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Research Paper

Estimation of Biomass Energetic Yield and Maintenance Energy of Growth of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* on Diesel Oil

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Abstract-*Biodegradation data for the growth of Stock pure and mixed cultures of Pseudomonas aeruginosa and Pseudomonas fluorescens on diesel oil have been analyzed using the concept of true energetic yield and maintenance energy based on re-parameterized Pirt's Model. The true biomass energetic yield, η_{max} was estimated as 0.230, 0.380 and 0.190 for P. aeruginosa, P. fluorescens and their mixed culture, respectively. Also, the maintenance requirements, M_e (day^{-1}) obtained were 0.588, 0.634 and 0.551 for P. aeruginosa, P. fluorescens and their mixed culture, respectively, the result of this work showed that relatively higher percentage degradation result from organism with lower true biomass yield and maintenance requirement and also reflected the possibility of a synergy between the two organisms since the mixed culture gave lower values of biomass yield and maintenance requirement when compared with the values obtained for the pure cultures.*

Keywords: Pseudomonas, Biomass yield, Maintenance energy, Diesel oil.

Introduction

The application of a biotechnological process involving microorganisms (biodegradation) has become a gradually increasing area of study in biochemical engineering, because of its increasing potential of solving the problems of hydrocarbon pollutants in the environment. This process of remediating and restoring the contaminated environments is known as Bioremediation. Bioremediation has become an accepted technology for restoration of hydrocarbon contaminated sites [11] because of its cost-effectiveness and eco-friendly nature [7]. As a result of this, hydrocarbon utilizing microorganisms play important role in the remediation or restoration of hydrocarbon contaminated environments and in hydrocarbon transformations in the petrochemical industry. A large number of microbial species (bacteria, cyanobacteria, fungi, algae, protozoa) which are capable of utilizing hydrocarbon as source of carbon and energy have been identified and isolated [3,4, 5].The most important species of oil degraders belonged to the genera of bacteria among which are Acetobacter and Pseudomonas [3,4].

Pseudomonas has been widely studied because of their amazing metabolic diversity within the genus and is widely distributed in nature. Previous research works on degradation of organic compounds probably contain more citation on Pseudomonas strains (*P. aeruginosa* and *P. fluorescens*) than any other bacteria genus. However, literature is sparse concerning the abilities of *P. aeruginosa* and *P. fluorescens* to degrade hydrocarbons. Most of the experiments have been carried out on the activities of microbes at the biological level such as measurement of respiration, enzymic activities, and microbial counts, but not much information regarding the material and energy balances such as substrate utilization product formation, growth and energetic yields and maintenance energy [2,11,19,20]. These parameters are the underlying phenomenon in converting organic compounds to various products.

Moreover, the capability of microorganisms to degrade hydrocarbon can be measured through cell growth, substrate utilization, and product formation. The true biomass yield (a growth yield) and maintenance requirement are equally important parameters used to characterize the growth of a microbe on any medium [1,19]. From bioenergetics point of view, the better microorganism for biodegradation should have higher true biomass energetic yields and less energy for maintenance followed by higher rate of substrate utilization [11,13,20].

There have been several reports on diesel oil spills in the environment, and the process of their bioremediation has not been fully developed due to high content of poly-nuclear molecules in the diesel oil, which have made it very refractory to microbial attack [6]. The objective of this study is to evaluate the true biomass yield and maintenance energy

of *P. aeruginosa* and *P. fluorescens* on diesel oil using pure and mixed cultures.

Overview of Method of Estimation of True Yield and Maintenance Coefficient

When the products formed are viewed as essential for the generation of Adenosine triphosphate, (ATP), necessary for the growth and maintenance of the microorganisms, then the electrons which are transferred from the substrate to the products are viewed as being expended to provide energy for growth and maintenance [12, 14].

Pirt (1965) has identified a model which relates growth yields and the specific growth rate, and this model for the growth process which is based on the Monod kinetic equation is dependent on the substrate uptake. This Monod' model is that makes more biological as well as mathematical sense (Solomon *et al.,* 1994) as shown

$$q_s = \frac{q_s^{\max} S}{(k_s + S)} \qquad 1$$

Based on the assumption that for a given amount of biomass the maintenance energy is constant and independent of the growth rate (Pirt, 1982), the rate of energy consumption is given by

$$q_s = \frac{\mu}{\gamma_{x/s}^{\max}} + M_s \qquad 2$$

The equation can be re-parameterized in energetic terms using the concept of available electron balance as follows [9,10,18,19]. From this equation, estimates of true biomass yield and maintenance energy can be made.

$$\frac{\mu}{\eta} = \frac{\mu}{\eta^{\max}} + M_s \qquad 3$$

Material and Methods

This section discussed the basic materials and also the method used in carrying out the experiment. Stock culture of *P. aeruginosa* and *P. fluorescens*, which are coded National Collection of Industrial Bacteria, Scotland, NCIB950 and 3756 respectively were obtained from Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. Diesel oil was obtained from African Petroleum Filling Station in Ile- Ife Nigeria. Equipment used include New Brunswick Environment Controlled Incubator shaker, Astell Heavson Model 19/ 94 SBV autoclave, Gallenkamp Centrifuge of 5 x 10^3 rpm capacity and M2Ol Visible Spectrophotometer Camspec Cambridge.

The culture media was composed according to Zhang *et al.* (2005). The compositions of this mineral salt medium were (gl^{-1}) Na_2HPO_4 $12H_2O$ (3.0), KH_2PO_4 (3.0), $MgSO_4$ (0.2), $FeSO_4$ $7H_2O$ (0.001), $CaCl_2$ $2H_2O$ (0.1), NaCl (1.0), KCl (1.0), $NaNO_3$ (4.0) and 2.0 ml of trace element solution of whose composition (gl^{-1}) was H_3BO_3 (0.15), $ZnSO_4$ $7H_2O$ (0.75), $NaMoO_4.2H_2O$ (0.05), $MnSO_4$ H_2O (0.75), $CuSO_4.5H_2O$ (0.075), $FeCl_3 6H_2O$ (0.08) and $CoCl_2.6H_2O$ (0.08).

From the stock culture obtained, a loop of cells was transferred to 100 ml conical flask containing 50 ml mineral salt medium and 1%(v/v) of diesel oil as sole carbon source. This was done separately for each organism and incubated in rotary shaker (200 rpm) for 32 hours at 30^0C to obtain primary cultures. The secondary culture were prepared by transferring 25 ml of the cultures to 25 ml fresh medium and incubated in 100 ml conical flasks in the rotary shaker (200 rpm) for 14 hours at 30^0C. Inoculums for the experimental runs were taken from the secondary cultures, with the view that exponential growth phase is fully established. The biodegrading abilities of these pseudomonas species on diesel oil, in pure and mixed cultures were tested in 250 ml mineral salts medium, containing 3-5% (v/v) diesel oil, and 50 ml of inoculums. The set ups, which were in duplicate, were incubated at 28^0C on a New Brunswick Gyratory Shaker at 200 rpm for 14 days. Samples were withdrawn at two days interval for analysis to obtain the dry biomass concentration and concentration of the diesel oil. The average values specific growth rate and biomass energetic yield of the data were tended kinetic models to obtain the parameters such as required for this study.

Results and Discussion

Microbial Growth and Biodegradation of Diesel Oil

From the experimental report it was observed that the two microbes, *P. aeruginosa* and *P. fluorescens* grew and degraded the diesel oil within the condition of the experiment. Moreover, it is, also observed from the specific growth rate and the specific rate of substrate consumption which were calculated for each of the organisms and their mixed culture that the specific growth rate, μ generally decreases as the specific rate of substrate consumption q_s, decreases. This is shown in Figure 1–3. This is an indication that the growth rates of the organisms were direct consequence of substrate consumption. This is a required condition [9,1017,18,19] to tend the data obtained to the models for the analysis of the true biomass growth yield and maintenance energy.

Biomass True Yield and Maintenance Requirement of the Organisms

The estimation of the true yield and maintenance energy are obtained using equation (3). The regression of the plots of inverse of biomass energetic yields, η against the inverse of the specific growth rate, μ yielded the true biomass energetic yield, and maintenance energies. The result for the analysis is presented in Table 1.

It was observed from Table 1 that the higher the value of the biomass energetic yield, the higher the maintenance energy requirement. This was so since the yield factor is strongly influenced by the substrate requirement for cell maintenance as explained by Abbot and Clamen (1973). The η_{max} is independent of the growth rate. Both the η_{max} and M_e depend on the species being considered [18]. It could be explained therefore, that there are more available electrons which are not transferred to oxygen but assumed incorporated into biomass yield in *P. fluorescens* than in *P. aeruginosa* and their mixed culture [18]. This resulted in higher value of η_{max} for *P. fluorescens* than *P. aeruginosa* and their mixed culture. But more available electrons are transferred to oxygen rather than biomass in the mixed

culture of *P. aeruginosa* and *P. fluorescens*, resulting in a lower value of η_{max} for the mixed culture.

The synergetic metabolism, usually obtained from mixed culturing, has induced a non-stressful metabolism of the substrate (diesel oil). This was indicated in the smaller value of maintenance energy requirement obtained for the mixed culture of *P. aeruginosa* and *P. fluorescens* on diesel oil [8]. However, as obtained in this result, *P. fluorescens* has more stressful metabolism (larger value of M_e) than *P. aeruginosa* in the biodegradation of diesel oil.

Conclusion

The growth rates of the organisms on the diesel oil are observed in the setups to be as a direct consequence of substrate consumption. The results obtained during the analysis of the yield factor and the maintenance energy showed that though *P. aeruginosa* had less stressed degradation of the diesel oil when compared to *P. fluorescens* but their mixed culture outperformed the pure cultures. The mixed culture gave the lowest value of the maintenance energy which could be an indication of the least stressful metabolism in the degradation of the diesel oil.

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Table 1: **Estimates of True Biomass Yield and Maintenance Coefficient for the Growth of *P. aeruginosa*, *P. fluorescens* and their mixed culture.**

Parameters	*P. aeruginosa*	*P. fluorescens*	Mixed culture
True biomass energetic yield, η^{max}	0.230	0.380	0.190
Maintenance Energy, M_e (day^{-1})	0.588	0.634	0.551

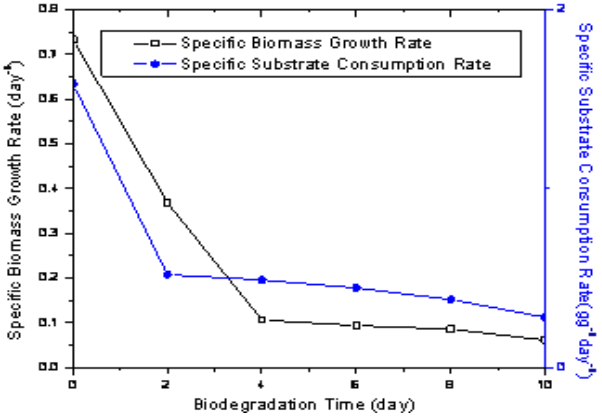

Figure 1: Plot of Specific Biomass Growth rate and Substrate Consumption rate of P. aeruginosa on Diesel Oil

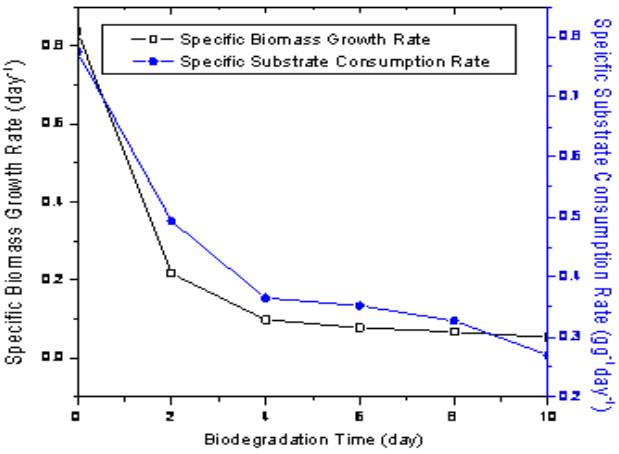

Figure 2: Plot of Specific Biomass Growth rate and Substrate Consumption Rate of P. fluorescens on Diesel Oil

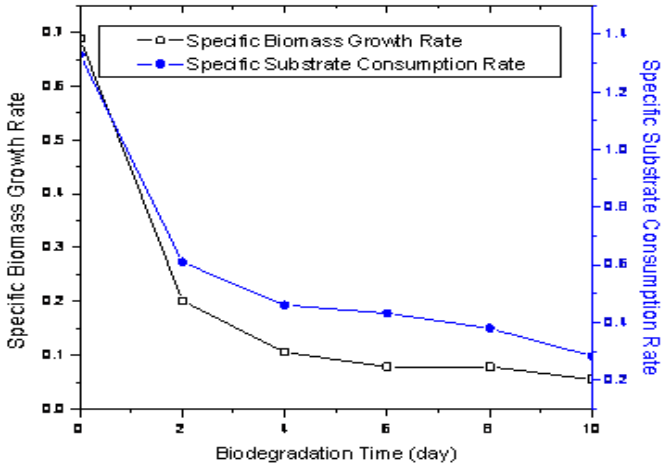

Figure 3: Plot of Specific Growth and Substrate Consumption Rate of Mixed culture of P. aeruginosa and P. fluorescens on Diesel Oil