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MODELLING THE RATES OF BIODEGRADATION, NITROGEN FIXATION AND UPTAKE OF FIXED NITROGEN DURING BIOREMEDIATION OF CRUDE OIL CONTAMINATED

ENVIRONMENT

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Abstract: Development of a biodegradation model for crude oil, which is a mixture of thousands of hydrocarbon compounds, involves numerous biochemical pathways when compared with degradation of individual hydrocarbons. The influence exerted by some factors such as nutrient limitations, particularly combined nitrogen compounds during biodegradation is well known. Hence the interpretation of the role of nitrogen fixing bacteria in the functioning of any oil polluted ecosystem requires an assessment of the relative efficiency of the uptake of the mineralized or fixed nitrogen compound and the estimate of the nitrogen-supplying potential of the diazotrophic bacteria in that environment. Formulating a mathematical model may be of great importance in this interpretation, and in evaluating the success of such bacterial consortium in treatment plants.

Key words: Bioremediation, crude oil, nitrogen fixation, mathematical modelling

Symbols and abbreviations used

Ec [%] - enhanced capacity in bioremediation

F [-] - total cellular activity of the diazotroph

K [-] - nitrogen fixation rate constant

Ks [mgTPH.d-1] - pseudo first-order degradation rate constant
Ms [g] - mass of soil sample assayed

n [-] - number of cellular macromolecules analysed for toxicity effect

Nt [mgNH4+] - total fixed nitrogen compound in the mass of soil sampled
r [mgd-1] - rate of degradation of crude oil contaminant in the soil

S [mg TPH g-1 soil] - concentration of crude oil remaining at a given time > 0

So [mg TPH.g-1 soil] - concentration of crude oil at time, t = 0
t [d] - time in days

TPH [-] -total petroleum hydrocarbon in crude oil

 [-] means no unit

Introduction

Crude oil and petroleum exhibit a complex formulation with variable composition from one oil field to another (Institute of Petroleum, 1983). Studies on their degradation as a mixture are difficult to interpret because of the vast arrays of compounds present. Synergistic or antagonistic interactions may not be ruled out during biodegradation. The relative importance of such phenomena as co-oxidation (Dalton and Stirling, 1982) and cometabolism (Beam and Perry, 1974; Harmer, 1992) cannot be overemphasized. Temperature, pH, nutrient application, particularly inorganic nitrogen compounds and phosphorus are important factors that also control biodegradation of crude oil. Many studies have shown that nitrogen and phosphorus limit the biodegradation of hydrocarbons (Atlas and Bartha, 1973), but phosphorus requirement is about one-sixth that of nitrogen (Bouwer, 1992). The technology of nutrient application to oil polluted environment is an innovative approach in environmental biotechnology. It is employed for optimization of the conditions that maximizes the contaminant-degrading potential of the indigenous microbial population. This was applied in the episode of the popular Exxon Valdez oil spill at Price Williams Sound, A.K, in which degradation rate was increased without increase in toxicity (Pritchard and Costa, 1991). Enhanced biodegradation may involve supplemental application of nitrogen, phosphorus or adapted hydrocarbonoclastic bacteria to concentration of oil on land or water.

Microbial nitrogen fixation is currently becoming an attractive innovation in bioremediation of oil polluted environments. Through the addition of post-harvest leaves and stems into oil polluted mesocosms, Paerl et al. (1996), co-optimised nitrogen fixation and petroleum hydrocarbon degradation. Also bioremediation of polluted soil was accelerated by seeding contaminated sites with adapted *Azotobacter sp* (Onwurah, 1996).

This paper considers simulation modelling of crude oil degradation rate using a consortium of hydrocarbonoclastic bacteria species and adapted, compatible diazotrophs, within crude oil spill environment. It is hoped that this may stimulate scientific innovation of designing bioreactors and competent consortium of diazotrophs and hydrocarbonoclastic bacteria for bioremediation of petroleum and crude oil contaminated soil or water.

**BIODEGRADATION MODEL**

In order to accurately predict the duration of a bioremediation process, it is necessary to model bacterial growth and substrate removal kinetics under sub-optimal conditions. Biodegradation patterns of Bonny light crude oil in nutrient media and oil-contaminated soil-microcosms innoculated with consortia of Azotobacter vinelandii/Pseudomonas spp, and Azotobatcter vinelandii/Bacillus spp were found to approximate pseudo first-order kinetics with respect to a constant cell number of the hydrocarbonoclastic bacteria (Onwurah, 1999; Onwurah and Nwuke, 2004). Therefore the biodegradation rate can be modelled as

 r = KsXS -------------------------------- (1)

where

r = the degradation rate of the total petroleum hydrocarbons.

Ks = pseudo first-order degradation rate constant, (d"1)

X = total bacterial cell protein (d-1)

S = concentration of crude oil remaining (g-1). At steady-state, uptake of find nitrogen by the hydrocarbonoclastic bacteria for biosynthetic activity is in equilibrium with the rate of crude oil degradation. Such an equilibrium assumption has been adapted for similar experiment (Folsom *et al*., 1990). Hence the rate of biodegradation of the petroleum hydrocarbons by the hydrocarbonoclastic bacteria at steady state of nitrogen uptake from the soil cores is given:

 $\frac{dS}{dt}=\frac{-KsXS}{(1+ Nt/Ms)}$ ---------------- 2

where:

Nt = total available nitrogen compound (fixed) in the given soil. Ms = mass of soil sample analysed Integrating Equation (2) above gives

S = S0 exp *A1*(3)

Where

 *A1* = $\frac{-KsXS}{(1+ Nt/Ms)}$

Since X, Ms are known parameters and Nt could be evaluated by the Kjeldahl method (Jackson, 1964), Ks and S0 can be analysed by fitting Equation (3) to concentrations of the crude oil (S) against time (d) by non-linear regression analysis. The estimate of the pseudo first-order rate constant (Ks) will be the primary basis of comparing or evaluating degradations Kinetics among different bacterial consortia supplemented with fixed concentration of combined nitrogen compound.

Inclusion of enhancement capacity (Ec) into Equation (2)

When inorganic nitrogen is limiting in the soil or oil polluted environment, inoculating with adapted diazotroph could provide the required nitrogen for growth and proliferation of the hydrocarbonoclastic bacteria (Onwurah, 1996). It should be noted that pure cultures of Azotobacter spp do not degrade or show any significant growth in Azotobacter medium containing crude oil as the only carbon source (Onwurah, 1998). But when supplemented with mannitol as alternative carbon source, growth was established as well as degradation of some components of the crude oil (Onwurah, 1996). However, metabolic intermediates of crude oil degradation by hydrocarbonoclastic bacteria can support the growth of adapted Azotobacter (Gottshalk, 1984; Walker and Harris, 1970; Onwurah, 1999). In this case, Azotobacter utilizes the secondary metabolites as the primary substrates for growth while co-metabolizing other components of the petroleum hydrocarbon mixtures (Onwurah, 1999). Therefore, insight into the reaction kinetics involving adapted diazotrophs can be obtained by comparing the rates of crude oil transformation by the pure strain of the hydrocarbonoclastic bacteria supplemented with adequate nitrogen compound with that of the consortium of the hydrocarbonociastic and diazotrophic bacteria (Onwurah and Nwuke 2004).

For the consortium of hydrocarbonoclatic and diazotrophic bacteria (under very low level of inorganic nitrogen compound), biodegradation rate was enhanced due to cometabolism by the diazotroph (Onwurah, 1999; Onwurah and Nwuke 2004), relative to pure culture of the hydrocarbonoclastic bacteria supplied with adequate N-compound. To account for this increase in biodegradation rate, the concept of enhancement capacity (Ec) is introduced into the Monod Kinetic model. The Ec takes care of the cometabolic activity of a fixed cell density of the diazotroph at a given density of the hydrocarbonoclastic bacteria. This model can be simplified to speudo first-order kinetics when compatible bacteria species perform cometabolic activity in the obligate presence of substrate augmenting hydrocarbonoclastic bacteria, thus

 *t* = $\frac{A2}{Ks \left(\frac{So}{A2} \right)Ec-X}$ *In* $\frac{Nt A2 EcX }{(Nt + A2EcX- So)So}$ --------------- 4

where:

A2 = (1 + N,/Ms)

Equation (4) is reminiscent of the product toxicity kinetic model that accounts for a decrease in degradation rate during metabolism of a mixture of trichloroethylene and chloroform, by resting cells (Alvarez - Cohen and McCarty, 1991). For effective and continuous degradation of the petroleum hydrocarbons by the hydrocarbonoclastic bacteria, continuous supply of fixed nitrogen is essential and this will be accomplished by the compatible diazotrophic bacteria present or/and those inoculated into the polluted environment. Excreted nitrogen compounds associated with A. vinelandii include aspartic acid and traces of hydroxylamine (Hoberg, 1936).

**Consideration of microbial nitrogen fixation in bioremediation**

Since fixed nitrogen supply by the diazotroph is assimilated rapidly during bioremediation of oil polluted environment, total nitrogen (Nt) will be based on the nitrogen fixing potential of the diazotrophic bacteria within the given environment when there is no external supply and its shortage will impede biodegradation rate. Hence analysis of the total nitrogenase enzyme protein within a given (soil) sample or cell density of the diazotroph, will enable the evaluation of the nitrogen supplying potential of the soil cores. Nitrogen fixation by *Azotobacter* is calculated using the equation described earlier (Onwurah 2000).

Nt=K.F ----------------------------------------- (5)

where

Nt = total nitrogen fixed as ammonium ion at time (t) for the soil sample.

 K = nitrogen fixation rate constant

= (specific nitrogenase activity of crude protein extract)/n

n = number of cell components (parameters of the diazotroph analysed for toxicity.

F = total cellular activity of the diazotroph under crude oil pollution.

The total cellular activity (F) which is related to the entire cellular biochemistry of the diazotroph could be evaluated from the toxic effects of the crude oil, at a given concentration, on DNA, protein and lipid components of the *Azotobacter* cell (Onwurah, 2000). Thus in all possible cases of bioremediation involving nitrogen fixing bacteria consortium and cometabolism, enhancement capacity can be evaluated with Equation (4), while Equation (5) provides for the calculation of the potentially fixed nitrogen compound in soil core inoculated with diazotrophs. An important assumption here is that nitrogen compound uptake rate by the hydrocarbonoclastic bacteria is in rapid equilibrium with the fixation rate by the diazotroph, during steady - state biodegradation of the crude oil.

Concluding remarks

A general bioremediation model involving microbial nitrogen fixation, uptake of the fixed nitrogen compounds and biodegradation of hydrocarbons in oil polluted environment is hereby proposed. The model also has a component referred to as enhancement capacity (Ec), which can be attributed to the nitrogen metabolism and cometabolic activity of a compatible diazotrophic bacteria within the environment of the oil-polluted soil.

Equation 4 (the model) was developed from observation, semi-empirical and theoretical approaches. The last concept was based on "thought experiment". It is a deterministic, simulation model, which employs well-accepted empirical equations patterned through calibrated coefficients that describe the system, and can meet special and temporal requirements. However, the data from which the model was developed were culled from previous experiments (Onwurah, 1999; 2000; Onwurah and Nwuke, 2004). The equation significantly satisfied other data points culled from some of these experiments, though not presented here. The equation, though may not be a recipe for biodegradation rate determination, or for nitrogen fixation and uptake rates, it explains that such a tripartite relationship is possible in a closed or controlled system such as a bioreactor.

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