Research Article

Statistical Modeling of Environmental Factors on Microbial Urea Hydrolysis Process for Biocement Production

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Calcium carbonate is a widely used raw material by many industries. It can be precipitated through microbial process within soil pores as cementitious bonding agent between grains for geotechnical applications. It is called microbially induced calcium carbonate precipitation (MICP). Designing an appropriate biogrout material for injection into soil is essential for controlling the amount, type, time, and place of the biocement production within pores. For this purpose, understanding the active reactions and the kinetics of bacterial growth and urea hydrolysis is necessary. A conductometric method and spectrophotometry were used in this study to, respectively, monitor the urea hydrolysis reaction progress and bacterial growth in \( S. \) pasteurii-inoculated urea-NB-NH\(_4\)Cl solution at different level of the environmental factors that are initial cell concentration, urea concentration, and temperature. Variation in conductivity of the solution versus logarithmic scale of time was depicted as microbial ureolysis characteristic curve (MUCC) through which lag duration, specific rate, and potential of urea hydrolysis at each condition were obtained. Central composite face-centered (CCF) design, which is one of the response surface methodologies, was employed to statistically fit polynomial models explaining the bacterial growth and the characteristics obtained from MUCCs in terms of the environmental factors and their interactions. An optimization analysis based on the urea-normalized responses was also carried out.

1. Introduction

Calcium carbonate is a widely used raw material by many industries. It can be precipitated in an aqueous calcium rich environment by mediation of microorganisms as catalyst. This process is called "microbially induced calcium carbonate precipitation" (MICP) which is a kind of biocementation. Injecting an appropriate biogrout material (or treatment solution) into soil can provide the MICP as a cementitious bonding agent between grains in the pores. Geotechnical engineering application of the MICP in soil pores for ground modification has been a concern of many studies since the middle of the last decade [1–13]. Among many different MICP processes, urea hydrolysis process has been more favorable due to energy efficiency [11] and ubiquity of urease enzyme-producing microorganisms [14]. Regulation and estimation of the amount, type, time, and place of this biocement production are necessary for application of the ureolytic MICP technique in soil engineering. For this purpose, understanding the active reactions and the kinetics of bacterial growth, urea hydrolysis, and calcium carbonate precipitation is essential.

Based on the recent insights, within the MICP process, the microbial urease enzyme accelerates the ammonium carbamate production by urea degradation. The ammonium carbamate is decomposed into ammonium and bicarbonate ions through a nonenzymatic and buffer-dependent reaction [15]. Higher ammonium increases the pH of the medium. Concentrated carbonate ions also start precipitating as CaCO\(_3\) in the presence of calcium ions at pH = 8.3 up to 9 [16]. The pH is then reduced back to neutral during precipitation [5]. \( S. \) pasteurii is urease-active bacteria which have been more focused in the studies.

Temperature, pH, urea concentration, calcium ion concentration, initial cell concentration, presence of other microorganisms, ionic strength of precipitation solution, existence of other types of ions (e.g., Ni\(^{2+}\), Na\(^{+}\), and Mg\(^{2+}\)), oxygen availability, type and concentration of nutrient
sources (i.e., protein and nitrogen sources, vitamins), pre-treatment, and mutating of bacterial cells are the factors influencing the kinetics of bacterial growth, its urease activity, and calcium carbonate precipitation. Many studies were performed to investigate the effect of one or some of these factors on S. pasteurii [5, 9, 16–25].

The kinetic studies in this subject use acid-base titration, ammonium ion selective electrode, spectrophotometric method, calorimetry, and electrical conductometry to monitor the reaction progress [15]. Conductometry which is an inexpensive, robust, easy-to-use, and continuous-measuring assay method was applied in this study. This method is more compatible with civil and geotechnical engineering applications.

Most of the studies investigating the kinetics of the microbial urea hydrolysis system have been conducted based on initial measurements through which the further progress is estimated. Actually the ureolysis rate is considered equal to the initial rate in this method. This method is true under the assumption of the first-order kinetics for ureolysis rate [17, 26]. In the present study, the extended measurement method was employed to check the soundness of the assumption at different environmental conditions.

Obtaining the maximum rate and amount of urea hydrolysis and calcium carbonate precipitation were the main concern of almost all the kinetic investigations in the literature, regardless of minimizing the amount of nonhydrolyzed urea and ammonium byproduct. A urea-normalized measurement was carried out in the current study to evaluate the variation of maximum rate and amount of urea hydrolysis along with amount of nondegraded urea in the system.

Except a few studies which applied advanced statistical methods for optimization of S. pasteurii growth condition [22] and calcium carbonate precipitation rate [27], the traditional one factorial method was used in the kinetic studies on MICP. The traditional method is only able to interpret the effect of an individual factor regardless of its possible interactions with other influencing factors. Such a method is usually costly and time consuming as well. In order to overcome the drawbacks of the conventional methods, response surface methodology (RSM) which is an efficient statistical method [28, 29] was employed in this study. Central composite face-centered (CCF) design, which is one of the designs describing the response surface, was used to fit a second-order model relating each response with the effect of initial cell concentration, urea concentration, and temperature. The responses were the bacterial growth, lag duration, urea hydrolysis potential, and specific rate of urea hydrolysis. Optimum condition at which the combination of the specific rate and potential of urea hydrolysis is maximized was also obtained. This paper presents the findings of such a multiresponse kinetic study using RSM, which has not been found elsewhere in the literature.

2. Materials and Methods

2.1. Bacterial Culture Medium. The urease producing bacteria used throughout the study were S. pasteurii (DSM33) grown in yeast extract-ammonium-Tris liquid medium. The medium was prepared by dissolving 20 g/L yeast extract and 10 g/L ammonium sulfate into 0.13 M Tris buffer solution (Trizma base, pH 9) separately. The solutions were then autocalved at 121°C for 20 minutes and mixed afterward. 200 mL of the mixture was inoculated with the bacteria and incubated in a 1000 mL flask for around 70 hours at 30°C and 200 rpm shaking speed to reach the desired cell concentration (OD_{600} = 1.4 equal to 1.2 × 10^9 cells/mL). It was stored at 4°C for further usage, not more than a week. The same but abiotic (without microbe) medium was also incubated in parallel to control the contamination.

2.2. Colony Counting and OD Measurement. Serial dilution method was used to find the cell concentration in the liquid growth medium. It was obtained by counting the single colonies grown on solid medium which has the same recipe as bacterial culture solution as well as 1.5% agar. Optical density of the bacterial solution at the wavelength of 600 nm (OD_{600}) was also measured using spectrophotometer. The OD_{600} value of the solution with known cell concentration (obtained from the serial dilution method) was later used to prepare bacterial solution with the same concentration.

2.3. Electrical Conductography of Microbial Ureolysis Process. The electrical conductometric method was used to monitor the microbial enzymatic urea hydrolysis reaction progress. A probe was dipped into S. pasteurii-inoculated urea-NB-NH_4Cl solution in order to simultaneously measure the temperature and electrical conductivity (E.C.) at a given constant temperature and 200 rpm shaking speed. The solution consisted of 3 g nutrient broth (NB), 10 g ammonium chloride, 2.12 g sodium bicarbonate, and varied amount of urea per liter of distilled water. The pH of the solution was adjusted to 6.5 for all the experiments. The initial concentration of bacterial cell in the solution was adjusted to be 10^6, 10^7, and 10^8 cells/mL. The bacterial solution taken for inoculation was earlier centrifuged (at 4000 rpm for 15 minutes) and the supernatant was also replaced with the fresh urea-NB-NH_4Cl solution. Pellets were mixed in the fresh solution using vortex mixer. The centrifugation process was found to have negligible effect on bacterial cell loss by counting the bacterial cells in the solution using serial dilution method before and after centrifugation. Parallel to each test, a noninoculated control solution was also observed. Since electrical conductivity of the solution was found to be temperature-dependent per se and the solution was taking some minutes to reach the given constant temperature, the electrical conductivity readings were corrected for various temperatures by using the related graphs (see Figure 1). These graphs were obtained through recording the electrical conductivity of noninoculated solution at different temperatures. Plotting the electrical conductivity changes caused by microbial activity versus logarithmic scale of time for each run resulted in a characteristic curve, which is called microbial ureolysis characteristic curve (MUCC), from which the responses investigated in the present study (except Δ(OD_{600}))
were graphically obtained (Figure 2). The MUCC is actually an indirect kinetic analysis result of the microbial urea hydrolysis process. Urea hydrolysis pattern can be determined through applying transformation to MUCC. The transformation function is the calibration curve relating the conductivity to ammonium concentration. Considering the first-order linear calibration curve [15,18], the transformation function is a constant conversion coefficient. It means the urea hydrolysis pattern is equal to MUCC multiplied by the constant coefficient.

Bacterial growth (Δ(OD₆₀₀)) was measured through spectrophotometry of the solution at the wavelength 600 nm. In order to eliminate the effect of color changes caused by various levels of degraded urea in the solutions, the solutions were earlier centrifuged and the supernatants were replaced with normal saline solution.

### 2.4. Design of Experiments (DOE)

A series of CCF designed experiments with one repetition has been carried out to investigate the effects of three factors (Table 1) and their interactions on the responses (Table 2). Each factor was considered at three levels of maximum, mid-level, and minimum which were coded as +1, 0, and −1, respectively. The CCF design contained 4 factorial points, 6 star points, and 3 center points. The design matrix was presented in Table 3. All the tests were run randomly and the data obtained from the repeated runs were averaged. Urea hydrolysis potential and specific urea hydrolysis rate are the main responses which were studied for optimization.

### 2.5. Statistical Data Analysis

The multiple regression calculations were carried out to fit a polynomial model to each response. Inverse transformation was applied to all the responses as it results in more realistic fit for asymptotic systems like biological systems [30]. The "Design-Expert" software (Stat-Ease, Inc., USA) was utilized for the analyses. The program calculates the effects for all model terms using the analysis of variance (ANOVA). A statistically significant model was detected through comparing statistics such as \( P \) value, lack of fit, and \( R^2 \)-squared values for each model. As a higher order model explicitly maximizes accuracy, the highest order model with \( P \) value less than 0.05, insignificant lack of fit, and reasonable agreement between adjusted \( R^2 \)-squared and predicted \( R^2 \)-squared (within 0.2 of each other) values was finally selected as a representative model. Normal probability plot and residual plots provided by the software were examined to check the assumptions underlying the data analysis and model fitting.

### 2.6. Optimization

A multiple response method called desirability [31] was used to find the optimum condition at which the most desirable combination of the main responses occurs. Potential and specific rate of urea hydrolysis were selected as the main responses. The optimization method which
Table 2: Responses investigated and their definitions.

<table>
<thead>
<tr>
<th>Responses</th>
<th>Unit</th>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial growth</td>
<td>—</td>
<td>Δ(OD&lt;sub&gt;600&lt;/sub&gt;)</td>
<td>Difference between initial and final optical density of the bacterial cell suspension at the wavelength 600 nm. It represents the bacterial cell growth for each experiment.</td>
</tr>
<tr>
<td>Urea hydrolysis potential</td>
<td>mS·cm&lt;sup&gt;-1&lt;/sup&gt;·M</td>
<td>P&lt;sub&gt;U&lt;/sub&gt;</td>
<td>The proportion of the maximum change in electrical conductivity of the solution to the initial urea concentration for each experiment, (ΔE.C.)/[U]&lt;sub&gt;0&lt;/sub&gt;.</td>
</tr>
<tr>
<td>Lag duration</td>
<td>min.</td>
<td>T&lt;sub&gt;lag&lt;/sub&gt;</td>
<td>Time corresponding to the intersection between tangent lines of lag phase and log phase for each experiment.</td>
</tr>
<tr>
<td>Specific urea hydrolysis rate</td>
<td>mS·cm&lt;sup&gt;-1&lt;/sup&gt;·min&lt;sup&gt;-1&lt;/sup&gt;·M</td>
<td>r</td>
<td>Rate of urea hydrolysis per initial urea concentration, r&lt;sub&gt;U&lt;/sub&gt;/[U]&lt;sub&gt;0&lt;/sub&gt;, r&lt;sub&gt;U&lt;/sub&gt; was considered as urea hydrolysis rate at log phase, (r&lt;sub&gt;U&lt;/sub&gt;/r&lt;sub&gt;U&lt;/sub&gt;)&lt;sub&gt;lag&lt;/sub&gt;, when (r&lt;sub&gt;U&lt;/sub&gt;/r&lt;sub&gt;U&lt;/sub&gt;)&lt;sub&gt;lag&lt;/sub&gt; was negligible (around 0.15 or less); otherwise it was taken as secant rate, T&lt;sub&gt;lag&lt;/sub&gt; (see Figure 2).</td>
</tr>
</tbody>
</table>

Table 3: Design matrix and corresponding observed Δ(OD<sub>600</sub>).

<table>
<thead>
<tr>
<th>Test number</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Δ(OD&lt;sub&gt;600&lt;/sub&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.416</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>−1</td>
<td>1</td>
<td>0.134</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0.348</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>−1</td>
<td>0.808</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0.271</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.381</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>−1</td>
<td>0</td>
<td>0.573</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0.128</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>10</td>
<td>−1</td>
<td>0</td>
<td>0</td>
<td>0.34</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>1</td>
<td>−1</td>
<td>0.325</td>
</tr>
<tr>
<td>12</td>
<td>−1</td>
<td>1</td>
<td>1</td>
<td>0.051</td>
</tr>
<tr>
<td>13</td>
<td>−1</td>
<td>−1</td>
<td>−1</td>
<td>1.165</td>
</tr>
</tbody>
</table>

Figure 2: Graphically obtaining the urea hydrolysis potential, lag duration, and specific urea hydrolysis rate; for example, in the above curve which is related to test number 11 (see Table 3), (ΔE.C.)<sub>max</sub> = 67.5 mS·cm<sup>-1</sup>; P<sub>U</sub> = (ΔE.C.)<sub>max</sub>/[U]<sub>0</sub> = 67.5/1 = 67.5 mS·cm<sup>-1</sup>·M<sup>−1</sup>; T<sub>lag</sub> = 280 min; (r<sub>U</sub>/r<sub>U</sub>)<sub>lag</sub> = 0.036 mS·cm<sup>−1</sup>·min<sup>−1</sup>; r<sub>U</sub> = 0.034 mS·cm<sup>−1</sup>·min<sup>−1</sup>; (r<sub>U</sub>)<sub>0</sub> = 0.048 mS·cm<sup>−1</sup>·min<sup>−1</sup>; (r<sub>U</sub>)<sub>0</sub>/(r<sub>U</sub>)<sub>lag</sub> = 1.33 > 0.15 (not negligible) so r<sub>U</sub> = P<sub>U</sub> = 0.034 mS·cm<sup>−1</sup>·min<sup>−1</sup>; r = r<sub>U</sub>/[U]<sub>0</sub> = 0.034/1 = 0.034 mS·cm<sup>−1</sup>·min<sup>−1</sup>·M<sup>−1</sup>.

3. Results and Discussions

In this study, the interdependent kinetics of bacterial growth and urea hydrolysis within the microbial ureolysis process were monitored by utilizing conductometry of the solution at different environmental conditions. The outputs versus log-time were presented as MUCC. Urea hydrolysis potential (P<sub>U</sub>), lag duration (T<sub>lag</sub>), and specific rate of urea hydrolysis (r) were the responses obtained from MUCC of each experiment. The environmental variables investigated in the present study were initial cell concentration (A), urea concentration (B), and temperature (C). The dependence of the environmental variables and their interactions on the aforementioned response as well as bacterial growth (Δ(OD<sub>600</sub>)) was evaluated using the RSM with CCF design. P<sub>U</sub> and r were considered the responses for the optimization.

Microbial ureolysis characteristic curves (MUCCs) of all the experiments were presented in Figure 3. It was observed that all the curves follow a similar pattern as bacterial growth curve including four phases: lag phase, log phase, stationary phase, and decline phase (Figure 4). At lag phase, there is not a considerable amount of urea hydrolysis. The microbial urea degradation exponentially increases at log phase. It then drastically drops at the end of the log phase where the stationary phase starts. It finally begins to decline after a period. Excluding the last phase, the curves can be presented as modified logistic functions. They reveal that the microbial urea hydrolysis process which is a function of simultaneous contribution of bacterial growth and urease generation by
cells is mainly governed by bacterial growth within the range of the study. The same growth-base logistic pattern for the kinetics of calcite precipitation through microbial ureolysis process had been proposed by Stocks-Fischer et al. [16]. Such a pattern can be attributed to the starting pH less than 8 [21].

Bacterial growth was examined for each experiment by measuring $\Delta (\text{OD}_{500})$ of the solutions. Highest and lowest bacterial growth were, respectively, observed at minimum and maximum level of both urea concentration and temperature. The RSM analyses exhibited that bacterial growth is more significantly affected by urea concentration and temperature and their interactions (Table 4; Figure 5). It indicated the inhibitory effect of higher temperature and urea concentration on bacterial growth.

Urea hydrolysis potential ($P_U$), which was defined as the maximum conductivity change to the initial urea concentration, is one of the factors expressing the cost and ecological efficiency of the process. In this study, it was detected that a lower proportion of available urea was hydrolyzed at the utmost level of urea concentration, although a greater quantity of degraded urea was obtained in the solution containing further initial urea concentration. It was statistically figured out that urea concentration had quadratic effect on urea hydrolysis potential (Table 5). The urea level corresponding to the maximum potential can be attributed to an optimum hydrolysis potential (Table 5).

Table 4: Summary of ANOVA for the bacterial growth model fit.

<table>
<thead>
<tr>
<th>Source</th>
<th>Mean square</th>
<th>$F$-value</th>
<th>$P$ value, prob. $&gt; F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>8.292E − 003</td>
<td>645.87</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>7.188E − 006</td>
<td>0.34</td>
<td>0.8053</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9994</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adj. $R^2$</td>
<td>0.9974</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pred. $R^2$</td>
<td>0.9938</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.V. %</td>
<td>0.69</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Summary of ANOVA for the urea hydrolysis potential model fit.

<table>
<thead>
<tr>
<th>Source</th>
<th>Mean square</th>
<th>$F$-value</th>
<th>$P$ value, prob. $&gt; F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>1.228E − 008</td>
<td>2105.28</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>5.578E − 012</td>
<td>0.88</td>
<td>0.5933</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9995</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adj. $R^2$</td>
<td>0.9991</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pred. $R^2$</td>
<td>0.9988</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C.V. %</td>
<td>0.14</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$$\frac{1}{(\Delta (\text{OD}_{500}) + 1.55)} = 0.51 - 1.115E^{-3} \cdot A + 0.039 \cdot B$$

$~$
Figure 5: Response surface plots of the mutual effect of the factors on bacterial growth.
Table 6: Summary of ANOVA for the lag duration model fit.

<table>
<thead>
<tr>
<th>Source</th>
<th>Mean square</th>
<th>F-value</th>
<th>P value, prob &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>1.356E−010</td>
<td>876.45</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Lack of fit</td>
<td>1.476E−013</td>
<td>0.87</td>
<td>0.5952</td>
</tr>
</tbody>
</table>

$R^2$ = 0.9989

Adj. $R^2$ = 0.9977

Pred. $R^2$ = 0.9898

C.V. % = 0.21

$$\frac{1}{(T_{1_{ag}} + 5E + 3)} = 1.912E - 4 + 6.700E - 6 + A$$
$$- 1.444E - 6 + B + 9.226E - 6 + C$$
$$- 6.239E - 6 + AC + 1.688E$$
$$- 6 + BC - 2.253E - 6 + C^2$$

Duration of lag phase, as a time point at which the urea hydrolysis starts increasing exponentially, was graphically obtained from MUCC of each experiment. The corresponding conductivity of $T_{1_{ag}}$ in MUCC represents the minimum amount of hydrolyzed urea at the onset of log phase. It manifested that a minimum limit of urease enzyme should be produced to trigger the exponential degradation. Actually the lag phase corresponded to the time required for pH shift caused by urea degradation [18]. So, starting pH of the medium is a determining parameter on manifestation that a minimum limit of urease enzyme should be produced to trigger the exponential degradation. Actually the lag phase corresponded to the time required for pH shift caused by urea degradation [18]. So, starting pH of the medium is a determining parameter on the bacterial affinity for urea. Evaluation of the specific urea degradation rate using RSM analyses indicated the quadratic effects of the initial cell concentration and urea concentration on $r$ (Table 7). The specific rates were detected to be between 0.001 mS·cm⁻¹·min⁻¹·M⁻¹ and 0.56 mS·cm⁻¹·min⁻¹·M⁻¹ which would be equivalent to 0.00066 h⁻¹ and 0.37296 h⁻¹, respectively, based on the conversion factor demonstrated after Whiffin [18]. It was depicted that the maximum rates occurred around the mid-level of initial cell concentration. Comparing the response prediction surfaces of $r$ and $r_{ij}$, it was also observed that higher urea concentration and temperature led to reduction in $r$ while they increased $r_{ij}$ (Figure 8 and Figure S1 in Supplementary Material available online at http://dx.doi.org/10.1155/2015/340930). In other words, higher proportion of available urea degraded per unit time at lower temperature and urea concentration while the amount of degraded urea per unit time decreased. Regarding initial specific urea degradation rate (Figure 9) and $r$, it was shown that a greater initial rate does not necessarily represent a higher rate of urea degradation. Therefore, initial rate measurement cannot be an appropriate index for affinity of the bacteria for urea except at the condition with too short lag duration (starting pH around 8). Initial specific rate was detected to be mainly affected by initial cell concentration.

3.1. Statistical Analysis and Optimization. The summary of statistical analysis of each response was presented in Tables 4–7 and S1. Multiple regression analyses were applied to fit a model to the results of CCF designed experiments for each response. Statistical significance of each model, parameter estimates, and lack of fit were checked using ANOVA (F-test). Considering the significance level of 5%, the values of $P < 0.05$ and $0.05 < P < 0.10$ were, respectively, accepted as significant and marginally significant [32]. The model presented for each response exhibited $R^2$ equal to 0.99 which is much greater than the minimum acceptable value of 0.60 for the RSM [33]. It means that the model can cover 99% of the possible occurring responses. Moreover adjusted $R^2$, which is another statistic confirming the significance of a model, was determined to be bigger than 0.99 for all the models in this study [33, 34]. It indicated that the models can explain 99% of the variation around the mean of the responses. Prediction $R^2$ value obtained was larger than 0.97 implying the high goodness of all the models in prediction of a response value, as it is also within 0.2 of adjusted $R^2$. Coefficient of variation (C.V. %) of 1.37% and less confirmed the RSM and reproducibility of its results. Smaller coefficient of variation shows more closeness of the predicted values to the actual ones (see the predicted versus actual curves in Figure S2).

Variation of desirability function in terms of the environmental factors was described in Figure 10. It was shown that about 97% desirability (optimum point) was achieved at the mid-level of initial cell concentration (10⁷ cells/mL)
Design-Expert software
Factor coding: actual
Original scale
Urea hydrolysis potential
$X_1 = A$: initial cell concentration
$X_2 = B$: urea concentration
Actual factor
$C$: temperature = −1

Design-Expert software
Factor coding: actual
Original scale
Urea hydrolysis potential
$X_1 = A$: initial cell concentration
$X_2 = B$: urea concentration
Actual factor
$C$: temperature = 0

Design-Expert software
Factor coding: actual
Original scale
Urea hydrolysis potential
$X_1 = A$: initial cell concentration
$X_2 = B$: urea concentration
Actual factor
$C$: temperature = 1

Figure 6: Response surface plots of the mutual effect of the factors on the urea hydrolysis potential.
Figure 7: Response surface plots of the mutual effect of the factors on the lag duration.
Figure 8: Response surface plots of the mutual effect of the factors on the specific urea hydrolysis rate.
and lowest level of urea concentration (0.1 M) and temperature (20°C). It was predicted to acquire $\Delta (\text{OD}_{600}) = 1.307$, $P = 86.9 \text{ mS cm}^{-1} \cdot \text{M}^{-1}$, $T_{\text{lag}} = 469 \text{ min}$, and $r = 0.70 \text{ mS cm}^{-1} \cdot \text{M}^{-1} \cdot \text{min}^{-1}$ under the optimum environmental condition. Some experiments including the optimum point and a control medium were run in duplicate in order to check the validity of the result. The control medium was chosen under the same condition of test number 4 from the CCF
Figure 10: Desirability variation at different levels of the factors.
Table 8: Observed and predicted responses at the optimum environmental condition.

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>Δ(OD_{600})</th>
<th>P_{LT}</th>
<th>T_{Log}</th>
<th>r_{U}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Observed</td>
<td>0</td>
<td>-1</td>
<td>-1</td>
<td>1.346</td>
<td>87.7</td>
<td>510</td>
<td>0.082</td>
</tr>
<tr>
<td>Predicted</td>
<td>1.307</td>
<td>86.9</td>
<td>469</td>
<td>0.070</td>
<td></td>
<td></td>
<td></td>
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design. The results of the experiment verified the predicted value (Table 8). The desirability versus environmental factors detected that increasing the temperature and urea concentration causes desirability reduction. It was also shown that an initial concentration around 10^7 cells/mL is more desirable at a given temperature and urea concentration. Generally, it is worth mentioning that the conditions favoring the urease generation of S. pasteurii, quantity of hydrolyzed urea, and urea hydrolysis rate inhibited the cell growth, urea hydrolysis process. They provide useful information on designing an appropriate biogrouting material with environmental conditions in order to facilitate controlling the amount, type, time, and place of biocement production within soil. It was generally found that the conditions forcing the bacteria to produce more urease enzyme and higher quantity of urea degradation suppress the bacterial growth, potential, and specific rate of urea hydrolysis. The finding is in agreement with the nature of all the biological systems in which the organisms do not work properly and efficiently under the condition which is provided for slaving not growth.

A new illustration fashion of conductometric kinetic study on microbial ureolysis process was developed. In this fashion the conductivity was shown versus logarithmic scale of time. The semilogarithmic way of description signalized the initial rate of urea hydrolysis and lag duration which was often neglected in the common kinetic curves. The curves revealed that the initial urea hydrolysis rate cannot always be an appropriate index for evaluation of the potential urease activity of the bacteria at a given condition. It was also found that all the curves follow the same pattern as microbial growth curve.

The statistical analysis employed in this study was detected to be a robust method to evaluate the effect of the mentioned environmental factors and their interactions on the investigated responses of the microbial urea degradation process.

### 4. Conclusions

Results obtained through statistical investigation of effect of the environmental factors on microbial ureolysis process in this study assist a better understanding of the MICP process. They provide useful information on designing an appropriate biogrouting material with environmental conditions in order to facilitate controlling the amount, type, time, and place of biocement production within soil. It was generally found that the conditions forcing the bacteria to produce more urease enzyme and higher quantity of urea degradation suppress the bacterial growth, potential, and specific rate of urea hydrolysis. The finding is in agreement with the nature of all the biological systems in which the organisms do not work properly and efficiently under the condition which is provided for slaving not growth.

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### Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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