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# Civil Engineering Mathematical prediction of the compressive strength of bacterial concrete using gene expression programming

Hassan Amer Algaifi <sup>a,\*</sup>, Ali S. Alqarni <sup>e</sup>, Rayed Alyousef<sup>c</sup>, Suhaimi Abu Bakar <sup>b,\*</sup>, M.H. Wan Ibrahim <sup>a,\*</sup>, Shahiron Shahidan <sup>a,\*</sup>, Mohammed Ibrahim <sup>d</sup>, Babatunde Abiodun Salami <sup>d</sup>

<sup>a</sup> Faculty of Civil and Environmental Engineering, Universiti Tun Hussein Onn Malaysia, 86400 Parit Raja, Johor, Malaysia

<sup>b</sup> School of Civil Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 Johor Bahru, Johor, Malaysia

<sup>c</sup> Department of Civil Engineering, College of Engineering, Prince Sattam Bin Abdulaziz University, Al-Kharj 11942, Saudi Arabia

<sup>d</sup> Center for Engineering Research, Research Institute, King Fahd University of Petroleum and Minerals, Dhahran 31261, Saudi Arabia

<sup>e</sup> Department of Civil Engineering, College of Engineering, King Saud University, Riyadh 11421, Saudi Arabia

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## ABSTRACT

The impact of microbial calcium carbonate on concrete strength has been extensively evaluated in the literature. However, there is no predicted equation for the compressive strength of concrete incorporating ureolytic bacteria. Therefore, in the present study, 69 experimental tests were taken into account to introduce a new predicted mathematical formula for compressive strength of bacterial concrete with different concentrations of calcium nitrate tetrahydrate, urea, yeast extract, bacterial cells and time using Gene Expression Programming (GEP) modelling. Based on the results, statistical indicators (MAE, RAE, RMSE, RRSE, *R* and  $R^2$ ) proved the capability of the GEP 2 model to predict compressive strength in which minimum error and high correlation were achieved. Moreover, both predicted and actual results indicated that compressive strength decreased with the increase in nutrient concentration. In contrast, the compressive strength increased with increased bacterial cells concentration. It could be concluded that GEP2 were found to be reliable and accurate compared to that of the experimental results. © 2021 THE AUTHORS. Published by Elsevier BV on behalf of Faculty of Engineering, Ain Shams Uni-

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# 1. Introduction

In recent years, microbial calcium carbonate has become a hotspot for research in construction engineering. It is regarded as a promising innovative technique to extend the life span of cement-based structures. This technique is used to self-heal the concrete pores and inevitable microcracks by the  $CaCO_3$  precipitation from the metabolic activity of different bacterial species such as sulphate reduction bacteria [1], ureolytic bacteria [2–4], nitrate

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reduction bacteria [5,6] and oxidation of organic bacteria [7–9]. For example, ureolytic bacteria produce a urease enzyme that breaks down urea into carbonate ions. These carbonate ions react with calcium ions resulting in the precipitation of calcium carbonate surrounding the bacterial cells, as shown in Equation (1).

$$CO(NH_2)_2 + 2H_2O + Ca^{2+ \frac{urease}{\rightarrow}} 2NH_4^+ + CaCO_3$$
(1)

In the same regard, the compressive strength of bacterial concrete has been extensively examined to assess bacteria-based self-healing efficiency in the literature. This is because concrete strength is considered a critical parameter that reflects a concrete mix's consistency and material ingredients. The compressive strength test directly relates to the quality and properties of concrete [10,11]. As such, compressive strength approach has been widely utilised to evaluate the mechanism of bacteria-based selfhealing in concrete, incorporating bacteria and related chemical compounds in the literature. However, research on optimising bacterial concrete strength factors such as urea, calcium, nutrient, and bacterial cells concentration is still required to deeply understand the reason behind either an increase or decrease the compressive







<sup>\*</sup> Corresponding authors.

*E-mail addresses*: hassanamer@uthm.edu.my, enghas78@gmail.com (H.A. Algaifi), aalqarni@ksu.edu.sa (A.S. Alqarni), r.alyousef@psau.edu.sa (R. Alyousef), suhaimibakar@utm.my (S.A. Bakar), haziman@uthm.edu.my (M.H.W. Ibrahim), shahiron@uthm.edu.my (S. Shahidan), ibrahim@kfupm.edu.sa (M. Ibrahim), salami@kfupm.edu.sa (B.A. Salami).

strength. Finding and analysing the optimum chemical concentration is necessary to promote calcium carbonate precipitation inside the cement-base matrix.

For example, Bundur et al. [12] reported that bacterial mortar's compressive strength improved (specifically, at 7, 28 and 56 days) compared to the control mix. The improvement in compressive strength was attributed to the precipitation of calcium carbonate, which filled the pores and thus enhanced the concrete microstructure. Also, Andalib et al. [13] concluded that the maximum increase in compressive strength was recorded at a bacterial cells concentration of  $30 \times 10^5$  cfu/mL, and beyond this value, the compressive strength decreased. Similarly, Durga et al. [14] demonstrated that compressive strength increased at high cells concentration of  $10^8$  cells/mL. In the same regard, the most significant increase in compressive strength was achieved at  $10^6$  cells/mL [15–17].

Moving away from bacterial cells to calcium source. Luo and Oian [18] investigated the effect of calcium sources and bacterial spores concentration on bacterial concrete's compressive strength. Based on their finding, concrete strength was improved with an increase in calcium lactate and bacterial spore from 1% to 3%. In contrast, strength decrease was observed with the increase in calcium formate and calcium nitrate. Also, Vaezi et al. [19] found that the strength of bacterial mortar improved by 21% compared to the control mix at an optimum concentration of bacterial cells (10<sup>5</sup> cells/mL) and calcium lactate (1%). This result is similar to Vijay and Murmu [20], who stated that the maximum improvement of bacterial concrete strength was recorded with the addition of 0.5% calcium lactate and bacteria. In the same context, urea and yeast extract's impact on compressive strength of mortar was also investigated by Schreiberová et al. [21]. According to their results, a notable drop in compressive strength was recorded with the addition of yeast extract. In contrast, an increase in compressive strength was noticed from the addition of urea.

From another perspective, compressive strength predictions are very important as they dramatically reduce both cost and time. This fact has prompted researches to develop a mathematical model that successfully predicts the strength of different types of concrete, however, there is not yet a predictive equation or code provisions for determining the compressive strength of bacterial concrete. Such mathematical methods have been created using artificial neural networks [22–30], neuro-fuzzy networks [31–33] and Gene expression programming (GEP) [34-36]. In particular, GEP has gained a great deal of interest in predicting concrete properties as it more efficiently explores relationships between parameters than traditional regression methods. For example, compressive strength of high performance concrete was successfully predicted using GEP with a high correlation and minimum error [37]. In addition, other properties of cementitious materials such as lightweight concrete [38], normal concrete [39] and geopolymer concrete [40] were also predicted using the GEP technique.

Considering the above, it can be inferred that the compressive strength of bacterial concrete can be either positively or negatively affected according to the concentration of urea, calcium, yeast extract, and bacterial cells. Also, the existing literature experimentally explored the impact of each parameter on compressive strength. Moreover, no research has been carried out on developing mathematical equations to predict bacterial concrete strength. Hence, the GEP model was developed to predict the compressive strength of bacterial concrete. To achieve the aim of the present study, 69 experimental tests were conducted under different concentrations of calcium (20 – 500 mM), urea (2–40 g/L), yeast extract (0.5 – 20 g/L), bacterial cells ( $10^6 - 2 \times 10^7$  cells/mL) and time (7, 14 and 28 days). Then, a novel predictive equation for determining the compressive strength of bacterial concrete was developed using GEP.

# 2. Materials and methods

### 2.1. Bacterial strain

*Lysinibacillus sphaericus* bacteria strain Hass 1 species was used to achieve the aim of this study. This bacterial strain was isolated from a soil sample from Universiti Teknologi Malaysia (UTM) grounds in our previous works. The partial DNA sequence (16S rRNA) from the isolate bacteria was deposited in the gene bank under the accession number MG928532. Also, *Lysinibacillus sphaericus* was tested for its ability to hydrolyse urea and precipitate microbial calcium carbonate. Specifically, it has proved its ability to heal the concrete crack width of 0.4 mm in our previous work [4].

## 2.2. Bacterial solution

For the bacterial solution preparation, Luria Bertani (LB) broth was first made by mixing 10 g tryptone, 5 g yeast extract, and 10 g sodium chloride in 1 L of distilled water. The pH of the solution was kept constant at 7.0. Then, the LB was autoclaved at 121 °C for 20 min and subjected to environmental cooling before use. Consequently, bacterial cells were prepared through several steps. First, a 100 mL flask filled with 30 mL LB was inoculated with the bacteria. Then, the bacterial culture was overnight incubated in a shaker (150 rpm) at 30 °C [41]. After that, the bacterial culture was kept in a cooling room (4 °C) for further use. It is interesting to note that the optimum bacterial cells concentration was measured as 2  $\times$  10<sup>8</sup> cells/mL.

#### 2.3. Concrete mix proportions

A total of 69 experimental tests were carried out to evaluate the effect of the bacterial solution and their related chemical elements on the concrete's compressive strength. The control concrete mixture ingredients were first designed to obtain the compressive strength grade of 30 MPa at 28 days based on the method set by the Department of Environment (DOE) method. In achieving this aim, the required ingredients included 425 kg/m<sup>3</sup> cement, 235 L/ $m^3$ water, 1098 Kg/m<sup>3</sup> natural sand, 618 Kg/m<sup>3</sup> granite type 10 mm aggregates and 1.2% of Rheobuild 1100 admixture. These ingredients kept constant.

Next, the bacterial concrete mix was prepared similarly. However, different concentrations of calcium nitrate tetrahydrate (20 mM – 500 mM) and urea (2 – 40 g/L) were added to the mixing water. The target morality was calculated using Equation (3) [42]. Where  $V_1$  represented the stock solution volume, and  $C_1$  was the concentration of the stock solution. Moreover,  $V_2$  is the new solution volume, and  $C_2$  is the bacterial cells' concentration of the new solution. The bacterial nutrient (yeast extract) was also added at different concentrations of 0.5 – 20 g/L. Finally, the bacterial cells concentration of  $10^6 - 2 \times 10^7$  cells/mL using Equation (2) [43].

$$C_1 \times V_1 = C_2 \times V_2 \tag{2}$$

$$Molarity(M) = \frac{weight_{(g)}}{volume_{(I)} \times molar mass_{(g/mole)}}$$
(3)

Later, cubes sizes  $(100 \times 100 \times 100 \text{ mm})$  were cast after mixing. The target cubes were then cured after 24 h in a water tank and tested at 7, 14 and 28 d according to BS EN 12390-3:2009. The compressive strength test was conducted using a 3000 kN capacity automatic compression testing machine (NL Scientific) per BS 1881 part 116 (1983). It should be noted that the constituents materials

Table	1
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Limit of involved reaction parameters.

	Urea (g/L)	Ca (mM)	Nutrient (g/L)	Cells (cell/mL)
Min.	0	0	0	$egin{array}{c} 0 \ 2  imes 10^7 \end{array}$
Max.	40	500	20	

acted as the input parameters for the proposed model. In contrast, the output of the prediction model (*Y*) was the expected compressive strength of the bacteria concrete. Also, the range of the predicted Equation is presented in Table 1.

#### 2.4. Chemical analysis

Nessler reagent method was employed to evaluate the impact and extent to which the bacteria could hydrolyse urea. As well known, bacteria decompose urea into carbonate and ammonium. As a result, one mole of urea is hydrolysed via bacterial urease enzyme into two moles of ammonium, as shown in Equation (4) [44]. As such, the amount of urea hydrolysis is calculated by measuring the ammonium concentration in the bacterial solution using Equation (5) [4].

$$CO(NH_2)_2 + H_2O \rightarrow CO_3^{-2} + 2NH_4^+ + 2H_2O$$
 (4)

urea hydrolysis<sub>(mg/L)</sub> = 
$$\frac{NH_{4(mg/L)}^{+} \times 60_{(g/mole)}}{2 \times 18_{(g/mole)}}$$
(5)

The bacteria strain was first cultured in Lysogeny broth (LB) with different urea concentrations to implement the Nessler reagent test. Four experimental tests were used at urea concentration of 50, 100, 333, 500 and 666 mM. Moreover, the bacterial solution was statically incubated at 30 °C. Then, 0.1 mL was collected and subjected to Nessler Method 8038 test in the Centre for Water Security and Environmental Sustainability (IPASA-UTM) [45]. In particular, a 50 mL centrifuge tube was filled up with 25 mL distilled water to dilute the bacterial solution. After that, three drops of polyvinyl and mineral stabiliser (each) were added to the solution. Using a pipette, 1 mL Nessler reagent was taken and mixed into the solution for one minute. Subsequently, 10 mL of the solution was transferred to the targeted cuvette in the UV–Vis spectrophotometer (Hach, DR5000 Model), which measured the amount of ammonium in the solution (mg/L).

#### 2.5. Gene expression programming GEP method

Gene Expression Programming (GEP) is an advanced machine learning technique that explores experimental data relationships. The application of GEP in civil engineering, specifically in concrete technology, is still in its infancy. In general, the process of GEP

mimics biological evolution and human genetics. As is well known, each individual has 46 unique chromosomes, half of which come from each parent [46]. The chromosome is composed of long chains of warped DNA sequences in the form of billions of genetic nucleotides composed of Cytosine (C), Guanine (G), Adenine (A), and Thymine (T) that encode all hereditary information. A specific region of human DNA is, called a gene, of about 20,000 to 30,000 genes [47]. The gene is divided into a non-coding region (introns) and a coding region (exons) according to the arrangement (sequence) of nucleotides [48]. The coding region is active and responsible for giving instructions for protein production activities, while the non-coding region does not. Due to several influential factors, gene sequences in the chromosome might be changed or modified by several operations, such as mutations shown in Fig. 1 [49]. As such, it can be said that the chromosome acts as a typical answer or perfect solution that provides deep insight into the information required for human characteristics according to variations in gene sequences.

Similarly, the simulated chromosome is a predictive solution representing a predicted equation for bacterial concrete strength and its involved parameters in this study. The initial two chromosomes (solution) are first created using an expression tree and later translated into mathematical expressions. It should also be noted that the simulated chromosome contains at least one gene with a fixed length [50]. Inspired by the human gene, the simulated gene also consists of a head (encoded function) and tail (non-encoded function) exposed to modification. The head is denoted by a constant, variable, and function, while the tail is only represented by variables and constants [51]. Also, suppose the initial solution (chromos) does not meet the fitness function requirements; in that case, it is changed and reproduced using genetic operators (mutation, crossover, and deletion), allowing old chromosomes to evolve into a new generation of offspring. This loop was repeated until a generation (new offspring with optimum values) was found with minimum errors and high correlation between the actual and predicted results as shown in Fig. 2.

A sample example was described to virtually optimise the principle Equation dependent on two variables (v, r) to understand better the genetic operators and coding procedure used in GEP. According to virtual data from experimental works, data optimisation initially began in expression trees and mathematical formula. Fig. 3 shows the structural expression tree for parents 1 and 2,



Fig. 1. Genetic codes and mutation operators of the human chromosome.



Fig. 2. The process of the gene expression programming [37].

which represent the initial solution. It can be seen that parent 1 (chromosome) contained one gene with 6 genetic codes. The genetic codes of parent 1 contained three genetic codes (-,  $\times$ , sin) that were regarded as an active coding function (head) and three codes that were related to non-coding functions (r, v, b). Similarly, parent 2 also had 6 genetic codes, of which one was considered non-coding (r). Moreover, the tree structure for the solution was read and translated using Karva language, starting from the top of the tree from left to right. The tree structure was written as a mathematical formula, as shown in Equations (6) and (7), which represents parents 1 (Y) and 2 (X). The two factors affecting the principle were denoted by r and v, while a and b were kept constant.

$$Y = (r - v) \times \sin(b) \tag{6}$$

$$X = \frac{v}{\sqrt{r} + b} \tag{7}$$

These predictive equations were exposed to fit functions such as mean error and correlation coefficient. If they did not meet the target requirements, their genes were altered and reproduced to create a new generation (offspring) through mutation, crossover, and addition. For example, Fig. 3 shows the parent genes' mutation in which another mathematical function replaced one random node (genetic code). In the same regard, the right side of the expression tree of parent 1 was replaced by another node from parent 2 to create a new generation. This new generation was evaluated and modified until it fit the target goal of the predictive Equation.

# 2.6. Proposed GEP model

The GEP model assessed the relationship between involved reaction parameters and strength evolution for the first time for bacteria-based self-healing. Also, an empirical formula for the compressive strength of bacterial concrete was constructed to reduce cost and time. The result of the mathematical formula was regarded as an output (*Y*) of the proposed model. The input variables included time (do), urea (d1), calcium (d2), yeast extract (d3), and bacterial cells (d4). 69 bacterial concrete mixes were created to provide actual data on bacterial concrete strength, as discussed in Section 2.3. GeneXproTools 5.0 software was used to develop the study model. Collected data was divided into the training phase (85% of data) and the validation phase (15% of data). It should be noted that more than 10 models were constructed in this study, and all models were tested using different genetic operators such as mutation and inversion to obtain an empirical equation that effectively estimates bacterial concrete strength. The models were also checked using different chromosomal characteristic values such as gene, linking function, head size, etc., as shown in Table 2.

In the same context, the performance of the proposed model was evaluated in both the training and validation stages using several statistics indictors. For example, Mean Square Error (MSE) was used to quantify the degree of difference between the predicted concrete compressive strength and the experimental results. The disparity between the desired output and the predicted output was minimal when the MSE value was close to zero. In other words, a more efficient model was achieved when a smaller MSE value was obtained. The MSE formula is expressed, as shown in Equation (8) [52].



Fig. 3. Virtual basic example of an expression tree.

Table 2						
Parameter	settings	for	the	GEP	algorithm	ı.

Models	No. of chromosomes	Headsize	GenesNo.	Fitness function	Mutation	Inversion	Constant per gene	No. of function
GEP 1	10	8	3	RMSE	0.0012	0.0055	10	n <sup>a</sup>
GEP 2	30	8	3	Absolute error with SR	0.0012	0.0055	10	n <sup>a</sup>
GEP 3	30	8	2	RMSE	0.0012	0.0055	10	n <sup>a</sup>
GEP 4	30	6	3	Absolute error with SR	0.0012	0.0055	10	n <sup>a</sup>
GEP 5	30	8	3	RMSE	0.02	0.0055	10	n <sup>a</sup>
GEP 6	30	8	3	RMSE	0.0012	0.10	10	n <sup>a</sup>
GEP 7	30	8	3	RMSE	0.0012	0.0055	10	n <sup>b</sup>
GEP 8	30	8	3	RMSE	0.0012	0.0055	10	n <sup>c</sup>
GEP 9	30	8	3	RMSE	0.0012	0.0055	10	n <sup>d</sup>
GEP 10	30	8	3	RMSE	0.0012	0.0055	10	n <sup>e</sup>

<sup>a</sup> n are+, -, ×, /, squrt, exp, pow 10, ln, Inv,  $x^2$ ,  $x^{(1/3)}$ , min2, max2, ave2, not.

<sup>b</sup> n are+, -,  $\times$ , /, squrt.

<sup>c</sup> n are+, -,  $\times$ , /, squrt, exp, pow 10, ln.

<sup>d</sup> n are+, -, ×, /, *squrt*, exp, *pow* 10, ln, *lnv*,  $x^2$ ,  $x^{(1/3)}$ .

<sup>e</sup> n are+, -, ×, /, squrt, exp, pow 10, ln, Inv,  $x^2$ ,  $x^{(1/3)}$ ,  $x^3$ , sin, tan.

Similarly, Root Mean Square Error (RMSE) was also used as a statistical estimator as it provides the average error, as shown in Equation (9) [53]. The coefficient of determination  $(R^2)$  was also used as a statistical estimator to evaluate the results' strength. Also,  $R^2$  provided insight into the degree of fit between the network output and the collected experimental data expressed in Equation (10) [52]. The best fit for the actual compressive strength of the bio-concrete and the predicted results were occurred by increasing the value of the determination coefficient, which is usually in the range of 0-1. Other statistical indicators were also used to assess model performance, such as Absolute Error (MAE) [54], Relative Absolute Error (RAE), Mean Root Relative Squared error (RRSE) [55] and Correlation Coefficient (*R*), as expressed in Equations (11), (12), (13), and (14) respectively. The equations below  $Y_A$  are the experimental result for concrete strength, and  $Y_P$  is the model's concrete strength. The average value of the predicted results was termed Y<sub>Pmean</sub> mean, and the number of experimental runs was represented by N.

$$MSE = \frac{1}{N} \sum_{i=1}^{N} (Y_P - Y_A)^2$$
(8)

$$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (Y_P - Y_A)^2}$$
(9)

$$R^{2} = \frac{\sum_{i=1}^{N} (Y_{A} - Y_{P_{mean}})^{2} - \sum_{i=1}^{N} (Y_{P} - Y_{A_{mean}})}{\sum_{i=1}^{N} (Y_{A} - Y_{P_{mean}})^{2}}$$
(10)

$$MAE = \frac{1}{N} \sum_{i=1}^{N} |(Y_P - Y_A)|$$
(11)

$$RAE = \frac{\sum |(Y_P - Y_A)|}{\sum |(Y_P - \frac{1}{N} \sum Y_A)|}$$
(12)

$$RRSE = \sqrt{\frac{\sum (Y_P - Y_A)^2}{\sum (Y_P - \frac{1}{N} \sum Y_A)^2}}$$
(13)

$$R = \frac{(N \sum Y_A Y_P - \sum Y_A \sum Y_P)^2}{\left(N \sum Y_A^2 - (\sum Y_A)^2\right) \left(N \sum Y_P^2 - (\sum Y_P)^2\right)}$$
(14)

# 3. Results and discussion

### 3.1. Prediction equation of bacterial concrete strength

Based on the results, a new predictive compressive strength equation for bacterial concrete was developed from model GEB 2. The GEB 2 model was found to be the best model in terms of computational performance. In addition, the solution for the GEB 2 model (the predicted Equation) was represented by a chromosome composed of 3 genes (Sub-ET 1, Sub-ET 2, and Sub-ET 3), as shown in Fig. 4. Each gene might involve one or more independent variable (d), constant (c), and mathematical operation (+, /, etc.).

GEP 2 expression tree was then mathematically formulated as shown in Equation (15), (16), (17), and (18), in which Y denotes the predicted compressive strength of the bacterial concrete. In addition, the independents variables urea, calcium, nutrient and cells concentration are represented by  $d_1$  (g/L),  $d_2$  (mM),  $d_3$  (g/L) and  $d_4$  (cells/mL) receptively. Moreover, the time value is denoted by  $d_0$  (days), and  $c_1$  is constant (4.03325632496109).

$$Y = Y_1 + Y_2 + Y_3 (15)$$

$$Y_1 = \ln(d_2) + \ln(d_1) + \min(\max(d_3, d_1), (c_1 - d_3))$$
(16)

$$Y_2 = \frac{1}{\left(d_3 - \left(\ln(d_o) + \frac{1}{d_o}\right)\right) \times d_3} \tag{17}$$

$$Y_{3} = \max\left(d_{o}, \frac{d_{o} + \ln\left(\frac{d_{2}+d_{4}}{2} \times (d_{2}+d_{4})\right)}{2}\right)$$
(18)

## 3.2. Model performance

All developed models were evaluated using statistical indicators. In general, it can be seen that the GEP 2 model was the best model, as shown in Table 3. Besides, the predicted equation for GEP 2 model involved all the influential parameters, which were 5 independent variables.

The strength of the final proposed model, that was generated based on 69 experimental tests, was evaluated using various validation methods, as shown in Table 3. Based on the results, the GEP 2 model showed its ability to predict the compressive strength with a low error and a high correlation shown in Figs. 5 (a) and (b). This is also consistent with the logical hypothesis. In particular, the efficiency and accuracy between the predicted and actual



Fig. 4. The expression tree of the predicted GEB 2 model.

results were achieved when the correlation coefficients (R) were greater than 0.8, and the value of error was minimum. In addition, Pearson correlation and p-value also proved the model's reliability to predict the compressive strength. The p-value was less than 0.05, while the Pearson correlation was 0.93, as shown in Fig. 3 (a). It can be inferred that data used (69 experimental test), which were used to generate the final GEP model, was enough. This fact is in line with Fallahpour et al. [56], who developed accurate ANFIS, MEP and GEP models using 29 datasets in which minimum error and high correlation were obtained.

Similarly, Nematzadeh et al. [55] also developed a GEP model using 36 experimental data to predict the compressive strength of concrete incorporating corrugated steel fibers and recycled PET chips exposed to elevated temperature. Based on their finding, the derived GEP equation showed its ability to effectively predict the strength in which a high coefficient of determination  $R^2$  (0.98) value and small RMSE and MAPE error values were achieved. In the same context, Gandomi and Roke [57] suggested that the actual data and independent variable ratio is three for the accepted model. The ideal model is preferable to having a ratio greater than five. In this study's model, the ratio between the actual data of compressive strength and their involved parameters was 23.

In the same context, different statistical check, namely,  $R^{2}$ , was also taken into account to verify the developed present model.  $R^{2}$  is an important criteria that provides insight into the fitness level between the actual and predicted results of the proposed model. Shahmansouri et al. [11] demonstrated that both the predicted and actual results could be considered close results if  $R^{2}$  is greater than 0.7, and the accuracy is improved when it approaches 1.  $R^{2}$  was found to be 0.8–0.9 in previous research on genetic expression

Table 3

RRSE 0379 0.639 0.436 0 5 0 2 0.625 1.031 0.464 0727 0 301 0.510 0.681 1.073 0 5 4 4 0.855 0.433 0.437 0.603 0.706 0.288 0.466

Statistical parameters for predicted compressive strength of bacterial concrete.							
Models	phase	No. of variables	R	$R^2$	MAE	RMSE	RAE
GEP 1	training	3	0.928	0.862	1.359	1.777	0.342
	validation		0.827	0.865	1.539	2.206	0.715
GEP 2	training	5	0.930	0.865	1.32	2.04	0.334
	validation		0.920	0.846	1.01	1.56	0.472
GEP 3	training	2	0.792	0.627	1.975	2.933	0.497
	validation		0.388	0.151	1.962	3.270	0.912
GEP 4	training	5	0.897	0.805	1.625	2.177	0.409
	validation		0.781	0.609	1.507	2.305	0.701
GEP 5	training	5	0.954	0.911	0.997	1.412	0.251
	validation		0.871	0.758	1.079	1.616	0.501
GEP 6	training	5	0706	0.577	2.525	3.192	0.636
	validation		0.457	0.209	2.659	3.402	1.236
GEP 7	training	4	0.844	0.712	1.910	2.551	0.481
	validation		0.591	0.350	1.852	2.711	0.861
GEP 8	training	4	0.904	0.818	1.601	2.030	0.403
	validation		0.930	0.865	1.066	1.387	0.495
GEP 9	training	4	0.838	0.703	2.381	2.827	0.599
	validation		0.851	0.724	1.823	2.241	0.847
GEP 10	training	4	0.959	0.919	1.037	1.351	0.261
	validation		0.903	0.816	1.244	1.478	0.578



Fig. 5. GEP 2 model efficacy (a) correlation (b) error.

programming. For example, the predictive model's performance for ferrosialate geopolymer strength exhibited an  $R^2$  value between 0.88 and 0.92 [58]. The generated present model also had a high  $R^2$  value of 0.893 and 0.851 for the training and validation processes, respectively, as shown in Fig. 7. Hence, it can be inferred that the proposed model is accurate in predicting the compressive strength of bacterial concrete. This is also proved by the closeness of the fitting line for  $R^2$  and the consistency between the experimental data and predicted results shown in Fig. 7. In other words, the predicted results were close to actual data on bacterial concrete strength. Indeed, this fact is also consistent with the actual mechanism and evolution of bacterial compressive strength. As is known, bacteria, urea, calcium and nutrient are added to the concrete matrix. At early age, concrete matrix involves pores, microcracks, and interconnectivity pores which facilities the movement of these chemical ions. Fig. 6 shows the movement of these chemical ions towards the concrete pore. Then, bacteria located inside the concrete pore started to hydrolyse urea into carbonate ions, while the calcium ions stick into the bacterial cell wall due to charge differences (see Fig. 6).

Consequently, calcium carbonate is precipitated and deposited on the bacterial cell wall owing to the chemical reaction between calcium and carbonate ions. This process continues until filled the concrete pores and microcracks with calcium carbonate and thus enhances the concrete strength. However, the improvement of concrete strength is not high in which its value about 10% compared to that of the control concrete without bacteria. This is because the bacteria have a limited capacity to hydrolyse urea, as discussed in Section 3.3.2. This mechanism is similar to the output of the proposed model. The predicted results showed that the bacterial concrete strength improved with the increase of urea concentration up to 20 g/L (333 mM). Beyond this value, there is no significant change in strength. In addition, the predicted result indicated that the bacterial concrete strength increased up to 10% compared with the control mix.

#### 3.3. Parametric analysis of concrete strength (CS)

The effect of the involved parameters on the compressive strength of bacterial concrete was simulated using GEP. One parameter was varied during the analysis, and the rest were fixed at a constant value. According to the results, the effect of bacterial cells had a crucial role in precipitating calcium carbonate and improving concrete strength. This is discussed in detail in the flowing section.



Fig. 6. Evolution of bacterial concrete strength.



Fig. 7. Curve fitting of the GEP 2 model.

# 3.3.1. The effect of bacterial cells and nutrient concentration on CS

The impact of bacterial cells concentration on concrete strength was measured and evaluated using both experimental and predicted results. It can be seen that there is good agreement between the experimental results and the predictions of the GEP model. Fig. 8 (a) shows that the enhancement of concrete strength increased with a concomitant increase in bacterial cells concentration on both serious. This positive finding can be attributed to two reasons. The first is that the bacterial cells act as a micro filler material that fills concrete pores and micro-cracks inside the concrete matrix. The second is that the bacterial cells play an essential role in calcium carbonate precipitation by producing urease enzyme. By increasing the number of bacterial cells, more urease enzymes will be produced. In turn, this urease enzyme hydrolyses urea to carbonate, which reacts with calcium to form CaCO<sub>3</sub> on the cells surface. This calcium carbonate will flocculate and encapsulate the ureolytic bacteria, forming a large bacterial aggregate over time [59].

Consequently, the evolution of the bacterial aggregate is responsible for plugging cracks or capillary pores inside the matrix. This outcome enhances the concrete characteristics, which is in line with previous works. For example, Okwadha and Li [60] stated



Fig. 8. Predicted and actual evolution of concrete strength as a function of (a) bacterial cells concentration and (b) yeast extract concentration.

that a greater calcium carbonate deposition is linked to a higher bacteria cell concentration. In another study, microbial calcium carbonate precipitation enhanced concrete strength by filling concrete pores and micro-cracks inside the concrete matrix using another *Bacillus* bacterial species [61].

In contrast, increasing yeast extract concentration significantly decreased the compressive strength of bacterial concrete, as shown in Fig. 8 (b). At yeast extract concentrations greater than 5 g/L, compressive strength significantly declined. This is because a high concentration of yeast extract may hinder the cement hydration reaction. Similar results were also reported by Bundur et al. [12], who stated that the incorporation of 20 g/L yeast extract had a negative impact on concrete strength. Similarly, the predicted output and experimental data for yeast extract concentration were similar. In addition, only a slight improvement in compressive strength was achieved when the concentration of yeast extract was lower than 3 g/L. As such, it can be said that the contribution of yeast extract concentration to concrete strength enhancement was not significant.

## 3.3.2. The effect of urea and calcium concentration on (CS)

The compressive strength of bacterial concrete was also examined at different concentrations of urea and calcium. Fig. 10 (a) clearly shows the relationship between concrete strength enhancement with increased urea concentrations. Both experimental and predicted results showed a high degree of similarity. Concrete strength gradually increased with increased urea concentrations. Urea is an essential material because it acts as a source of carbonate ions in the presence of ureolytic bacteria. In particular, ureolytic bacteria produces urease enzymes, which intracellularly hydrolyse urea into carbamate and ammonia, as shown in Equation (19) [44]. Subsequently, the hydrolysis of carbamate was continued to induce an extra mole of carbonic acid and ammonia, according to Equation (20). After that, these compounds would develop into two moles of ammonium and hydroxide ions and one mole of bicarbonate, as shown by Equations (21) and (22). Finally, the pH level will rise and disrupts the bicarbonate equilibrium. As a result, carbonate ions would form.

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \tag{19}$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \tag{20}$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^-$$
 (21)

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \tag{22}$$

It can be seen that with more carbonate ions, more calcium carbonate is precipitated. This positive action is in good agreement with Balam et al. [2], who stated that the improvement of bacterial concrete depends on the available amount of urea as it is an essential source of calcium carbonate. Moreover, the optimum urea concentration for improving concrete strength was 20 g/L, as, beyond this value, an insignificant increase in concrete strength was observed. This could be due to limited ureolyric activity, which denoted the hydrolysis of urea by bacterial urease enzyme inside the concrete matrix. Indeed, the bacteria might reach its capacity to hydrolyse urea even with the increase of urea concentration. This fact is consistent with our experiment test, as shown in Fig. 9. It can be seen that the ureolytic activity (urea hydrolysis) increased with an increase of urea concentration up to 333 mM (20 g/L). Beyond this value, the amount of urea hydrolysis was not significant.

In the same regards, different dosages of calcium concentration were also used to acquire a maximum compressive strength with optimum concentrations of urea, yeast extract, and bacterial cells. The most significant improvement of concrete strength was achieved at calcium concentrations of 250 mM, as shown in Fig. 10 (b). Beyond this, the efficiency of bacterial activity was decreased. In other words, excessive amounts of calcium did not result in high bacterial cells inside the concrete matrix, preventing them from producing urease enzymes. This fact adversely affects





Fig. 9. Evolution of ureolytic activity under different concentration of urea.

Fig. 10. Predicted and actual evolution of concrete strength as a function of (a) urea concentration (b) calcium concentration.



**Fig. 11.** Predicted and actual evolution of concrete strength as a function of time (7, 14, 28 days).

the formation of calcium carbonate, which is consistent with Zhang et al. [62], who found that calcium concentrations of more than 60 mM reduced ureolytic activity and hindered the growth of bacteria. Similarly, the GEP model proved its ability to predict bacterial concrete strength at different calcium concentrations with reasonable accuracy compared to the experimental results.

#### 3.3.3. The effect of time on (CS)

The evolution of compressive strength of concrete was also investigated at intervals of 7, 14 and 28 days, as shown in Fig. 11. In the first week, the concrete strength enhancement rate was higher than the later stages in both the experimental and predicted results. This is attributed to differences in the porosity of the concrete matrix between the early and later stages. In the early stages, the movement of involved chemicals such as nutrients, calcium, and urea is smooth since the concrete matrix is still porous compared to later ages. These chemicals are necessary to implement the reaction that produces calcium carbonate. This result is in good agreement with Nain et al. [15], who stated that the bacteria faced difficulties to get the required amounts of calcium, urea, and nutrients in later stages due to the decreasing of concrete porosity over time. Kadapure et al. [63] also stated that the maximum value of CaCO<sub>3</sub> precipitation could be achieved during the early stages. This is because the bacteria's viability is affected in the later stages due to decreased concrete pores that host the bacteria. Specifically, bacterial cells may not acquire enough space to be accommodated as the concrete becomes denser over time. In addition, this fact exposed the bacterial cells to compression [64]. Therefore, encapsulation or a bacterial carrier technique is recommended to preserve bacterial longevity inside a concrete matrix over the long term. Researchers have recently attempted to evaluate the efficacy of said techniques for bacteria concrete use [65– 69].

# 4. Conclusion

In recent years, microbial calcium carbonate products have been widely examined and recognised as a sustainable strategy for improving concrete compressive strength. However, no studies have developed an empirical equation to predict the compressive strength of bacterial concrete. As such, in this study, A Gene Expression Programming (GEP) was used to predict the compressive strength of bacterial concrete under different concentrations of calcium, urea, yeast extract, and bacterial cells. Based on the results, the proposed GEP 2 model proved its ability to predict bacterial concrete strength with values similar to the experimental results. Also, the evolution of strength gain depended significantly on the number of bacterial cells, while increasing the nutrient concentration negatively affected strength. It can be concluded that the results of the proposed model relatively matched the experimental data.

# **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**Hassan Amer Aljaifi** obtained his first degree of civil engineering at Mosul university (Iraq-2001), master (2011-2013) and PhD at UTM (2016-2019) from UTM. He has an extensive practical experience (12 years) in the field of structural engineering; in particular, concrete engineering, analysis and design of structural elements. NOW, he is post-doctoral fellow at UTHM, Malaysia.