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Optimum concentration of *Bacillus megaterium* for strengthening structural concrete

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HIGHLIGHTS

- *Bacillus megaterium* was introduced in concrete to obtain optimum concentration of bacteria.
- Microbial calcite precipitation was quantified using conventional techniques.
- Higher strength of structural concrete was obtained.

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ABSTRACT

Five different cell concentrations of *Bacillus megaterium* (10×10^5 to 50×10^5 cfu/ml) were introduced in structural concrete to achieve the optimum concentration of bacteria. The significant increase in the strength was obtained in the case of 30×10^5 cfu/ml at different ages. The strength of highest grade of bacterial concrete had improved (24%) as compared to lowest grade (12.8%) due to calcification mechanism. Microbial calcite precipitation was quantified using X-ray diffraction analysis, visualized by scanning electron microscopy and analyzed by energy dispersive spectrometer. It was found that the optimum concentration of *B. megaterium* had a positive effect on high strength structural concrete.

1. Introduction

Currently the application of concrete is continuously increasing throughout the world due to its basic ingredient availability. It is obvious that cement as the main ingredient of concrete, has higher environmental impact on global warming since 10% of total CO_2 emission is due to cement industry. Therefore the making of sustainable concrete is one of the immediate requirement for environmental justification. Hence, it has become necessary that studies in relevance of using natural materials gain more attention.

Admixtures with filling capacity can be added to concrete to improve its characteristics. There are some natural waste materials which have greater potential as partial replacement of ordinary Portland cement to manufacture less porous concrete. Construction problems and mix design complexity using waste materials are reasonable evidence for the need of another type of concrete. Bacterial concrete is a novel research domain and can be used for cementitious materials that cure itself automatically using the mechanism of bio-mineralization. The idea of introducing bacteria in concrete, is to precipitate calcite in pores and tiny cavity areas.

The presence of pores and micro-cracks in the hydrated cement paste can highly influence the concrete characteristics and it can provide a path through which moisture, chlorides, carbon dioxide and other aggressive agents can penetrate. Mostly the micro-cracks without suitable and immediate attention can expand, thus causing the deterioration and weakening of the concrete strength.

Based on literature, in almost all of the previous studies, the concentrations of bacteria or number of colonies are not mentioned as the ingredient and bacteria are usually purchased from the culture collection centers [1–11]. In this study, different cell concentrations of bacteria were introduced in concrete to obtain

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optimum concentration of bacteria which can increase its strength significantly. Bacteria was directly isolated from the tropical environment and its different concentrations were extracted based on the appropriate serial dilution factor.

Bacillus is a type of bacteria that can produce as a binding filler material to decrease concrete capillary pores to improve its strength and durability. There are some species of *Bacillus* that produce urease enzyme to precipitate calcite associated with bio-mineralization [1,3,12]. It was found that the bio-mineralization process will not interfere with the setting time of concrete [13]. Hence any concrete mix design standards can be used for bacterial concrete.

Based on the bio-mineralization mechanism ($CaCO_3$ precipitation), this new technique can significantly reduce the maintenance cost required for bacterial concrete due to its longer life span growth, which will subsequently reduce atmospheric CO_2 emission to help in partially reducing global warming thereby reducing the demand for cement. In accordance to the research of Wu et al. research (2012), the equations show a sequence of biochemical reaction that occurs to form calcium carbonate in cementitious material with the help of ureolytic bacteria [14].

The current study demonstrates that bio-mineralization mechanism in cementitious materials can be an appropriate technique to improve the structural concrete characteristics. Hence, in order to obtain greater ability to realize the effects of bacteria in concrete, investigation on its characteristics was found to be necessary.

2. Experimental programme

2.1. Materials

In this study, ordinary Portland cement (OPC) complied with Type I of Portland cement in accordance to Malaysian Standard MS 522 (2007) and ASTMC150–05, 2005 was used. The chemical composition of OPC (mass%) is shown in Table 1. Fine sand with a fineness modulus of 2.8 in saturated surface dry condition, 10 mm aggregates, and normal water as mixing agent was applied during the experiment to prepare concrete specimens. The components of concrete were calculated based on DOE method in accordance to the British standard [15].

2.1.1. Sieve analysis and DO meter output

The 10 mm coarse and fine aggregate sieve analysis results are shown in Table 2 and 3 for comparing with grading standards based on BS 882: 1992 specification. However the cumulative percentage of fine aggregates passing through 0.6 mm sieve was about 40.8%, the cumulative percentage of coarse aggregates retained on 5 mm sieve was around 92.04%. It was found that coarse aggregate sieve analysis result complies with British standard in presence of single-sized 10 mm aggregate, whereas the sand sieves analysis data is as per the requirements of BS in zone M.

Normal tap water was applied in making of structural bacterial and ordinary Portland cement concrete. The water quality is based on the amount of dissolved oxygen (DO) (less than 60% or over 125%) are assessed to be as inadequate or poor quality, PH ranging from 6 to 8 is also recommended. The results obtained for mixing water using DO meter is shown in Table 4. It is seen that the amount of DO and PH used was around 63% and 6.5 respectively. Therefore, the water quality used in this study for making concrete was in acceptable range.

2.1.2. Concrete mix design results

Concrete mix design was carried out to obtain the desired strength of structural concrete based on the DOE method of British standard. Table 5 demonstrates the concrete mix proportions for different grades of structural concrete. The normal type of cement (OPC) with two types of aggregate (crushed and uncrushed) and a different ratio of W/C (0.38–0.53) with free water quantity (213.34 kg/m^3) was applied to achieve the desired compressive strength.

Table 1
The chemical composition of OPC (mass%).

Si O_2	Al_2O_3	Fe_2O_3	CaO	MgO	Na_2O	K_2O	P_2O_5	LOI*
43.1	5.0	2.6	46.0	1.1	0.2	0.5	0.2	1.3

* Loss on ignition.

Table 2
Coarse aggregates (10 mm) sieve analysis.

Sieve size (mm)	Weight of aggregate retained (gram)	Percentage of aggregate retained (%)	Cumulative percentage of aggregate retained (%)	Cumulative percentage of aggregate passing (%)
14	0	0.00	0.00	100.00
10	738	14.76	14.76	85.24
5	3864	77.28	92.04	7.96
2.36	350	7.00	99.04	0.96
Pan	48	0.96	100.00	0.00

Sum = 5000 g.

Table 3
Fine aggregate (sand) sieve analysis.

Sieve size (mm)	Weight of sand retained (gram)	Percentage of sand retained (%)	Cumulative percentage of sand retained (%)	Cumulative percentage of sand passing (%)
5	6.0	1.20	1.20	98.80
2.36	49.0	9.80	11.00	89.00
1.18	112.0	22.40	33.40	66.60
0.6	129.0	25.80	59.20	40.80
0.3	108.8	21.76	80.96	19.04
0.15	68.0	13.60	94.56	5.44
Pan	27.2	5.44	100.00	0.00

Sum = 500 g.

2.2. Isolation and identification of bacteria

The soil sample (1 g) collected from a silty clay location was suspended in 10 ml of nutrient broth containing (peptone 5.0 g/L, yeast Extract 3.0 g/L, distilled water) in a sterile conical flask. The flasks were placed in water bath (100 °C for 10 min) equipped with incubator shaker at 37 °C for 24 h to create spore forming bacteria respectively. After sufficient growth, a loopful of nutrient broth containing soil sample was streaked onto nutrient agar plates containing (peptone 5.0 g/L, yeast Extract 3.0 g/L, agar. agar 12.0 g/L, distilled water). Eventually pure colonies were obtained using streak plate technique. In this study, calcium lactate (80 g/L) and urea (20 g/L) were added to the culture medium for the purpose of bio-mineralisation [8,16]. The bacterial identification was carried out as to identify the unknown bacteria present in the culture medium. The procedure for the bacterial selection and identification includes phase I and II of the study as shown in Fig. 1.

2.3. Mix proportion of specimens

The mix design proportion can be specified as the procedure for selecting appropriate ingredients of concrete such as cement, aggregate, water and calculating their ratio as to attain the desired strength. This process comprises of two parts, the first purpose was to obtain the required strength and the second purpose was to make concrete in a most economical way. For bacterial concrete, the required amount of bacteria is introduced to the concrete with mixing water. The application of bacterial cells in mixing water could be the easiest way to cause bio-mineralization especially for calcite deposition in cement based materials. Because of the presence of the microorganism, mixing on low volume is recommended.

Carbon, nitrogen, vitamins and other material are the significant ingredients of nutrient broth, which the microorganisms require for their metabolism. However, excessive nutrient broth will have a negative impact on the mechanical characteristics of concrete due to its chemical effect; so appropriate amount of nutrient broth is needed for its metabolism. According to Andrew et al. (2012), the most economical and efficient percentage of liquid culture medium applied to concrete was 5%, which can improve its designed compressive and flexural strength [17]. In this current study also five percent of mixing water as bacterial broth medium culture was introduced for making bacterial concrete. The biomass concentrations was obtained by repeated serial dilutions, repeated spread and streak plate techniques to fix as desired.

2.4. Micro-structural investigation on bacterial concrete

The micro-structural investigation was carried out as to confirm the appropriate concentration of bacteria that is required for making concrete through the testing of compressive and flexural strength. In order to obtain the relationship between the