RELATIONSHIPS OF TEMPERATURE AND HUMIDITY TO THE BIODEGRADATION OF PETROLEUM HYDROCARBONS IN SOILS

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Abstract. This work focused on monitoring CO₂ production, microbial growth and residual hydrocarbon concentration during bioremediation experiments performed on laboratory soil microcosms. A natural soil was artificially contaminated with hexadecane and adjusted with inorganic nutrients to stimulate biodegradation. Microbial growth, CO₂ production and residual hexadecane were periodically monitored at different soil water contents ranging from 0.15 to 0.25 g water g⁻¹ of dry soil and at different temperatures ranging from 20 to 25 °C. The results showed that the humidity had a greater effect on microbial activity and contaminant degradation than the temperature. The study established the experimental regression equation of temperature and humidity to the hexadecane mineralization rate, an important parameter in assessing the ability to convert organic carbon into inorganic carbon. The difference between the results of the hexadecane mineralization rate obtained from the experiment and calculated from the regression equation is not too high, from 2% to 20%.

Keywords: microbial activity; soil; petroleum hydrocarbons; biodegradation; experimental regression equation.

Classification numbers: 3.1.1, 3.3.1, 3.4.3.

1. INTRODUCTION

Bioremediation is currently being applied as a solution for the treatment of petroleum hydrocarbons in contaminated sites [1]. Previous studies have mainly focused on the conditions that affected the activity of microorganisms involved in hydrocarbons biodegradation process in different soil types and different geographical and climatic conditions. These conditions can be listed as: pH, temperature [2]; microbial consortium [3]; oxygen content, nutrients (nitrogen, phosphorus), humidity, salinity [4], nature and concentration of contaminants [5]. In aerobic bioprocesses which aliphatic hydrocarbon removal is involved, environmental factors such as water content and temperature may greatly influence microbial activities. Metabolic status of the microorganisms depends on soil physico-chemical conditions. High soil water content is in general considered to enhance nutrient diffusion and microbial movement, thus accelerating the microbial activity and mineralization. However, in aerobic processes, higher water contents may
create unfavourable conditions for oxygen transfer. Regarding the temperature, this is the factor that affects the rate of metabolic reactions in the soil, and also affects the development of microorganisms. Measurements of carbon dioxide (CO$_2$) produced from hydrocarbons biodegradation process are important in evaluating biomass and activity of soil microorganisms, as well as decomposition of soil organic matters [6, 7].

In the study, hexadecane was chosen as a model compound for petroleum hydrocarbons. CO$_2$ production was measured based on acid/base titration method in order to assess the effect of temperature and humidity on the biodegradation process, thereby establishing the experimental regression equation of temperature and humidity to the hexadecane mineralization rate, an important parameter in assessing the ability to convert organic carbon into inorganic carbon.

### 2. MATERIALS AND METHODS

#### 2.1. Soil characterization

Soil samples were collected from a natural field (garden soil) of Hanoi suburb (March 2019), then the samples were sieved at 2 mm and stored in the dark at 4 °C before being used. Initial soil parameters were determined: soil water content of 15 % calculated from weight loss on drying at 105 °C for 24h (ASTM D2980-71); total organic carbon TOC = 20.5 g/kg soil (TCVN 6642:2000); total nitrogen NTK = 1.69 g/kg soil (TCVN 6498 : 1999); and orthophosphate P$_2$O$_5$–P = 0.10 g/kg soil (TCVN 8661 : 2011).

#### 2.2. Experimental and microcosms set-up

The biodegradation tests were performed in laboratory microcosms, consisting of Schott Duran bottles 500 mL, containing 50 g of soil. Hexadecane was added to each bottle and shaken well to make soil contaminated by 5.8 mg/g-dry soil. The C:N:P ratio of 100:10:1 was adjusted by adding external N and P sources in the form of (NH$_4$)$_2$SO$_4$ and KH$_2$PO$_4$ [7]. The soil microcosms, prepared as described above, were used for gas measurements (CO$_2$ production) during the 14-days bioremediation experiment. A tube filled with 10 mL of 0.5 M KOH solution, placed into each bottle, was used as alkaline trap to fix CO$_2$. KOH solution was removed from the tubes and renewed daily in all microcosms. CO$_2$ production was determined by acid/base titration of this solution using hydrochloric acid 0.1 M HCl and some drops of phenolphthalein solution as indicator.

The study conducted 2 series of experiments under different conditions of temperature (20 °C; 25 °C and 30 °C) and humidity (15 %, 20 % and 25 %). Non-contaminated soil (biotic microcosms) and abiotic microcosms (containing 0.02% w/w of sodium azide (NaN$_3$)) were used as controls.

The study used the experimental planning method to establish the experimental regression equation of temperature and humidity to the hexadecane mineralization rate, including 4 steps [8].

**Step 1**: Determining the objective function, affecting factors, and coordinates of affecting factors in the dimensionless coordinate system. The objective function is the hexadecane mineralization rate $\frac{m_{C(CO_2)}}{m_{C(hexadecane)}}$. The values of temperature and humidity were changed from 20 to 30 °C and 15 to 25 %, respectively.

The based level and ranges of changes were determined by equation:
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\[ Z_j^0 = \frac{Z_j^{\text{max}} + Z_j^{\text{min}}}{2} \quad (j = 1, 2) \]  

\[ \Delta Z_j = \frac{Z_j^{\text{max}} - Z_j^{\text{min}}}{2} \quad (j = 1, 2) \]

where \( Z_j^{\text{max}} \) are upper level coordinates; \( Z_j^{\text{min}} \) are lower level coordinates; \( Z_j^0 \) are base levels; and \( \Delta Z_j \) are ranges of changes.

The study converted the real variable \( Z_j \) into the dimensionless variable \( x_j \) according to the equation:

\[ x_j = \frac{Z_j - Z_j^0}{\Delta Z_j} \quad (j = 1, 2). \]

In the dimensionless coordinated system, upper level coordinates are \((+1)\), lower level coordinates are \((-1)\) and 0 is the origin of the coordinate system.

**Step 2**: Developing experiment plan. The experiment was performed with 2 affecting factors, so the number of experiments were \( 2^2 = 4 \), and 3 experiments at the base level.

**Step 3**: Determining the experimental regression equation and calculating the coefficients.

The regression equation has the form:

\[ \tilde{y} = b_0 + b_1 x_1 + b_2 x_2 + b_{12} x_1 x_2 \]

where \( b_0, b_1, b_2, b_{12} \) are regression coefficients calculated by the bellowed equations:

\[ b_0 = \frac{1}{N} \sum_{u=1}^{N} x_{0u} y_u \]

\[ b_j = \frac{1}{N} \sum_{u=1}^{N} x_{ju} y_u , \quad (j = 1, 2) \]

\[ b_{12} = \frac{1}{N} \sum_{u=1}^{N} x_{1u} x_{2u} y_u \]

where \( N \) is the number of experiments.

The study examined the meaning of \( b \) coefficients based on the Student Standard

\[ S_{b_j} = \frac{S_{th}}{\sqrt{N}} \]

where \( S_{th} \) is the recursive variance.

\[ S_{th}^2 = \frac{\sum_{o=1}^{m} (\tilde{y}_{oi} - \tilde{y}_o)^2}{m-1} \]

\( m \) is the number of experiments at the base level \((m = 3)\); \( y_o \) are values determined based on the experiments; \( \tilde{y}_o \) are values calculated based on the regression equation.

The Student Standard was calculated based on the equation:

\[ t_j = \left| \frac{b_j}{S_{b_j}} \right| \]

where \( S_{b_j} \) is the standard deviation of \( b_j \).
If \( t_j > t_b \), \( b_j \) is meaning; and if \( t_j < t_b \), \( b_j \) is not meaning.

**Step 4:** Checking the appropriateness of regression equation based on the Fisher Standard

\[
F_n = \frac{S_n^2}{S_{tt}^2}
\]

where \( S_n^2 \) is compatible variance determined by the equation:

\[
S_n^2 = \frac{1}{f_n} \sum (\tilde{y}_u - \bar{y}_u)^2
\]

\( F_n \) is the degree of freedom corresponding to the compatible variance (\( F_n = N - L \)); \( L \) is the number of meaning coefficients.

If \( F_n < F_b \), the regression equation appropriates to the compatible variance (\( F_n = N - L \)); \( L \) is the number of meaning coefficients.

If \( F_n > F_b \), the regression equation does not appropriate to the experiment.

3. RESULTS AND DISCUSSION

3.1. Effect of temperature and humidity on cumulative CO\(_2\) production

![Figure 1. Cumulative CO\(_2\) production at different temperatures.](image1)

![Figure 2. Cumulative CO\(_2\) production at different humidities.](image2)

Through the experiments, cumulative CO\(_2\) production or hexadecane mineralization calculated as a percentage of hexadecane initial concentration was divided into 3 phases, shown in Fig. 1 and Fig. 2. The first phase (from 0 to 3 days), cumulative CO\(_2\) production was low. This phase may correspond to the lag phase when the indigenous microbial population adapts and responds to the source of hexadecane. The second phase (from 3\(^{th}\) to 9\(^{th}\) day), cumulative CO\(_2\) production was significantly higher corresponding to the “exponential” phase. During the last phase (after the 9th day), the CO\(_2\) production rate was no longer as high as in the second phase for all experiments.

The results showed that when the temperature increased, the CO\(_2\) production rate also increased accordingly. However, in the range of temperature from 20 °C to 30 °C, the CO\(_2\) production rate or the biodegradation of hexadecane in the soil increased insignificantly. The cumulative hexadecane mineralization rates during 14 days were 60 %, 63 % and 70 % at 20 °C, 25 °C and 30 °C, respectively. The amount of CO\(_2\) formed during hexadecane biodegradation were 8.27, 9.05, 9.64 mg/g dry soil at 20 °C, 25 °C and 30 °C, respectively (Fig. 1)

Similar changes were observed in the series of experiments when assessing the effect of
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humidity. However, in the range of humidity from 15 % to 25 %, the CO₂ production rate increased significantly. The cumulative hexadecane mineralization rates during 14 days were 47 %, 62 % and 73 % at humidity of 15 %, 20 % and 25 %, respectively. The amount of CO₂ formed during hexadecane biodegradation were 6.2, 8.9, 10.5 mg/g dry soil at humidity of 15 %, 20 % and 25 %, respectively (Fig. 2). This is also quite consistent with previous studies when changing the humidity, the cumulative hexadecane mineralization during 14 days ranged from 40 to 70 % [9]. The curve obtained from the abiotic control showed that CO₂ production by physic-chemical processes such as evaporation and sorption is almost zero (less than 3 %), which means that the mineralization of hexadecane is indeed due to biodegradation. Similarly, the CO₂ production of the biotic control (non-contaminated soil) was negligible, which indicates that the mineralization of organic matter in the soil is insignificant. It is consistent with the initial values of TOC, NTK and P₂O₅–P which are insignificant compared with the hexadecane content added to the soil sample. The results showed that the humidity has a greater effect on carbon dioxide (CO₂) produced from hydrocarbons biodegradation process and it is an important indicator in evaluating microbial activity and contaminant degradation [6, 7] than the temperature.

3.2. Experimental regression equation of temperature and humidity to the hexadecane mineralization rate

The values of real variables Zᵢ, the dimensionless variable xⱼ and objective function (or hexadecane mineralization rate) were presented in Table 1.

| No | Real variable values | Coding values | Values of objective function (%) |
|----|----------------------|---------------|---------------------------------|
| 1  | 20 (°C) 15 (%)       | -1 -1 +1     | 46.73                           |
| 2  | 30 (°C) 15 (%)       | +1 -1 -1     | 64.54                           |
| 3  | 20 (°C) 25 (%)       | -1 +1 -1     | 72.89                           |
| 4  | 30 (°C) 25 (%)       | +1 +1 +1     | 80.75                           |
| 5  | 25 (%) 20 (%)        | 0 0 0        | 62.63                           |
| 6  | 25 (%) 20 (%)        | 0 0 0        | 60.54                           |
| 7  | 25 (%) 20 (%)        | 0 0 0        | 63.03                           |

Table 2. Regression coefficients.

| Regression coefficients | bⱼ  | tⱼ | t₀.05₂ | Meaning (Y/N) |
|-------------------------|-----|----|--------|---------------|
| b₀                      | 66.2275 | 99.0559 | 4.3    | Y             |
| b₁                      | 6.4176  | 9.5986  | 4.3    | Y             |
| b₂                      | 10.5925 | 15.8431 | 4.3    | Y             |
| b₁₂                     | -2.4875 | 3.7205  | 4.3    | N             |

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Based on Table 1, the regression equation was determined with coefficients presented on Table 2. The recursive variance and the compatible variances were determined by the equation Eq. 8 and Eq.11: \( S_{\text{th}}^2 = 1.7880; \) \( S_{tt}^2 = 24.7506; \) \( S_{bj} = 0.6685. \)

The experimental regression equation of temperature and humidity to the hexadecane mineralization rate was determined \( Y = 66.2275 + 6.4176x_1 + 10.5925x_2. \)

Where \( Y \) is the objective function that represents the hexadecane mineralization rate; \( x_1 \) is coding value of temperature; and \( x_2 \) is coding value of humidity.

The study examined the appropriateness of the regression equation by the equations Eq.10 and Eq.11: \( F_{\text{th}} = 13.9023. \)

Based on the Fisher standard table with \( P = 0.05 \), the study had \( F_{\text{th}} = 18.51 \), so the regression equation appropriated to the experiment.

\[ Table 3. \text{Values of hexadecane mineralization rate from the experiments and calculating from the regression equation.} \]

| Humidity (%) | Temperature (°C) | Values of hexadecane mineralization rate | Difference (%) |
|--------------|-----------------|----------------------------------------|----------------|
|              | Experiments     | Regression equation                    |                |
| 15 \((x_1 = -1)\) | 25 \((x_2 = 0)\) | 46.84                                  | 55.63          | 18.8           |
| 20 \((x_1 = 0)\)  | 30 \((x_2 = +1)\)  | 70.67                                  | 72.64          | 2.8            |
| 20 \((x_1 = 0)\)  | 20 \((x_2 = -1)\)  | 63.56                                  | 59.81          | 5.6            |
| 25 \((x_1 = +1)\) | 25 \((x_2 = 0)\) | 73.85                                  | 76.82          | 4.0            |

Table 3 shows the correlation between experimental results and values of hexadecane mineralization rate calculated from the regression equation.

The difference between the results of the hexadecane mineralization rate obtained from the experiment and calculated from the regression equation is not too high, from 2% to 20%.

\[ 4. \text{CONCLUSIONS} \]

The study established the experimental regression equation of temperature and humidity to the hexadecane mineralization rate, an important parameter in assessing the ability to convert organic carbon into inorganic carbon. The results showed that the humidity has a greater effect on microbial activity and contaminant degradation than the temperature. The regression equation was \( Y = 66.2275 + 6.4176x_1 + 10.5925x_2. \)

Setting up the experimental empirical regression equation enables quick assessment. Additional research is needed to determine the influence of water content and temperature in microbial activities and contaminant degradation within a range of soil textures and structures. Further studies are also necessary to determine the applicability of these results in field conditions with microbial degradation processes may be more complex, followed by the online application of monitoring soil pollutants.
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