using equation (23), one can determine the overall rate of reaction by measuring the change in concentration of S with time in a batch bioreactor. It is also necessary, for effective reaction system, adequate temperature control is required so as to maintain isothermal system at the desired temperature. Under these condition, in a steady state a component material balance can be presented as:

$$F(S_{in} - S_t) + V_c R_S = 0 \qquad (24)$$

where F is volumetric flow rate for both the influent and the effluent.

S_{in} is influent substrate concentration
S_t is concentration of substrate at any time.

From equation (24) the rate of formation can be determined as

$$R_s = \frac{F}{V_c}(S_t - S_{in}) = D(S_t - S_{in}) \qquad (25)$$

where $\frac{F}{V_c}$ = D = dilution rate.

RESULTS AND DISCUSSION

The results obtained from the investigation are presented in tables and Figures. The evaluation concept to determine the functional parameters is shown in equation (26) below, which is known as the general material balance for the investigation:

$$\begin{bmatrix} \text{mass of heavier} \\ \text{hydrocarbon} \\ \text{mixture} \\ \text{introduced into} \\ \text{to the bioreactor} \end{bmatrix} = \begin{bmatrix} \text{mass of lighter} \\ \text{hydrocarbon} \\ \text{produced in the} \\ \text{bioreactor} \end{bmatrix} + \begin{bmatrix} \text{mass of water} \\ \text{produced in the} \\ \text{bioreactor} \end{bmatrix} + \begin{bmatrix} \text{mass of gas} \\ \text{production in} \\ \text{the bioreactor} \end{bmatrix} - \begin{bmatrix} \text{mass of} \\ \text{batch} \\ \text{reactor} \end{bmatrix}$$

(26)

where AHHC – reactor A heavier hydrocarbon concentration, ALHC – reactor A lighter hydrocarbon concentration, AGC - reactor A gas concentration, and AWC - reactor A water concentration. The result presented in Figure 1, illustrates the heavier hydrocarbon utilization which yielded lighter

hydrocarbon, water, gases and other components. From Figure 1, it is observed that decrease in the heavier hydrocarbon was observed with increase in lighter hydrocarbon, water, gases and other components concentration.
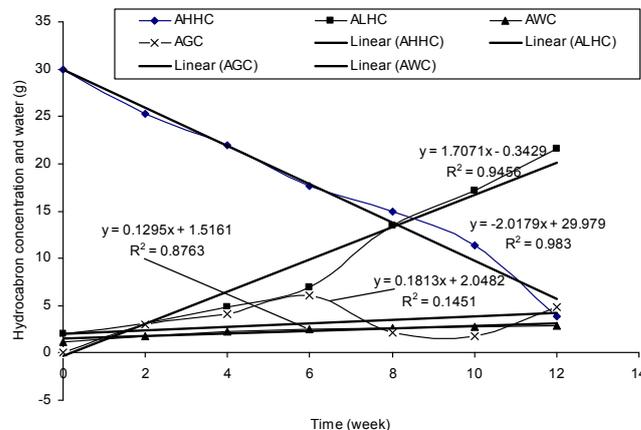
Figure 1: Graph of hydrocarbon concentration and water versus time for reactor (A)

The various in heavier hydrocarbon, lighter hydrocarbon, water, gases etc can be attributed to the variation in biomass concentration and time. The equation of the best fit and square root of the expressions are written as follows, for reactor A of heavier hydrocarbon concentration (AHHC), y = -2.0179x + 29.979, and R^2 = 0.983; for reactor A of lighter hydrocarbon concentration (ALHC) y = 1.707x – 0.3428 with R^2 = 0.9455; for reactor A of water concentration (AWC) y = 0.1813x + 2.0482 with R^2 = 0.1451 and for reactor A of gas concentration (AGC), y = 0.1295x + 1.5161 with R^2 = 0.8763. The microorganisms used for reactor A is known as *pseudomonas sp*.
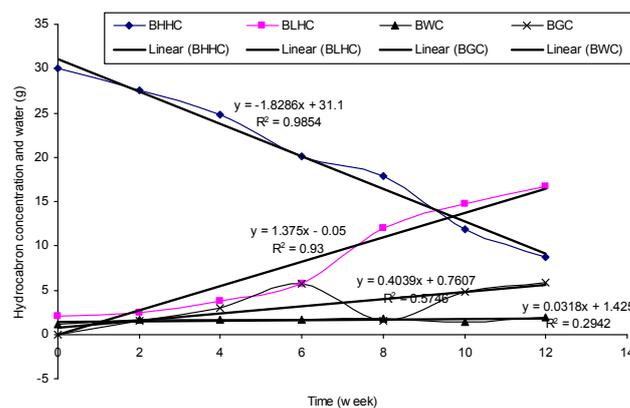
Figure 2: Graph of hydrocarbon concentration and water versus time for reactor (B)

where BHHC – reactor B heavier hydrocarbon concentration, BLHC – reactor B lighter hydrocarbon concentration, BGC - reactor B gas concentration, and BWC - reactor B water concentration. Figure 2, illustrate the relationship among heavier hydrocarbon, lighter hydrocarbon, gases, and water concentration with respect to time. The variation in the heavier, lighter hydrocarbon, gases and water can be attributed to the variation in biomass concentration and time. As the concentration of the heavier hydrocarbon decreases the concentration of lighter hydrocarbon, gases and water increase

Table 1a: Experimental analysis results of physiochemical parameters and microbial population of the different bioreactor

Time	Reactor	pH	TDS (mg/l)	OD (mS/cm)	BOD (mg/l)	COD (mg/l)	DO (mg/l)	CH (mg/l)	NT (mg/l)	PP (mg/l)	SP (mg/l)	RI (mg/l)	Microbial species	Microbial population
0	A B C D E F G	5.64	171.0	1.0	332.0	249.0	19.62	3.51	210.7	118.30	15.9	212.50	*Pseudomonas sp* *Streptococcus* *Estcherichia coli Chromobacerium* *Bacillus* *Flavobaclerium* *Micrococcus*	102×10^3
2	A	5.90	183.0	1.1	352.4	256.3	18.5	3.50	202.1	105.0	96.7	217.0	*Pseudomonas sp*	93.0×10^4
	B	5.80	180.6	1.0	341.5	240.1	22.7	3.04	210.2	114.6	113.0	212.8	*Streptococcus*	50.1×10^4
	C	5.81	194.3	0.9	347.1	250.8	19.41	3.48	209.5	118.10	114.5	220.0	*Estcherichia coli Chromobacerium*	53.7×10^4
	D	5.52	204.0	0.8	350.0	252.0	20.00	3.56	208.0	102.4	110.6	218.6	*Bacillus*	36.1×10^4
	E	5.00	173.0	1.3	338.3	261.8	19.55	3.71	205.7	99.5	108.1	220.6	*Flavobaclerium*	72.5×10^4
	F	5.87	172.5	1.1	337.2	250.1	18.87	2.86	207.4	117.0	113.8	218.9	*Micrococcus*	51.3×10^4
	G	5.33	198.1	0.8	340.7	263.0	19.53	3.47	207.1	116.3	98.7	219.5		40.7×10^4
4	A	4.53	236.0	1.6	360.0	268.0	18.1	3.60	183.4	93.7	90.4	235.0	*Pseudomonas sp*	102×10^{60v}
	B	5.10	200.0	1.5	353.5	254.3	25.0	3.00	199.0	102.1	100.0	227.4	*Streptococcus*	75×10^6
	C	5.30	245.0	1.2	348.6	255.1	19.02	3.10	193.5	81.5	946	225.0	*Estcherichia coli Chromobacerium*	77×10^6
	D	4.98	266.4	0.9	342.1	257.6	21.16	3.27	175.0	80.9	91.7	238.8	*Bacillus*	53×10^6
	E	5.05	196.3	1.0	346.1	260.0	19.00	3.85	188.0	71.7	85.3	241.9	*Flavobaclerium*	88×10^6
	F	5.72	201.5	0.9	341.3	258.0	18.31	3.12	186.7	96.1	92.5	239.3	*Micrococcus*	81×10^6
	G	5.80	233.8	1.2	346.0	271.0	20.4	3.31	190.0	94.5	69.0	246.0		63.0×10^6

Table 1b: Experimental analysis results of physiochemical parameters and microbial population of the different bioreactor

Time	Reactor	pH	TDS	OD	BOD	COD	DO	CH	NT	PP	SP	RI	Microbial species	Microbial population
6	A	4.30	257.3	1.3	362.5	263.0	17.3	17.3	2.47	142.5	91.0	230.1	*Pseudomonas sp*	271.0×10^6
	B	4.92	226.0	1.7	355.0	257.8	19.01	19.01	2.83	152.7	92.9	257.3	*Streptococcus*	145.7×10^6
	C	5.00	259.0	1.0	349.8	262.1	18.8	18.8	2.95	163.0	75.0	230.1	*Estcherichia coli*	201.0×10^6
	D	4.57	270.1	1.3	340.3	259.4	17.4	17.40	3.18	160.1	72.8	257.3	*Chromobacerium*	99.3×10^6
	E	4.73	204.7	1.2	348.0	265.2	16.95	16.95	3.42	175.6	60.1	250.0	*Bacillus*	227.0×10^6
	F	5.20	231.0	1.5	343.7	249.7	17.14	17.14	2.72	168.0	90.3	252.8	*Flavobaclerium*	212×10^6
	G	5.41	268.2	1.3	341.4	273.1	18.05	18.05	3.15	171.5	92.0	258.1	*Micrococcus*	75×10^6
8	A	4.27	267.4	1.2	360.9	253.4	17.20	2.55	96.4	70.1	74.0	252.1	*Pseudomonas sp*	71.0×10^7
	B	4.83	281.0	0.9	371.0	267.0	18.41	2.98	109.0	83.8	61.8	241.8	*Streptococcus*	43.8×10^7
	C	4.92	288.3	0.7	354.0	271.8	18.96	3.37	101.5	71.2	70.4	240.5	*Estcherichia coli*	54.1×10^7
	D	4.61	300.0	0.5	362.7	256.3	19.30	2.00	99.3	70.4	73.2	275.1	*Chromobacerium*	12.8×10^7
	E	4.54	246.1	1.1	350.8	268.9	15.50	1.96	110.0	52.7	52.7	268.0	*Bacillus*	65.0×10^7
	F	5.01	291.4	1.4	347.0	253.7	17.06	2.51	98.7	75.0	75.0	272.6	*Flavobaclerium*	63.5×10^7
	G	5.35	273.1	1.3	350.0	261.2	18.13	2.74	97.1	81.5	81.5	270.8	*Micrococcus*	40.2×10^7
10	A	4.20	273.0	1.10	381.3	288.5	17.63	2.40	52.6	43.7	52.1	293.7	*Pseudomonas sp*	24.3×10^8
	B	4.77	297.9	0.95	374.1	273.0	17.78	2.51	35.1	58.4	47.3	284.1	*Streptococcus*	12.8×10^8
	C	4.98	305.7	0.8	349.4	284.1	18.06	3.14	73.0	53.6	42.0	271.0	*Estcherichia coli*	18.0×10^8
	D	4.52	323.6	0.6	370.0	271.3	17.31	2.10	87.2	60.1	50.8	290.4	*Chromobacerium*	10.3×10^8
	E	4.37	275.0	0.96	358.6	269.7	16.53	2.00	105.3	31.0	40.7	282.9	*Bacillus*	20.7×10^8
	F	4.87	319.0	1.21	349.3	266.8	17.00	2.45	63.1	60.8	50.0	300.5	*Flavobaclerium*	19.6×10^8
	G	5.10	281.9	1.12	353.0	273.1	17.95	2.28	58.5	75.6	47.1	313.8	*Micrococcus*	11.3×10^8
12	A	4.13	312.7	1.5	396.0	297.3	17.04	2.27	20.71	28.3	18.6	371.6	*Pseudomonas sp*	172.4×10^8
	B	4.26	302.0	1.2	382.7	275.0	17.25	2.44	42.3	30.6	26.3	352.0	*Streptococcus*	130.2×10^8
	C	4.51	327.4	1.10	354.0	291.6	18.00	3.02	34.7	32.4	20.7	263.1	*Estcherichia coli*	142.0×10^8
	D	4.48	351.0	0.9	361.5	276.8	17.01	2.07	30.4	41.0	25.7	335.3	*Chromobacerium*	115×10^8
	E	4.21	377.0	1.3	358.1	274.3	16.42	1.95	58.6	39.7	30.1	366.2	*Bacillus*	163.7×10^8
	F	4.80	346.1	1.08	360.3	270.2	16.77	2.23	25.1	31.4	21.0	348.5	*Flavobaclerium*	154.1×10^8
	G	5.01	336.2	1.0	371.6	284.1	17.48	2.11	22.8	40.1	24.8	339.2	*Micrococcus*	125.3×10^8

Table 2a: Experimental analysis results for some useful component during heavier hydrocarbon degradation by microorganisms

Time (week)	Reactor	Heavier hydrocarbon concentration (mg)	Lighter hydrocarbon concentration (mg)	Water concentration (mg%)	Gas concentration (mg%)	Reaction weight (g)	Total reactor weight	Microbial species	Microbial population
0	A							*Pseudomonas sp*	
	B							*Streptococcus*	
	C							*Estcherichia coli*	
	D	30.0	2.00	1.20	0.00	15.00	48.2	*Chromobacerium*	102×10^3
	E							*Bacillus*	
	F							*Flavobaclerium*	
	G							*Micrococcus*	
2	A	25.3	3.1	1.80	3.00	15.00	48.2	*Pseudomonas sp*	93.0×10^4
	B	27.6	2.5	1.62	1.48			*Streptococcus*	50.1×10^4
	C	26.5	2.8	1.83	2.07			*Estcherichia coli*	53.7×10^4
	D	29.2	2.41	1.95	0.36			*Chromobacerium*	36.1×10^4
	E	25.8	2.9	1.75	2.75			*Bacillus*	72.5×10^4
	F	27.1	2.6	1.70	1.80			*Flavobaclerium*	51.3×10^4
	G	28.6	2.46	1.58	0.56			*Micrococcus*	40.7×10^4
4	A	21.9	4.9	2.30	4.10	15.00	48.2	*Pseudomonas sp*	102×10^{60v}
	B	24.8	3.7	1.67	3.03			*Streptococcus*	75×10^6
	C	24.1	4.1	1.83	3.17			*Estcherichia coli*	77×10^6
	D	25.5	3.5	2.41	1.79			*Chromobacerium*	53×10^6
	E	23.7	4.3	1.90	3.30			*Bacillus*	88×10^6
	F	24.5	3.9	1.73	3.07			*Flavobaclerium*	81×10^6
	G	25.2	3.8	1.63	2.57			*Micrococcus*	63.0×10^6

Table 2b: Experimental analysis results for some useful component during heavier hydrocarbon degradation by microorganisms

Time (week)	Reactor	Heavier hydrocarbon concentration (mg)	Lighter hydrocarbon concentration (mg)	Water concentration (mg%)	Gas concentration (mg%)	Reaction weight (g)	Total reactor weight	Microbial species	Microbial population
6	A	17.6	7.0	2.5	6.10			*Pseudomonas sp*	271.0×10^6
	B	20.1	5.7	1.73	5.67			*Streptococcus*	145.7×10^6
	C	20.3	6.3	1.85	4.75			*Estcherichia coli*	201.0×10^6
	D	21.9	5.0	2.67	3.63	15.00	48.2	*Chromobacerium*	99.3×10^6
	E	16.0	6.8	2.02	8.38			*Bacillus*	227.0×10^6
	F	19.4	5.9	1.81	6.09			*Flavobaclerium*	212×10^6
	G	22.8	5.3	1.70	3.40			*Micrococcus*	75×10^6
8	A	15.0	13.5	2.6	2.1			*Pseudomonas sp*	71.0×10^7
	B	17.9	12.0	1.81	1.49			*Streptococcus*	43.8×10^7
	C	18.5	12.5	1.90	0.3			*Estcherichia coli*	54.1×10^7
	D	18.7	11.6	1.78	1.12	15.00	48.2	*Chromobacerium*	12.8×10^7
	E	15.3	12.9	2.75	2.35			*Bacillus*	65.0×10^7
	F	17.8	12.3	1.88	1.22			*Flavobaclerium*	63.5×10^7
	G	18.2	11.5	1.81	1.69			*Micrococcus*	40.2×10^7
10	A	11.4	17.2	2.8	1.8			*Pseudomonas sp*	24.3×10^8
	B	11.8	14.8	1.86	4.74			*Streptococcus*	12.8×10^8
	C	13.6	15.3	1.96	2.35			*Estcherichia coli*	18.0×10^8
	D	12.9	15.0	2.81	2.49	15.00	48.2	*Chromobacerium*	10.3×10^8
	E	11.7	15.1	2.20	4.2			*Bacillus*	20.7×10^8
	F	12.0	14.0	1.90	5.3			*Flavobaclerium*	19.6×10^8
	G	12.5	14.6	1.92	4.18			*Micrococcus*	11.3×10^8
12	A	3.9	21.6	2.85	4.85	15.00	48.2	*Pseudomonas sp*	172.4×10^8
	B	8.7	16.7	1.92	5.88			*Streptococcus*	130.2×10^8
	C	7.3	17.5	2.01	6.39			*Estcherichia coli*	142.0×10^8
	D	10.1	16.0	3.42	3.68			*Chromobacerium*	115×10^8
	E	4.5	18.1	2.26	8.34			*Bacillus*	163.7×10^8
	F	8.4	17.2	1.95	5.65			*Flavobaclerium*	154.1×10^8
	G	9.6	16.5	2.28	4.82			*Micrococcus*	125.3×10^8

with increase in time. The equation of the best fit and the square root of the equation is shown in Figure 2. The microorganism used for conducting the investigation in reactor B is known as streptococcus.
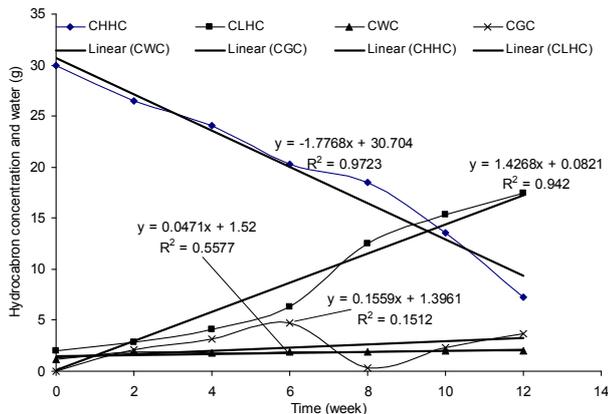
Figure 3: Graph of hydrocarbon concentration and water versus time for reactor (C)

where CHHC – reactor C heavier hydrocarbon concentration, CLHC – reactor C lighter hydrocarbon concentration, CGC - reactor C gas concentration, and CWC - reactor C water concentration.

The equations of the best fit and the square root of the equations were established as shown in Figure 3. From Figure 3 it is observed that the concentration of the heavier hydrocarbon decreases with increase in lighter hydrocarbon concentration, gas concentration and water concentration as the time increases. The variation in the concentration of the component as shown in Figure 3 can be attributed to the variation in time. The microorganism used in conducting the investigation is known as *Escherichia coli*.
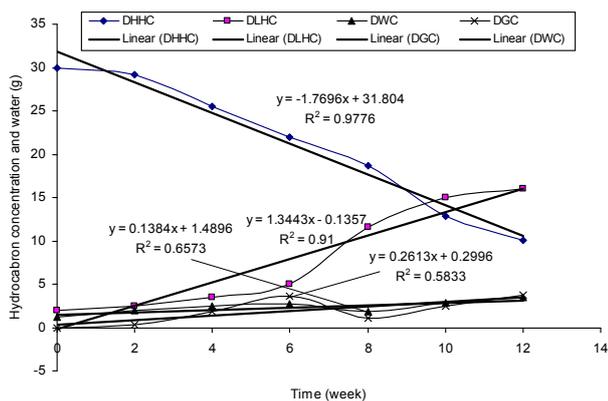
Figure 4: Graph of hydrocarbon concentration and water versus time for reactor (D)

where DHHC – reactor D heavier hydrocarbon concentration, DLHC – reactor D lighter hydrocarbon concentration, DGC - reactor D gas concentration, and DWC - reactor D water concentration.

Figure 4 illustrates the change in heavier hydrocarbon concentration with changes in time. The variation in the heavier, lighter hydrocarbon concentration, gas and water concentration can be attributed to variation in time. Decrease

in heavier concentration was observed with increase in lighter hydrocarbon, gas and water as well as increase in biomass concentration and time. The equation of the best fit and the square root of the equations were established as shown in Figure 4. The microorganism used in conducting the investigation is known as *chromobacterium*.
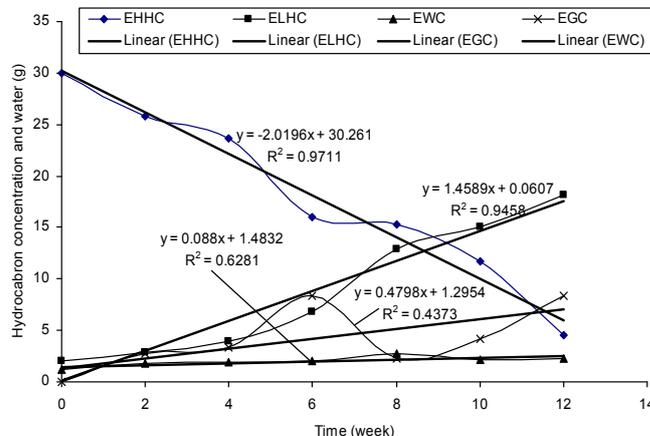
Figure 5: Graph of hydrocarbon concentration and water versus time for reactor (E)

where EHHC – reactor E heavier hydrocarbon concentration, ELHC – reactor E lighter hydrocarbon concentration, EGC - reactor E gas concentration, and EWC - reactor E water concentration.

The result presented in Figure 5 illustrates the variation in the heavier and lighter hydrocarbon, gases, water with variation in biomass concentration and time. The heavier hydrocarbon decreases with increase in the rate of production of lighter hydrocarbon, gases, water, and biomass concentration with increase in time. The equation of the best fit and the square root of the equation were established as shown in Figure 5. The microorganism used in conducting the investigation is known as *Bacillus*.
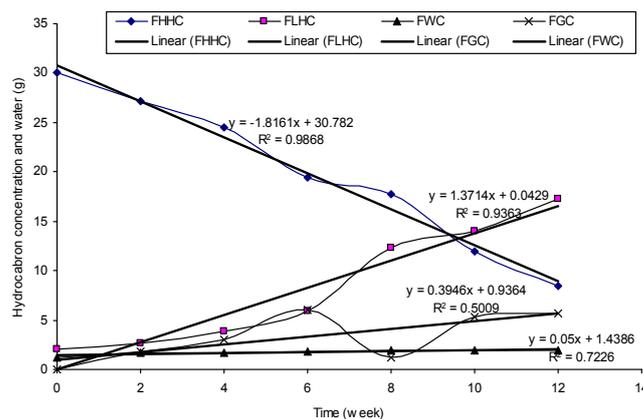
Figure 6: Graph of hydrocarbon concentration and water versus time for reactor (F)

where FHHC – reactor F heavier hydrocarbon concentration, FLHC – reactor F lighter hydrocarbon concentration, FGC - reactor F gas concentration, and FWC - reactor F water concentration. Figure 6, illustrate the relationship among heavier hydrocarbon, lighter hydrocarbon, gases, and water concentration with respect to time. The variation in the

heavier, lighter hydrocarbon, gases and water can be attributed to the variation in biomass concentration and time. As the concentration of the heavier hydrocarbon decreases the concentration of lighter hydrocarbon, gases and water increase with increase in time. The equation of the best fit and the square root of the equation is shown in Figure 6. The microorganism used for conducting the investigation in reactor B is known as *flavobacterium*.
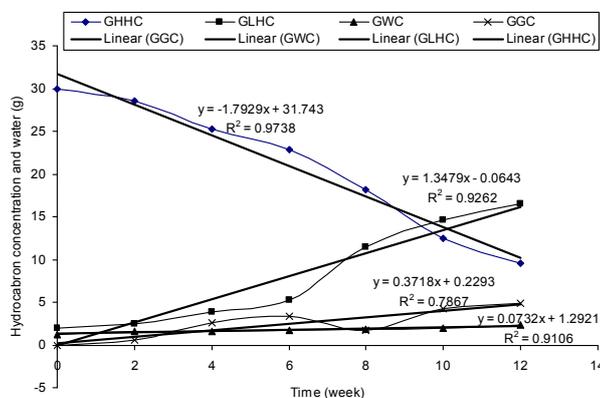
Figure 7: Graph of hydrocarbon concentration and water versus time for reactor (G)

where GHHC – reactor G heavier hydrocarbon concentration, GLHC – reactor G lighter hydrocarbon concentration, GGC - reactor G gas concentration, and GWC - reactor G water concentration.

Figure 7 illustrates the change in heavier hydrocarbon concentration with changes in time. The variation in the heavier, lighter hydrocarbon concentration, gas and water concentration can be attributed to variation in time. Decrease in heavier concentration was observed with increase in lighter hydrocarbon, gas and water as well as increase in biomass concentration and time. The equation of the best fit and the square root of the equations were established as shown in Figure 7. The microorganism used in conducting the investigation is known as *chromobacterium*.

Conclusion

The following conclusions were drawn from the investigation conducted such as:

- The various microorganisms isolated and identified from the oil wells in Niger Delta Area of Nigeria are capable of improving the dead oil wells.
- The degree of biomass build up in the reserviour depends on the concentration of the physicochemical properties of the well.
- The rate of production of the lighter hydrocarbon, gases and other useful components are dependent of biomass concentration as well as the heavier hydrocarbon concentration.
- The pressure rise changes due to biomass concentration with increase in time.
- Increase in biomass build up depends on the favourable conditions of the reservoir or oil well.

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