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<td>ONNWURA, N.E Ikechukwu</td>
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Integration of biodegradation half-life model and oil toxicity model into a diagnostic tool for assessing bioremediation technology

Method

Integration of biodegradation half-life model and oil toxicity model into a diagnostic tool for assessing bioremediation technology

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Abstract

An integration of a natural biodegradation half-life model and oil toxicity model has resulted in a precise, fine-tuned science-based technology for evaluating success of bioremediation strategies, particularly for crude oil-contaminated soil. By extension, this mathematical tool can be applied for impact assessment of crude oil on land at a given time. This Methods paper focuses on both the use of diazotrophic bacteria in evaluating toxicity of a given crude oil and natural biodegradation half-life of the petroleum hydrocarbon in the given ecosystem, the ultimate goal being the development of a diagnostic tool for best decision making in the allocation of limited financial resources and best options for bioremediation strategies.

Introduction

The field of environmental biotechnology has grown tremendously within the last decade, with a number of remediation efforts involving specialized or adapted microbial consortia, such as IBERS (blend of hydrocarbon-degrading microbes from IRS; Auburn, Washington), 'sRudornoins sp./Azotobacter vinelandi', and Bio-Degilec inoculums (Regenesis; San Clemente, California). Optimization in bioremediation is the primary goal of environmental biotechnology, and this involves evaluation of the attenuation process. For example, a novel bioaugmentation and molecular diagnostic tool for bioremediation of ethene-contaminated sites has been reported. More efforts towards the expansion of molecular approaches as comprehensive diagnostic and diagnostic tools for site prioritization, and remedial efforts to achieve site closure are needed. In order to reduce cost, effective operations must be selected from several alternatives, and this may require such tools as mathematical models relevant for operational units. Process engineers and scientists use such mathematical models to investigate complex, integrated biodegradation operations, without the need for extensive experiments. Such simulation models can be of great use at any stage of process development. From initial concepts through to design of final operations.

In our recent work, the integration of a bioremediation half-life into a bioremediation strategy that involves a consortium of a hydrocarbonoclastic bacterium and an adapted diazotroph provided the
required mathematical model for estimating the possible time frame for cleanup of petroleum hydrocarbon (PHC) contaminant in a soil ecosystem. One of the models provided the necessary combination ratio and concentration of inocula for scale-up of bioremediation in the case of larger oil spills. There is no well-known documented bioremediation effort in Nigeria, as was made evident when a community in the Mkpanak area of Cross River State was displaced because their farmland was rendered unproductive due to the impacts (specifically, sterilization) caused by crude oil spills. The availability of a proper diagnostic tool may enable optimal choices for bioremediation strategies for oil-contaminated agricultural land in terms of cost and reduced time of restoration for agricultural purposes.

This paper describes a diagnostic tool that may be employed or developed further into a mathematical software package for identifying the best bioremediation strategy for reducing both monitoring events and time for remediation. The importance of this becomes obvious when one considers the fact that bioremediation of PHC contaminants follows a first-order decay function.

Modeling

The diagnostic tool is derived from two models. One is the natural bioremediation model, which is given as

\[ C_t = C_0 e^{-Kt} \]  

where \( C_t \) and \( C_0 \) are the concentrations of the spilled oil at time \( t \) and \( t = 0 \) respectively, and \( K \) is the biodegradation rate constant. The other model is the oil toxicity model developed by Onwurah, given below

\[ T_i = \sum w_j V_j \]  

subject to \( V_j > 0 \) for \( j = 1, 2, \ldots, n \), where \( V_j \) represents the \( j \)th decision variable, which are DNA level (mg/l), protein level (mg/ml), and lipid peroxidation products (Malondialdehyde, or MDA, measured as mmol MDA/mg protein) of the indicator organism; \( w_j \) are the relative weightings assigned to these variables according to their unique properties. \( T_i \) is the inherent toxicity or toxicity index of the crude oil.

The integration of these two models as a diagnostic tool was based on the fact that, although crude oil is a repository of several hydrocarbon compounds, for modeling purposes it is assumed to be a bulk contaminant. Hence pulsed input, or a continuous step function, reminiscent of wave motion, was assumed. The effective concentration \( E_{50} \) of crude oil is the concentration, or quanta at which 44-50% nitrogen-fixing capacity of the indicator organism was lost. This is the minimum concentration of crude oil that will produce toxic effect on the indicator organism. Hence discrete concentrations or quanta that can give multiples of toxic effects at time \( t = 0 \) is given thus:

\[ C_{t=0} \]

This was significant only when the concentration \( C_t \) of the spilled oil at time \( t = 0 \) was equal to or greater than the \( E_{50} \) value. Hence, concentration of spilled oil less than the \( E_{50} \) value was regarded as no-observed-effect concentration (NOEC). Therefore impact \( E \) due to oil spill is proportional to the multiplicative of the \( E_{50} \) value of the concentration of the crude oil in the soil at a given time \( t \). If the concentration of the spilled oil at time \( t = 0 \) was \( C_0 \), where \( C_0 > E_{50} \), then

\[ E = A \cdot C_0 \]

whereby

\[ E = T_i \cdot C_0 / E_{50} \]  

\[ T_i = \frac{C_0}{E_{50}} \]  

where \( A \) is the proportionality constant for the crude oil and is the inherent toxicity index derived from cybernetic modeling with Azotobacter vinelandii as the indicator organism. \( E_{50} \) is also a constant for the crude oil at a given time, with respect to the indicator organism.

Substituting the two constants by combining them \( T_i / E_{50} \) as a single constant \( A \), Equation 1 becomes

\[ E = A \cdot C_0 \]  

Hence, when considering crude oil spills in similar ecosystems, it is reasonable to suggest that impact is proportional to the concentration of the spilled oil.

Based on the fact that the impact of the oil spill depends on its duration in the soil ecosystem, the biodegradation rate of the crude oil in that ecosystem becomes very important. If \( C_t \) is the concentration of the crude oil remaining in the soil compartment at time \( t > 0, \) then

\[ C_t = C_0 e^{-Kt} \]


Methods

\[
E = A \cdot C
\]

Substituting for \( C \), using the value in Equation 5:

\[
E = A \cdot C \cdot e^{-kt} 
\]

Taking log, of both sides of Equation 6

\[
\ln E = \ln A \cdot C \cdot e^{-kt} 
\]

or

\[
\ln E = \ln A \cdot C \cdot e^{-kt} 
\]

By definition, the rate constant \( K \) is the concentration of the crude oil lost per day through biodegradation. Equation 8 can then be used in monitoring bioremediation efforts at specified time-intervals, bearing in mind that \( A = T/K_{EC} \).

Materials and methods

Determination of natural biodegradation half-life constant (\( K \))

Crude oil spills were simulated on composite soil samples obtained from a depth of 0-15 cm of uncontaminated soil in the zoological garden of the University of Nigeria, Nsukka. This was divided into 2 parts: 17 portions of one part were mixed with 0, 50, 100, or 150 mg of Bonny light crude oil/kg soil, and this constituted the crude oil-contaminated soil microcosm X. Also, 17 portions of the composite soil sample were inoculated with varying densities of the consortium Psedomonas sp./Azotobacter vinelandii as previously described, before mixing with 0, 50, 100, or 150 mg crude oil/kg soil, and this constituted the amended oil-contaminated soil microcosms Y. Parallel controls were set up for X and Y as samples not contaminated with crude oil.

Four days after the crude oil spill simulation on the soil microcosms, samples were scraped from designated segments, and the remaining petroleum hydrocarbons (PHCs) were determined by the solvent extraction method described by Snell and Snell. The quantity of PHCs degraded by the microbial population in the soil microcosms was thereafter estimated. From this, the rate loss constant \( K \) for the PHCs in each soil microcosm was evaluated.

Evaluation of inherent toxicity and \( EC_{50} \) of the crude oil

Toxicity index \( T_I \) and \( EC_{50} \) (the concentration of the crude oil that results in 50% loss in nutrient cycle in the excised soil exemplified by 44-50% loss in nitrogen-fixation capacity of Azotobacter vinelandii) were determined for Bonny light crude oil using Equation 1 as earlier reported.

Results and discussion

The values of \( T_I \) and \( EC_{50} \) obtained for Bonny light crude oil were, respectively, 0.5 units and 1.5 (mg) of the crude oil in the culture medium; the rate loss constants \( K \) for the PHCs in each soil microcosms are shown in Table 1. With the above values, the impacts \( E \) of the simulated oil spill on the soil microcosms X and Y were calculated (Table 2) using the model given in Equation 8. Table 2 also shows the percentage bioremediation success achieved by the various microbial treatments. It may be noted from this table that impact of 50 g crude oil/kg soil sample of soil microcosm X at day 4 was 14.13 units, giving a reduction in impact of 15.14% relative to the initial

<table>
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<tr>
<th>Table 1. Total petroleum hydrocarbon loss rate constant in soil microcosms X and Y at Day 4 of application of Bonny Light crude oil</th>
<th>SOIL SAMPLE X</th>
<th>SOIL SAMPLE Y</th>
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<tbody>
<tr>
<td>S OL. SAMPLE X</td>
<td>LOSS RATE CONSTANT (K OA)</td>
<td>S OL. SAMPLE Y</td>
</tr>
<tr>
<td>(CRUDE OIL APPLIED (G/ KG SOIL))</td>
<td>(G/ D)</td>
<td>(G/ D)</td>
</tr>
<tr>
<td>0.07</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>0.14</td>
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<td>0.21</td>
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<td>0.35</td>
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Impact assessment (16.65 units). For soil microcosm Y (treated) that received the same concentration of crude oil, there was a reduction of impact from 16.65 units to 10.47 units, signifying a bioremediation success of 37.12% in 4 days. For portions of soil microcosms Y, which received increased concentrations of competent bacterial inocula as described elsewhere, the bioremediation success increased in spite of the increased concentration of the crude oil applied. This also brings to perspective the usefulness of this model in assessing the effectiveness of bioremediation strategies.

The validity of the model was also tested by applying it to a set of values (Table 3) obtained for a land treatment project for aged oily-sludge. The table shows the concentration of sludge added to the various plots and the exposure period (days) used in calculating the rate loss constants (K). Equation 8 was also employed for the data set given in this table in calculating the impact (E) of the sludge on the plots after 100 days. For this land treatment project, the bioremediation efficiency of the indigenous microbial population was also evaluated using the mathematical model as per Equation 8. The bioremediation efficiency of the plots that received 170 g sludge per kg of soil was 22.82%. When the sludge application increased to 9,500 g/kg soil, the efficiency decreased to 14.23%. This implies that sludge remains for a longer period because the biodegradative capacity of the microbial population within the given plots is being overwhelmed. In this case, it may become necessary to inoculate the plots with a higher concentration of the competent bacteria to reduce the quantity of sludge applied to the plots. This scenario again underlines the utility of the model.

Combining toxicity data from laboratory experiments and biodegradation data from field studies or microcosm experiments may provide a logical approach to empirically determine impact. Soil is a very complex system, and it is difficult to develop reliable mathematical models that govern the impacts of oil pollution. However, in the area of environmental pollution, such models can be constructed on the basis of reductionist philosophy, partial understanding of microscale subsystems, and an intuitive feel for the manner in which these subsystems may interact. Attempts at model estimation/validation were made using primary data obtained from the laboratory and microcosms experiments. Data from the farmland treatment project for aged oily-sludge was also used for model validation. The resulting impacts as calculated from the Equation 8 model were reasonable with respect to the in situ data set. This suggests that the model can be used to estimate or monitor effectiveness of bioremediation projects. The results obtained for the land farming treatment project provided the justification of assuming its holistic validity, though not from a deterministic perspective. Calculated impact of the crude oil spill on land was largely dependent on the crude oil's half-life in the soil compartment. If the spill is cleaned up within 24 hours, the impact or effect may be negligible or zero. The residence time or half-life of the crude oil as the soil compartment is a function of a number of parameters, such as biodegradiability for biodegradation rate constant, volatilization, hydrolysis, and chemical oxidation, though biodegradation is considered the major loss mechanism. This explains why the biodegradation model was considered an important component for impact assessment. Biodegradation rate depends on the presence of a competent microbial population that can metabolize or degrade the crude oil and hence reduce impact at a given time. Inherent oil toxicity, in spite of concentration effect, may also have a negative impact on the soil, particularly on nutrient cycling (nitrogen fixation). Other toxicity models that can be used together with the biodegradation model include the OilToxic model, which considers the effects of ambient temperature and exposure time on toxic units, and the Quantitative Structure-Activity Relationship (QSAR) model, though the latter has the disadvantage of requiring knowledge of the actual composition of the crude oil.

### Table 3: Impact values (E) of Sonsy Light crude oil at time t = 0 and t = 4 (d) for soil samples X and Y

<table>
<thead>
<tr>
<th>[CRUDE OIL APPLIED (GMS/SOIL)]</th>
<th>IMPACT (E) ON SOIL SAMPLE X</th>
<th>IMPACT (E) ON SOIL SAMPLE Y</th>
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<tbody>
<tr>
<td>Control No Treatment</td>
<td>t = 0</td>
<td>t = 0</td>
</tr>
<tr>
<td>1</td>
<td>14.60</td>
<td>14.60</td>
</tr>
<tr>
<td>2</td>
<td>33.33</td>
<td>33.33</td>
</tr>
<tr>
<td>3</td>
<td>45.95</td>
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Note: In the table, values in parentheses are exponential values. The table shows the concentration of sludge added to the various plots and the exposure period (days) used in calculating the impact (E) of the sludge on the plots after 100 days.
and concentrations of the spilled oil. The cybemetic and structured microbial processes approach to modeling toxicity of crude oil used in this study may be more suitable to it requires neither knowledge of the composition of the crude oil nor the effect of ambient temperature.

Conclusion

Simultaneous application of two models such as the natural bioremediation model and oil toxicity model can be quite useful in assessing the efficiency of biotreatment strategies, especially for petroleum hydrocarbon polluted sites. It may also be helpful as a tool for selecting among a given collection of microbial strains or consortia claimed to be effective for FIK degradation, thus reducing waste of resources and time, by assessing contaminant of various alternatives.

REFERENCES


