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INVESTIGATING THE GROWTH AND TRANSPORT OF MICROORGANISMS IN A MICROBE-FLOODED BOUNDED RESERVOIR

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ABSTRACT

Microbial Enhanced Oil Recovery (MEOR) is a family of processes that involves the use of selected microbes to achieve enhancement of oil production. The microbial system is carefully designed to produce bioproducts capable of making positive alterations to the inherent rock and fluid properties. The overall efficiency of this technology is hinged on the ability of the injected microbes to multiply and be transported deep into the subject reservoir. This work presents a study on the growth rate and transport of Bacillus Substillis in a bounded reservoir core model, incorporating mathematical models and experimental studies. Results of the experimental study indicate that nutrient concentration is the decisive factor in the growth of the choice bacteria. A Comparison of the growth rate predicted from the logistic growth model and that predicted by the Monod equation is also presented.

Keywords : Bacillus Substillis; MEOR; Bacteria growth;Monod

1 INTRODUCTION

The overall improvement of the enhanced stage of oil recovery has for long been of great interest to the oil industry. In microbial enhanced oil recovery (MEOR), the growth and transport of microorganisms has always played an important role in the oil recovery process. The growth of the microbes has to do with the ability of the microbes to feed and grow under bounded reservoir conditions, while transport involves the ability of the microbes to migrate to other parts of the reservoir under prevailing conditions [1]. The reservoir physicochemical properties in terms of salinity, pH, temperature, pressure are also vital considerations before the final choice of bacteria is made.

1.1 Growth of Microorganisms

Before a particular microorganism is used for MEOR, the bacteria must be able to grow and survive under reservoir conditions. A bacterium growth is very well dependent on nutrient concentration and is often divided into different phases [2].

Phase 1 is the lag phase in which there is little increase in cell concentration because the cells are adjusting to their new environment. The duration of the lag phase depends upon the growth from which the bacterium is taken relative to the reaction medium in which it is placed. Phase 2 is the exponential phase owing to the fact that cells' growth rate is proportional to the nutrient concentration. This phase comes to play after the bacteria have adjusted to their environment. Phase 3 is the stationary phase during which cells reach a minimum biological space where the lack of one or more nutrients limits bacteria cell growth. During this stage, growth rate is zero as a result in depletion of nutrients and essential metabolites. Phase 4 is the death phase where a decrease in live cell concentration occurs. This is as a result of the toxic by-product, harsh environment and/or depletion of nutrients.

Different techniques have been employed in the study of the growth kinetics of bacteria isolated from oil reservoirs. Nan and Tan [3] investigated such growth kinetics by inference from measured thermal power in relation to temperature and Sodium Chloride concentration. A noteworthy feature of this study is its use of the Verhulst model for bacteria growth kinetics, in contrast to the more common use of the Monod equation.

Bryant and Lockhart [4] assumed that the consumption of nutrient is proportional to the product of one bacterial and nutrient concentrations and that the growth rate of the bacteria is also proportional to the product of nutrient and bacterial concentrations. They showed, in particular, that the sum of these concentrations is constant. A similar study by Kim and Fogler [5] examined the formation of biofilm by inference from permeability changes, taking into account the erosion of biofilm due to high shear stresses associated with constricted flow channels. Nematic et al [6] considered permeability modification by microbial precipitation of calcium carbonate.

The influence of water flow on the spatial distribution of microbial growth (Pseudomonas strain) was also investigated by Thullner et al [7]. They found that microbial activity was limited by transverse mixing of the electron-donor (glucose) and acceptor (nitrate) nutrient streams. For this species, extracellular polymeric material was the dominant contributor to the bio-clogging process.

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Fundamental mathematical models for MEOR have been presented by Islam [8] and Zhang et al [9]. The paper of Islam [8] covered 3-dimensional mathematical formulation for bacterial transport. His model is presented as follows:

$$\nabla\left(\frac{C_{wb}KK_{rw}}{\mu_w B_w}\nabla\phi_w\right) + q_w C_{wb} = \frac{\partial}{\partial t}(\varphi S_w \rho_w C_{wb} + \sigma) \quad (1)$$

1.2 Transport of Microorganisms

The transport of microorganisms in bounded reservoirs governs many phenomena in MEOR. The transport process is governed by a host of complicated physical, chemical and biological phenomena such as adsorption, interaction between bacteria and substrate, and growth and decay of cells [10]. An accurate modeling of bacteria transport can be best achieved through an integration of laboratory data with mathematically developed data [8].

Zhang et al [9] made a 1-dimensional mathematical model for bacteria convection and a dispersion equation for species transport. The transport of species through the media is described by:

$$\frac{\partial(\varphi S_w C_s)}{\partial t} = -\frac{\partial(U_f C_j)}{\partial x} + \left(\varphi S_w K_D \frac{\partial c_j}{\partial x}\right) \quad (2)$$

where
U_f = Darcy flux
K_D = Dispersion coefficient
R_j = Net production rate for species, j

Zhang et al [9] also calculated biomass accumulation from a net result of retention, detachment and growth. The biomass detachment rate is a function of the biomass attached on the pore surfaces and the shear force applied between the fluids and the sessile phase.

2 Mathematical Models

2.1 Growth of Microorganisms using the logistic growth model

Assuming that the rate of nutrient consumption by microorganisms in the bounded reservoir is proportional to the product of the bacterial concentrations and the nutrient concentrations (i.e., the law of mass action), we have:

$$\frac{dN}{dt} = -KBN \quad (3)$$

where:
B = Bacterial concentration
N = Nutrient concentration
$\frac{dN}{dt}$ = Rate of nutrient consumption
K = Constant of proportionality which is the growth rate of bacteria per unit concentration of nutrient

Assuming also that the growth rate of the microorganisms (bacteria) in the bounded reservoir is proportional to the product of the nutrient concentration and the bacterial concentration:

$$\frac{dB}{dt} = KBN \quad (4)$$

where
dB/dt = growth rate of the microorganism
These assumptions are resolved to give:

$$B = C \cdot \frac{1}{1+\left(\frac{N_o}{B_o}\right)e^{-kct}} \quad (5)$$

$$N = C \cdot \frac{\left(\frac{N_o}{B_o}\right)e^{-kct}}{1+\left(\frac{N_o}{B_o}\right)e^{-kct}} \quad (6)$$

Thus, the bacterial concentration is limited by the nutrient concentration which is the essence of the logistic growth law.

2.2 Transport of Microorganisms using the Islam [8] model

The transport of microorganisms through porous media is described by the following equation:

$$\nabla\left(\frac{C_{wb}KK_{rw}}{\mu_w B_w}\nabla\phi_w\right) + q_w C_{wb} = \frac{\partial}{\partial t}(\varphi S_w \rho_w C_{wb} + \sigma) \quad (1)$$

where
c_{wb} = concentration of bacteria in the water phase
k = permeability
k_{rw} = relative permeability
μ_w = Water viscosity
B_w = Water formation volume factor
ϕ_w = Fluid potential
s_w = Water saturation
σ = Volume of fines deposit per unit initial pore volume

Bacteria or microorganism capture kinetics is given by:

$$\frac{\partial\sigma_{np}}{\partial t} = -\alpha(\mu_{np} - \mu_c)\sigma_{np} + \beta c \quad (7)$$

where
α=constant
μ = volume flux density
β=constant

From law of conservation of mass:

$$\begin{bmatrix}\text{Rate of accumulation of cells, g/s}\end{bmatrix} = \begin{bmatrix}\text{rate of cells entering, g/s}\end{bmatrix} - \begin{bmatrix}\text{Rate of cells leaving, } \frac{g}{s}\end{bmatrix} + \begin{bmatrix}\text{Net Rate of generation of core cells, g/s}\end{bmatrix} \quad (8)$$

Which is also stated as:

$$V\frac{dC_c}{dt} = v_o C_{co} - vC_c + (r_g - r_d)V \quad (9)$$

The corresponding substrate balance:

$$\begin{bmatrix}\text{Rate of accumulation of substrate, g/s}\end{bmatrix} = \begin{bmatrix}\text{Rate of substrate entering, g/s}\end{bmatrix} - \begin{bmatrix}\text{Rate of substrate leaving, g/s}\end{bmatrix} + \begin{bmatrix}\text{Rate of substrate generators of, g/s}\end{bmatrix} \quad (10)$$

Which is resolved to give:

$$V\frac{dC_s}{dt} = V_o C_{so} - VC_s + r_s V \quad (11)$$

Equation (11) can be rearranged to give the Rate of cell growth as:

$$r_s = \frac{dC_s}{dt} + \frac{1}{V}(vC_s - v_o C_{SO}) \quad (12)$$

The equation is resolved to obtain:

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$$r_g = \frac{C_c}{t} + r_d \quad (13)$$

Where r_g is the growth rate.

3 Materials and Methods

This work presents a practical investigation on the growth and transport of microorganisms in a bounded reservoir using laboratory models. The approach used for the modeling of the growth microorganism is the logistic growth model which is a linear logistic equation that applies a law of mass action to microbial populations. The modeling of the transport of microorganisms in bounded reservoirs makes use of the model presented by Islam in 1990 [8].

3.1 Laboratory Approach

A synthetic whole core sample was used as the bounded reservoir model. The core was simulated using cement to sand ratio of 1:25. A laser particle size analysis was also performed.

TABLE 1
CORE SAMPLE USED IN EXPERIMENT

Parameters	Values
Length of core	14cm
Depth of core	9cm
Width of core	14cm
Surface tension	40mN/m

The crude oil used as the Oleic phase was gotten from Ebocha field, Nigeria. The fluid parameters used in the experiment are given in Table 2 below.

TABLE 2
FLUID PARAMETERS USED IN THE EXPERIMENT

Parameters	Values
Initial oil saturation	0.87
Residual water saturation	0.13
Initial Oil Viscosity	1.9cp
Formation compressibility	0.000018psi^{-1}
Oil compressibility	0.00014psi^{-1}
Injection pressure	28psi
Water Injection rate	1.5cm^3/in.
Permeability	410mD
Initial porosity	0.15
Oil production rate	2cm^3/min

The flooding agent used was distilled water, autoclaved at a temperature of 125°C. Fluid injection was done using an injection syringe. The total volume of the syringe was 30ml and the injection rate was manually controlled. All system tubing was $\frac{1}{8}$ in. outer diameter.

A three-dimensional glass model was used as the reactor. The bioreactor was made a digester as it was air tight. Some researchers have employed image-analyzing techniques for the saturation measurement of micromodels. In this work, a computerized image processing system which consisted of a Computer and a scanner was used to monitor the advancement and subsequent effects of the bacteria on the oil-water fluid system.

The nutrient broth used was prepared by dispensing 13g of broth E powder in 1 litre of deionized water. The mixture was heated to dissolve the powder properly and subsequently sterilized by autoclaving at 121°C for 15min. Table 3 shows the composition of the nutrients.

TABLE 3
NUTRIENT SOLUTION USED IN THIS EXPERIMENT

Parameters	Values
Beef extract	1.0g/L
Yeast extract	2.0g/L
Peptone	5.0g/L
Sodium Chloride	5.0g/L

TABLE 4
BACTERIA AND NUTRIENT PARAMETERS

Parameters	Values
Bacterial Injection rate	0.5cm^3/min
Injected microbial concentration	0.8cell/ml
Bacterial viscosity	0.86cp
Gas produced	0.1ft^3/LBN
Nutrient injected	2.5lb/cu.ft.
Diffusion coefficient for bacteria	0.0055ft^2/min
Diffusion coefficient for nutrient	0.0083ft^2/min
Maximum growth rate	8.4 day^{-1}
Decay rate	0.22day^{-1}
Total volume of bacteria injected	30cm^3
Biomass, σ_{BM}	0.02
Permeability	821mD

The choice of bacteria for the purpose of this work was *Bacillus Subtilis*. The bacterium was collected using the Persian Type Culture (PFCC). *B. Subtilis* was cultured on liquid growth media A and B respectively at a concentration of 0.1g/L. The composition of each growth medium is presented in Table 5.

TABLE 5
COMPOSITION OF GROWTH MEDIUM

Composition	A	B
Sucrose	0	0.5
NH_4Cl	2.0	2.0
Glucose	5.0	0
Peptone	1.0	1.0
Meat infusion	5.0	5.0
Na_2HPO_4	20.0	2.0
Sodium Chloride	0.3	0.25

The bacteria culture was centrifuged at 2000rpm for 30min and collected at a stationary state. The culture was then suspended on autoclaved distilled water. The bacterial suspension was placed on a magnetic stirrer and allowed to mix at

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warm temperature for 8mins. The solution was centrifuged and washed once again with water. The cell density of the bacterial solution was adjusted to 0.8×10^7 cell/cm^3.

3.2 Experimental procedure

The procedure adopted for the experiment is outlined as follows:

a. The glass model (bioreactor) was sterilized using Xylene.
b. It was moist-dried by using self-indicating Silica gel.
c. The core was saturated with water until a desired connate water saturation was reached.
d. It was then saturated with crude oil.
e. The model was placed in an incubator at a steady room temperature of 25°C.
f. Air was then injected at a pressure of 28psi until no more fluid was produced on the effluent reservoir flowline.
g. Water was pumped at a pressure of 28psi until no more oil was produced at the effluent.
h. 11.3% of the pore volume of the mixture of bacterial solution and nutrient solution (50-50) was injected into the model.
i. The model was incubated aerobically for a period of 48hours at a steady ambient temperature of 25°C.
j. Following the solution period, one model was then water-flooded until no more oil was produced at the effluent.

4 Results

4.1 Bacteria Growth Rate

The population of the microbial system used grew to 10^8 times within 4 hours at full supply of nutrients. The equivalent to a maximum growth rate of 8.4 day^{-1}. The death rate of the bacteria increased, determined from population change during the period of 200-400hours at an average rate of 15.022day^{-1}. These results are shown in the table below.

TABLE 6
BACTERIA GROWTH RATE

Time, t (hrs)	Cell concentration, g/cm^3	Nutrient concentration, g/cm^3	r_d, g/day	r_g, g/day
0	0.8×10^{-9}	0.195	0	0
0.5	2.8×10^{-5}	0.145	0	5.6×10^{-3}
1.0	5.31×10^{-3}	0.116	0	5.31×10^{-3}
1.5	6.07	0.097	0	5.38
2.0	6.02	0.087	0.09	4.05
2.5	4.00	0.077	0.21	1.62
3.0	2.00	0.067	0.33	0.90
3.5	0.0	0.057	0.52	0.52
4.0	0.0	0.057	0.85	0.85

The growth rate of r_g for *Bacillus Substillis* was gotten from the model equation (13). Table 7 shows the growth rate calculated using Monod equation.

TABLE 7
BACTERIA GROWTH RATE USING THE MONOD EQUATION

Time, t hrs	Cell concentration, g/cm^3	Nutrient concentration, g/cm^3	r_d, g/day	r_g, g/day
0	0.8×10^{-9}	0.195	0	0
0.5	2.8×10^{-5}	0.145	0	1.10×10^{-5}
1.0	5.31×10^{-3}	0.116	0	3.35×10^{-3}
1.5	6.07	0.097	0	6.38
2.0	6.02	0.087	0.09	5.69
2.5	3.52	0.077	0.21	3.68
3.0	1.72	0.067	0.33	1.89
3.5	0.0	0.057	0.52	0
4.0	0.0	0.057	0.85	0

These results are best illustrated in the figures below.

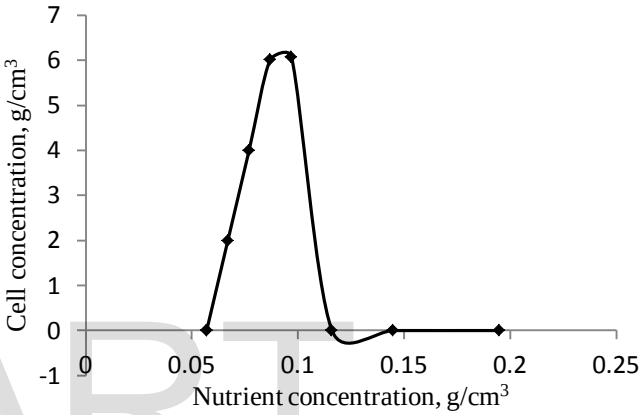

Fig. 1 Variation of cell concentration with nutrient concentration

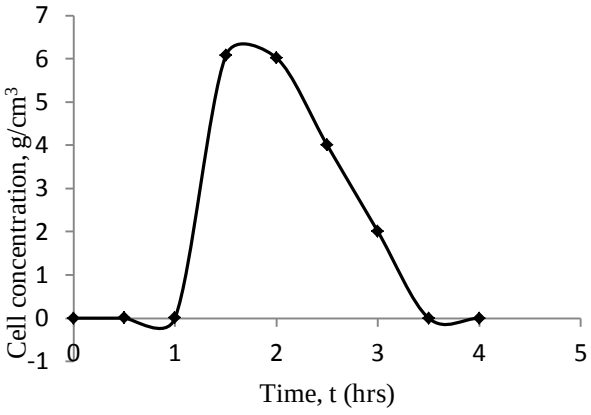

Fig. 2 Variation of Cell concentration with time

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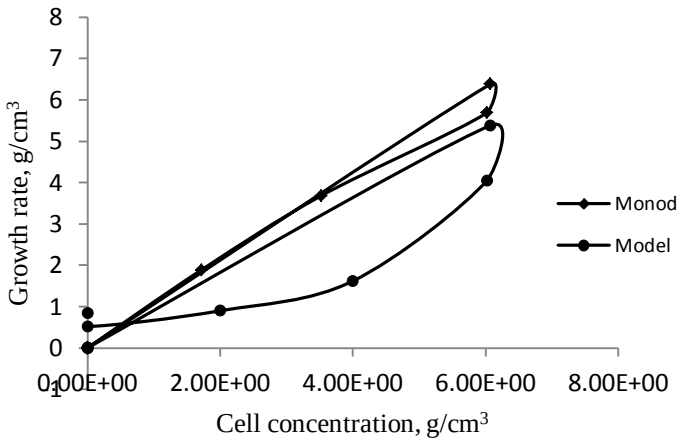

Fig. 3 Comparison of the Variation of growth rate with Cell concentration for the Model and the Monod equation

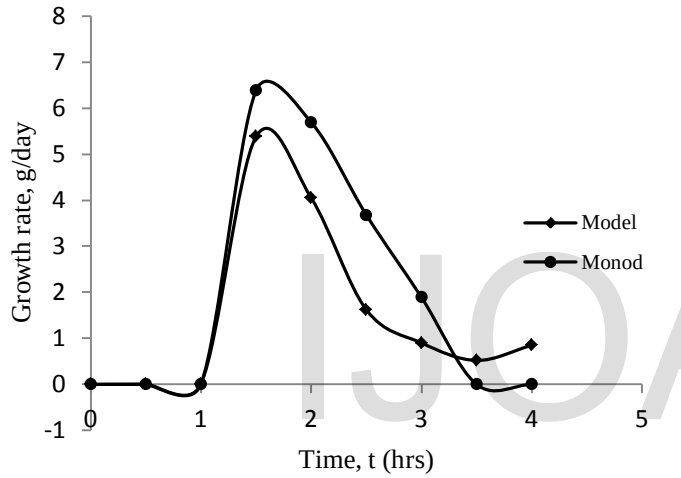

Fig. 4 Comparison of the variation of growth rate with time for Model and Monod equation

It is obvious from these plots that the bacteria growth rate is dependent mainly on nutrient concentration. The close correlation of the model results with the Monod equation is also observed.

4.2 Bacteria Transport Rate

The transport of the *Bacillus Substillis* was determined along the length of the core medium with time. After 4hours of incubation, high cell concentrations were observed and the cells had grown and migrated into other sections of the core. The mechanism of transport observed was a diffusion process.

5 Conclusion

Laboratory experiments were successful in determining some of the input parameters of microbial growth and transport system for one developed mathematical model. The results reveal a strong dependence of bacteria growth rate on nutrient concentration. The unusual complexity of microorganism growth and transport requires close coordination between laboratory mechanistic studies and mathematical model. As such, it is recommended that a simulator incorporating all the complex mechanisms and transport phenomena such as absorption, desorption, chemotaxis etc. The accuracy of such a simulator would be dependent upon the accuracy of the equations used and the level of assumptions involved.

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