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Dynamics Growth of *Bacillus Licheniformis* & *Alcaligeneseutrophus* Bacterial in Gasoline Contaminated Soil, Federal Institute of Industrial Research Oshodi, Lagos, Nigeria

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Abstract: Microbial growth and division are fundamental processes relevant to many areas of life science, of particular interest are homeostasis mechanisms, which buffer growth and division from accumulating fluctuations over multiple cycles. These mechanisms operate within single cells, possibly extending over several division cycles. Experimental studies to date have relied on measurements pooled from many distinct cells. The microbial population analysis was carried out through microbial plate count. By analyzing correlations along up to hundreds of generations, we find that the parameter describing effective cell size homeostasis strength varies significantly among cells. The mixed cultured of Bacillus licheniformis and Alcaligenes eutrophus bacterial were capable of growing at a rate such that the culture doubles in biomass every 2.302 days in Benzene, 2.180 days in Xylene and 2.032 days in Ethylene substrate. Bacillus licheniformis was capable of growing at a rate such that the culture doubles in biomass every 2.3255 days in Benzene and Ethylene while 2.207 days in Xylene. Alcaligenes eutrophus was capable of growing at a rate such that the culture doubles every 2.7305 days in Benzene, 2.398days in xylene and 2.3623 days in ethylene substrate. There was higher competition between consortium of two species than single species of the bacterial organism, the consortium of two species will also enhance the degradation of benzene in aromatic compounds contaminated soil. The Bacillus licheniformis and Alcaligenes eutrophus bacteria can be use for bioremediation of aromatic compounds contaminated soil such as benzene and xylene.

Keywords: *Bacillus licheniformis*, *Alcaligeneseutrophus*, Dynamics Microbiological Growth Kinetics

1. Introduction

'Dynamics' may be defined as a study of forces acting upon a body and in this sense, the 'dynamics of microbial growth' implies a study of those (environmental) forces that act either to promote or to impede the growth of microbes. Studies of microbial growth kinetics had concentrate largely on bacterial species and attention was directed mainly to the aspects of bacterial growth [4]. The capacity to grow and ultimately multiply is one of the most fundamental characteristics of living cells. In fact, with microorganisms it is generally the sole criterion that is used to assess whether or not such creatures are alive. Hence, by definition, a non-viable microbe is one which is incapable of increasing in size and number when incubated in an equitable growth-supporting environment for prolong period often time. The manipulation of quantitative data and their correlation with growth-associated processes frequently demands the

construction of mathematical equations popularly called 'models'. These range from the very simple to the exceedingly complex models. In this paper, connections are established between individual bacterial growth and division, the corresponding distributions of times to division, and population growth curves that in the long term can aid the quantification [6].

A number of modeling approaches for bacterial population dynamics have been built around the concept of times to division and particularly the first lag time to division of cell populations. The development of analytical techniques capable of measuring single-cell parameters such as cell length renewed interest in modeling single-cell growth. Experimental techniques for single-cell studies include turbidimetry, lithographic techniques and flow cytometry. Recently, time-lapse microscopy has been successfully applied to obtain quantitative information on colonial growth dynamics originated from single cells [1].

In this study we determine the dynamics microbial growth of *Bacillus lichenformis* and Alcaligenes eutrophus bacterial.

The objectives were;

(a) To isolate and identify bacteria associated with utilization of gasoline.
(b) To characterize the bacteria.
(c) To determine the Bacillus lichenformis and Alcaligeneseutrophus bacteria doubling time, specific growth rate in xylene, benzene and ethylene substrate.

2. Materials and Methods

The sandy soil used in this experiment was obtained from Federal Institute of Industrial Research Oshodi (FIIRO), Lagos State, Nigeria.1kg of soil sample was collected from two different spots in Federal Institute of Industrial Research Oshodi (FIIRO), Lagos State of Nigeria. Soil was sampled with the sampler Auger of 0-15cm depth. The sample was scrapped into sterile plastic bag and maintained in aseptic conditions. The sample was taken to (FIIRO) Biotechnology laboratory for analysis. On collection, stones and refuse were separated from the soil sample, further separation was carried out using 2 mm mesh size to remove the larger non-sandy fractions from the sandy.500gram of soil sample was spiked with 100ML of gasoline in ratio 5:1, evenly mixed with spatula and spread on a woody rack to dry for two days and it was sieved with a 2mm sieve in other to remove various sizes of stones and refuse [14].

Isolation of soil hydrocarbon degrading bacteria was performed by serial dilution method. 1gram of soil sample was weighed into a MCarthney (sample bottle) bottle and dissolved with 10ml distilled water. 1gram of soil sample was then serially diluted in sterilized distilled water to get a concentration range from 10^{-1} to 10^{-6}.

150ML of Minimal salt medium was put into 250ML conical flask and inoculated with 1gram of the soil sample. Gasoline was sterilized separately before it was added to the medium at 1.0% (v/v). A volume of 0.1ml of each dilution was transferred aseptically to MSA plates (Petri dish). The sample was spread uniformly. The culture was incubated with shaking at 120rev/min and was incubated at 30^{o}C for 7-12 days. The bacteria isolates was further sub cultured to obtain pure culture. Pure isolates on nutrient agar slants was then maintained at 4^{o}Cin a refrigerator to avoid overgrowth [15].

The gasoline was purchased from Mobil Oil filling station in Oshodi, Lagos. The distilled water used for dissolving the soil, washing apparatus was obtained in Federal Institute of Industrial Research Oshodi (FIIRO), Lagos, Nigeria. Hydrogen peroxide $(H_2O_2)3\%(^{W}/_{V})$, a product of Sigma Aldrich, USA, Kovac's reagents, a product of Sigma Aldrich, USA, and gram staining reagents, a product of Sigma Aldrich, USA, were bought from Rochester Silicate Limited chemical store in Mushin, Lagos, Nigeria for biochemical characterization of bacterial isolates [7, 11, 13]. Some of the sterile nutrient agar plates were exposed to air for 30 minutes in order to isolate microbial cultures from environment. The above mentioned procedure for isolation was followed. All morphologically contrasting colonies were purified by repeated streaking. Identification of the genus was based on morphological and biochemical characteristics for *Bacillus* species. All the tests were performed on liquid cultures in late-logarithmic phase.

The Alcaligenes eutrophus and Bacillus licheniformis bacteria was isolated and selected from the mixed cultured, purified and enriched in MSA medium which consist of $Mg_2SO_4.7H_2O$ 0.05g/ML, Na_2HPO_4 1.0g/ML, KH_2PO_4 0.265/ML, NH_4NO_3 2.0g/ML, K_2HPO_4 0.1g/ML, Agar 7.5g/ML, substrate 2% dissolved in 500ML H_2O [8,9].

The methanol solvent manufactured by Sigma Aldrich, USA, was used for extraction of gasoline from soil was bought from a chemical store in Lagos, Nigeria. The Nutrient Agar a product of Himedia laboratories pvt Ltd, used for supporting the growth of bacterial was purchased from Richmond Consultants store, Mushin, Lagos, Nigeria [10, 13].

The culture vessels were '250 ML.' Pyrex bottles with interchangeable stoppers. The 10 conical flasks of 250 ML (bioreactors) at room temperature slightly closed were designated D_1-D_{10} (aerobic). 15 gram of gasoline spiked soil was weighed into each of the bioreactor in a solution of Minimal Salt Medium (MSM) 150 mL and mixed properly at room temperature 30^{0}C ± 3^{0}C and Autoclave at 121^{0}C for 15 minutes. The bioreactor D_2-D_{10} (aerobic) were amended with 3 ML substrate, benzene D_2-D_4; xylene D_5-D_7 and ethylene D_8-D_{10} respectively after cooling and pure culture of Bacillus lichenformis/Alcaligeneeeutrophus bacterial. The bioreactor under treatment D_1 (control) was not amended with any biostimulating agent and microbes. In total, 10 microcosms were settled and incubated for 42 days. All bioreactors were mixed manually through hand stirrer once per week to enhance oxygenation and kept moist during the 42 days experimental duration. This set-up was monitored in aerobic condition, samples were withdrawn at intervals of one week for microbial population analysis through microbial plate count respectively [10, 12, 13].

3. Results and Discussion

Table 1. *Growth potential of hydrocarbon-utilizing bacteria isolatesfrom the gasoline contaminated soil.*

Bacterial Isolates	Xylene		Benzene		Ethylene	
	specific growth rate μ(/d)	Doubling time td(d)	specific growth rate μ(/d)	Doubling time td(d)	specific growth rate μ(/d)	Doubling time td(d)
Bacillus lichenformis & Alcalgeneseutrophs	0.317	2.181	0.301	2.302	0.341	2.032
Bacillus lichenformis	0.314	2.207	0.298	2.3255	0.298	2.3255
Alcalgeneseutrophs	0.289	2.398	0.2538	2.7305	0.301	2.3023

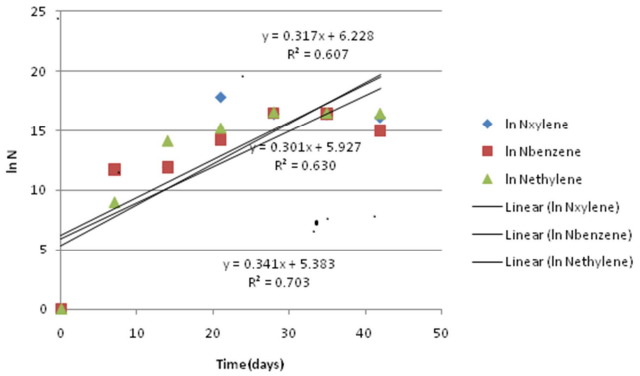

Figure 1. *Variation of In Nwith time for Bacilluslicheniformis and Alcaligeneseutrophus.*

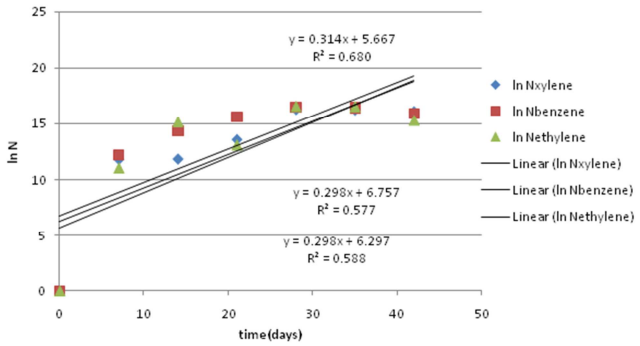

Figure 2. *Variation of In N with timeforBacilluslicheniformis.*

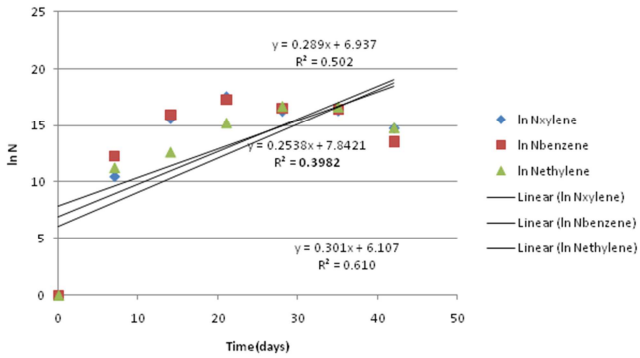

Figure 3. *Variation of In N with time for Alcaligeneseutrophus.*

From figures 1, 2 and 3; the number of organism present in a culture after t hour of incubation will be calculated from the graphs.

Bacillus licheniformis and *Alcaligeneseutrophus* in substrates;

$N_{Benzene}$ = slope =0.3010day^{-1}; μ= 0.01254hr^{-1}

$$\text{Slope} = \frac{0.693}{tD} \quad (1)$$

Where t_D is doubling time (day) or (hr); μ is called specific growth rate constant (day^{-1}) or (hr^{-1}).

$$t_D = \frac{0.693}{0.3010} = 2.302\text{days}$$

$N_{ethylene}$ = slope = 0.341day^{-1}; μ = 0.0142hr^{-1}

$$\text{Slope} = \frac{0.693}{tD} \quad (2)$$

$$t_D = \frac{0.693}{0.341} = 2.032\text{day.}$$

$N_{X\ ylene}$ = slope = 0.317day^{-1}; μ = 0.0132hr^{-1}

$$\text{Slope} = \frac{0.693}{tD} \quad (3)$$

$$t_D = \frac{0.693}{0.317} = 2.186\text{day.}$$

Average specific growth of the microbes in the substrates $= \frac{0.301+0.341+0.317}{3} = 0.3197\text{day}^{-1}$.

Bacillus licheniformis in substrate;

$N_{ethylene}$ = slope= 0.298day^{-1}; μ = 0.01242day^{-1}

$$\text{Slope} = \frac{0.693}{tD} \quad (4)$$

$$t_D = \frac{0.693}{0.298} = 2.3255\text{day.}$$

$N_{Benzene}$= slope = 0.298day^{-1}; μ =0.01242hr^{-1}.

$$\text{Slope} = \frac{0.693}{tD} \quad (5)$$

$$t_D = \frac{0.693}{0.298} = 2.3255\text{day.}$$

N_{Xylene}= slope = 0.314day^{-1}; μ = 0.01308hr^{-1}.

$$\text{Slope} = \frac{0.693}{tD} \quad (6)$$

$$t_D = \frac{0.693}{0.314} = 2.207\text{day.}$$

Average specific growth constant of microbe in substrates $= \frac{0.298+0.298+0.314}{3} = 0.3033\text{day}^{-1}$.

Alcaligeneseutrophus in substrates;

$N_{Ethylene}$= slope = 0.301day^{-1}; μ = 0.01254hr^{-1}

$$\text{Slope} = \frac{0.693}{tD} \quad (7)$$

$$t_D = \frac{0.693}{0.301} = 2.3023\text{day}.$$

$N_{Benzene}$ = slope = 0.2538day^{-1}; μ = 0.010575hr^{-1}.

$$\text{Slope} = \frac{0.693}{tD} \quad (8)$$

$$t_D = \frac{0.693}{0.2538} = 2.7305\text{day}.$$

N_{Xylene} = slope = 0.289day^{-1}; μ = 0.01204hr^{-1}.

$$\text{Slope} = \frac{0.693}{tD} \quad (9)$$

$$t_D = \frac{0.693}{0.289} = 2.398\text{days}.$$

Average specific growth of microbe in substrate (μ) = $\frac{0.301+0.2538+0.289}{3} = 0.2813\text{day}^{-1}$.

The figures 1, 2 and 3; illustrate that growth occurs primarily at the level of the cell. The mechanism by which growth occur is a cell division cycled at time and coordinate regulated. Although the rates at which bacterial are likely to grow in natural ecosystems is far below those exhibited by most laboratory cultures. The growth kinetics of mixed microbial populations, the simplified assumption was made that no interaction occurred cells other than competition for essential nutrient substances. The organisms of each species would accumulate at rates related to their exponential growth rate. This is known as the doubling time of the bacterial. The doubling time is the number of doubling of the initial populations over the course of the exponential growth phase. The extent to which the one species outgrows the other depends on the number of doublings that the environmental conditions can support.

The plotting of the natural logarithm of the number of bacterial above against the time of incubation yield a straight line whose slope will be numerically equal to $0.693/t_d$. This is found to be the case of many real bacterial cultures. The doubling time and the specific growth rate constant was obtained with the help of the graph. It is the rate of increase in cell numbers per unit of cell number. The doubling time and the specific growth rate constant for mixed culture of *Bacillus licheniformis* and *Alcaligeneseutrophus*, and their single strain from figures 1, 2 and 3 are all calculated above.

4. Conclusion

The study focused on dynamics microbial growth of *Bacillus licheniformis*and *Alcaligenes eutrophus* bacterial. This study show that *Bacillus licheniformis* and Alcaligenes eutrophus bacteria had highest doubling time of 2.302days in benzene substrate, Bacillus licheniformis bacteria had equal doubling time of 2.3255days in benzene and ethylene and Alcaligenes eutrophus bacteria had highest doubling time of 2.7305days in benzene. There was higher competition between consortium of two species than single species of the bacterial organism. The consortium of two species will also enhance the degradation of benzene in aromatic compounds contaminated soil. This study also shows that Bacillus licheniformis and Alcaligenes eutrophus bacteria can be use for bioremediation of aromatic compounds contaminated soil such as benzene and xylene. [2, 3, 10].

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