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ARTICLE

Evaluation of the Relationship between Bacteria Concentration and the Strength and Durability of Self-compacting Concrete Incorporating *Sporosarcina pasteurii*

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ABSTRACT

This research was carried out to evaluate the relationship between the incorporation of calcite precipitation bacteria, *sporosarcina pasteurii* using calcium lactate as nutrient source and the properties of calcined clay and limestone powder blended self-compacting concrete. Ten mixes were designed and designated S0 to S9 with S0 the control (without bacteria and nutrient) and S1 to S9 at varying bacteria and calcium lactate concentrations and the effect of the bacteria cell density and calcium lactate concentration on the compressive strength, sorptivity and tensile strength with age were evaluated using experimental program and statistical packages (ANOVA and post hoc tests). The result of both the experimental program and statistical evaluation shows that the incorporation of *sporosarcina pasteurii* and calcium lactate as nutrients had a positive impact on the properties of the ternary blended self-compacting concrete.

Keywords: Sporosarcina pasteurii; Calcium lactate; Ternary self-compacting concrete; Statistical evaluation; Strength and durability

1. Introduction

Self-compacting concrete is generally the more expensive and less environmentally friendly version of concrete in terms of the high cement content requirement [1]. Since the production of self-compact-

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ing concrete requires the use of large quantities of cement and fines, the former being expensive and eco-unfriendly, this leads to an escalation of the overall cost of construction using this very important construction material. Thus, the production of SCC using the required quantities of cement is not sustainable due to the fact that sustainability can only ensue with the use of cheaply and readily available local construction materials [2]. There is thus a compelling need to use readily available and relatively cheaper cementitious alternatives and fines (fillers) in the production of SCC while not compromising its flowability, filling ability, segregation resistance, and strength as well as durability characteristics of SCC. The use of calcined clay and Limestone powder will alleviate, to a large extent, the cost and environmental effect associated with SCC production, and they have been used successfully in normal vibrated concrete [3]. More so, the durability of SCC can be enhanced by an improvement of its pore structure by positively altering its permeation properties. One way this can be done is by the application of Microbial Induced Calcite Precipitation technology (MICP) in SCC, which could lead to an improvement in the pore characterization of SCC and hence its durability. The combined use of MICP technology via the application of calcite precipitation bacteria and the use of ternary blends of cement, limestone powder and calcined clay will solve multiple problems associated with SCC production and use, namely; environmental impact, economy, strength and durability characterization.

The use of statistics in solving engineering problems is well documented [4]. Statistical packages like ANOVA and post hoc packages like Tukey HSD, Scheffé, Holm and Bonferroni multiple comparison analysis tools can be used to evaluate the extent of the relationships between variables and this act to complement the results of experimental programs.

This research uses ANOVA from Microsoft Excel and post hoc packages from National Institute of Standards and Technology (NIST), US department of Commerce to evaluate the statistical relationship between the incorporation of bacteria in concrete and its effect on the properties of SCC.

2. Methodology

2.1 Preparation of cementation reagent

The following composition was used in the first instance in preparing the cementation reagent.

Nutrient broth (calcium lactate)
Urea: 20 gm
Ammonium chloride: 10 gm
Sodium hydrogen carbonate: 2.12 gm
Calcium chloride: 2.8 gm
Distilled water: 1000 mL

Calcium lactate was used as the nutrient source and it was dissolved in one litre of distilled or sterile water to produce a solution of desired concentration of 0.01 mol/L. Three different quantities of this solution were used corresponding to 0.5, 1.0 and 2.0% by weight of cement. The composition listed above was weighed and dissolved in 1 litre of distilled water in a conical flask and cotton flogged. The media were then sterile in an autoclave at a temperature of 110 °C for 10 minutes allowed to cool before use.

2.2 Bacteria isolation and inoculation

The prepared media are cotton flogged and sterilized in an autoclave at a temperature of 110 °C for 10 minutes, allow the media to cool completely before inoculation of the bacteria. The ureolytic bacteria (*sporosarcina pasteurii)* was isolated from fresh soil by sub-culturing in 1 L of sterilized nutrient bough and the media was incubated at 35 °C in an orbital shaker for 10 days at 125 rpm. The bacteria growth was determined in terms of optical density by measuring the rate of absorbance at a wavelength of 500 nm. The isolate was purified using the streak plate technique on nutrient agar and the bacteria isolate was identified. The quantification of the bacteria was carried out by using a spectrophotometer. A blank solution of 0.5 mL was placed in the spectrophotometer at a wavelength of 500 nm and the reading was taken. The blank solution was replaced by the bacteria solution of 0.5 mL at the same wavelength and the concentration of the bacteria was measured using the relation $y = 8.59\times10^7 z^{1.3627}$ where y is the bacterial concentration per mL and z is the reading at OD600. After the media have cooled, the conical flasks are labeled using masking tape then a standardized incubator of the bacterial isolate will be inoculated. A standardized incubator is a bacterial suspension whose turbidity is compared with that of the McFarland turbidity standard. The standard starts from a scale of 0.5 to 9, and each scale is representing a bacteria cell density. After inoculating the media with the standardized bacteria, the conical flasks were incubated in an incubator at a temperature of 37 °C for 24 hours before use. For this research, the bacterial cell density used corresponded to a McFarland turbidity scale of 0.5, 2.0 and 4.0.

2.3 Mix design

The method of mix design that is adapted for this work is based on the plastic viscosity of the SCC mix, which was first proposed by Karihaloo & Ghanbari and Deeb & Karihaloo [5,6]. It exploits the expression for the determination of the plastic viscosity of a heterogeneous material like SCC from the known plastic viscosity of the homogeneous component (in this case the cement paste). It is based on the micromechanical procedure developed by Ghanbari & Karihaloo [7].

For the self-compacting concrete incorporating *sporosarcina pasteurii* and calcium lactate, ten mixes were designed and designated S0 to S9 with S0 the control (without bacteria and nutrients) and S1 to S9 at varying bacteria and calcium lactate concentrations.

Table 1 shows the mix compositions used for developing the different mixes at different bacterial concentrations and nutrient content.

2.4 Strength characterization

The compressive strength of the self-compacting concrete incorporating *Sporosarcina pasteurii* was determined using 100 cubic millimeters of concrete cubes cured at 7, 14, 28 and 56 days in accordance with the provisions of BS EN 12390-3 [8]. For each mix, three cubes were used and the average value was taken, with a total of 120 cubes used in the determination of the compressive strength of the calcined clay and limestone powder blended SCC. Cement was replaced partially with 15% calcined clay and using limestone powder as filler.

The tensile strength was determined using a diameter of 100 by 200 cylinders at 7 and 28 days by means of the split tensile strength test by crushing the cylinders longitudinally on a compressive strength test machine in line with the provisions of BS EN 12390-6 [9]. Three specimens were used for each test and the average value was taken. A total of 60 cylinders were used in the investigation of the tensile strength of the self-compacting concrete incorporating *Sporosarcina pasteurii.*

Table 1. Mix designation and proportions for bio self-compacting concrete.

Mix	Bacteria Conc. Cfu/mL	Calcium lactate (% of cement)	Cement (kg)	Calcined clay (kg)	Superplasticizer (kg)	Limestone filler (kg)	Sand (kg)	Coarse aggregates
S0	-	-	11.56	2.04	0.083	4.1	25.9	28.3
S1	1.5×10^8	0.5	11.56	2.04	0.083	4.1	25.9	28.3
S2	1.5×10^8	1.0	11.56	2.04	0.083	4.1	25.9	28.3
S3	1.5×10^8	2.0	11.56	2.04	0.083	4.1	25.9	28.3
S4	1.2×10^9	0.5	11.56	2.04	0.083	4.1	25.9	28.3
S5	1.2×10^9	1.0	11.56	2.04	0.083	4.1	25.9	28.3
S6	1.2×10^9	2.0	11.56	2.04	0.083	4.1	25.9	28.3
S7	2.4×10^9	0.5	11.56	2.04	0.083	4.1	25.9	28.3
S8	2.4×10^9	1.0	11.56	2.04	0.083	4.1	25.9	28.3
S9	2.4×10^9	2.0	11.56	2.04	0.083	4.1	25.9	28.3

2.5 Permeation characterization

The permeation properties of the SCC were evaluated using sorptivity which was carried out to determine the susceptibility of the unsaturated concrete to the penetration of water through capillarity by determining the increase in the mass of the specimen resulting from absorption of water as a function of time when only one surface is exposed to water [10]. The test was carried out at 28 and 56 days for each mix to evaluate the short and long-term effects of the SCM and filler materials on the rate of water absorption through interconnected capillary poles. Three diameters 100 by 50 discs, cut from 100 by 200 concrete cylinder specimens, were used for each test and the average result was calculated. A total of 90 discs cut from 30 cylinders were used for the determination of sorptivity for Bio-SCC.

2.6 Statistical validation

The analysis of variance was carried out using statistiXL package in Microsoft Excel while the post hoc tests were carried out using engineering statistical packages from the National Institute of Standards and Technology (NIST), US Department of Commerce.

3. Results and discussion

3.1 Compressive strength relationship

The strength properties of the calcined clay and limestone powder blended self-compacting concrete incorporating *sporosarcina pasteurii* as calcite precipitation agent at different bacterial cell density and nutrient content was measured using compressive strength at 7, 28 and 56 days and tensile strength at 7 and 28 days. The result for the change in compressive strength with age of concrete, bacterial cell density and nutrient content is presented in **Figure 1**. It can be seen from **Figure 1** that the compressive strength changes with the bacterial density, nutrient concentration and age of concrete. Generally, the strength increases as the concrete ages, with the highest strength recorded at 56 days of curing. Also the SCC mix with bacteria and nutrients shows higher strength than the control (without bacteria). This is because, the bacteria, in the presence of moisture, use up the nutrient and deposits calcium calcite which also improves the strength development. This, in addition to cement hydration and the pozzolanic reaction accounts for the improved strength development with age. For a given bacteria concentration, using 0.5% calcium lactate maximized the compressive strength, with a decrease in the rate of strength

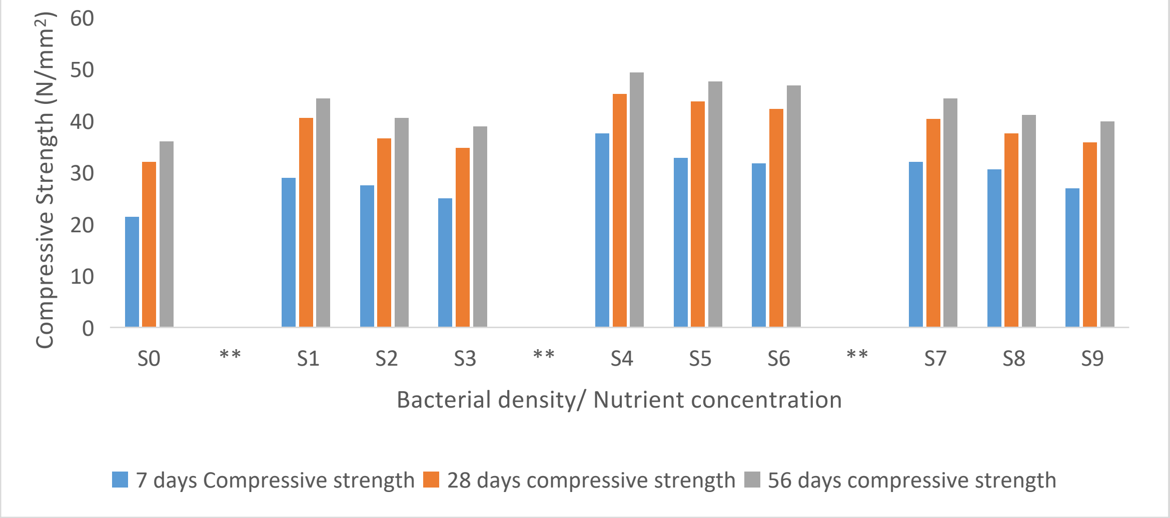

Figure 1. Relationship between bacterial concentration and compressive strength.

development with increasing calcium lactate content. The over-production of calcium carbonate crystals, which lowers the quality of the micro-structure resulted in a lower rate of strength gain at higher calcium content [11].

Also, materials added into concrete that do not contribute to the hydration process could impede strength development [10]. The gain in strength is due to the densification of the pore system due to the precipitation of calcium carbonate by the bacteria cells [11]. According to a recent study, it can be inferred that adding different bacterial concentrations leads to two different types of healing in concrete, namely, surface healing and inner matrix healing. At the surface region, as the availability of water is equal for all the samples, the precipitation is only dependent upon the bacterial concentration. Since a greater number of bacterial cells can precipitate a higher amount of calcite, the maximum amount of precipitation at the surface region of the mortar takes place at the highest cell concentration [12]. Thus, all the test results directly related to the surface region of the mortar, such as surface crack and pore healing and reduction in water penetration depth, exhibit better performance at the highest cell concentration (2.4×10^9 cfu/mL). However, the high calcite precipitation almost blocks the surface pores, and that leads to lower availability of water inside the mortar matrix. This is correlated with the result of research by Mondel & Ghosh, who state that there is an optimal bacteria cell concentration beyond which the strength could be adversely affected [13].

Also, the gain in strength from age 7 to 28 days is higher than that from age 28 to 56 days. This could be due to reduced hydration with age and reduced bacteria activity. Generally, bacteria activity reduces or even stops as the concrete environment gets more alkaline. Thus, with increased hydration and calcium hydroxide production, the concrete matrix gets more alkaline, thereby affecting the bacteria activity and hence calcium calcite production.

Analysis of Variance (ANOVA) was used to evaluate the relationship between the compressive strength, calcium lactate content and bacterial cell density as well as the age of concrete. The null hypothesis postulates that there is no significant relationship between the compressive strength, the calcium lactate content and bacterial cell density as well as the age of concrete. This hypothesis holds true when the p-value is less than 0.05 and the $F_{critical}$ is more than the F value, otherwise, the alternate hypothesis (there is a significant relationship between the variables) is adopted. The result is presented in **Table 2**.

Table 2. ANOVA showing the relationship between compressive strength and other variables.

Summary						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Bacteria Conc.	10	1.13E+10	1.13E+09	1E+18		
Calcium lactate	10	10.5	1.05	0.525		
7 days	10	294.3	29.43	20.45789		
28 days	10	388.6	38.86	17.87822		
56 days	10	428.7	42.87	18.19789		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**Fcrit**
Between groups	1.01E+19	4	2.53E+18	12.64045	5.64E-07	2.578739
Within groups	9.01E+18	45	2E+17			
Total	1.91E+19	49				

The result in **Table 2** gives an F value of 12.64045 which is higher than the $F_{critical}$ value of 2.578737. Also the p-value of 5.64×10^{-7} is less than 0.05. In fact, what it means is that there is a less than 0.0001% chance of the null hypothesis being true. The null hypothesis is therefore discarded and the alternate hypothesis is adopted. Namely there is a significant relationship between the parameters. The ANOVA result however does not tell us which parameters have a definite significant relationship. To identify that, a post hoc test is carried out to identify the extent of the relationship between the variables. The true relationship between the variables is determined using the post hoc test known as the Bonferroni and Holm multiple comparison tests, where A, B, C, D and E are the Bacteria Density, Calcium lactate (nutrient content), 7 days, 28 and 56 days' Compressive strengths respectively, and the result is presented in **Table 3**. It can be seen that there is a positive and significant relationship between the bacteria density and each of the other parameters as expected, with both the Bonferroni and Holm P-values less than 0.01 in those relationships. There are however no significant relationships between B and C, B and D, B and E, C and D, C and E as well as D and E, with the p-values well above 0.05. The result of the statistical tests agrees with the experimental result.

3.2 Sorptivity relationship

The relationship between the rate of permeation of substances through the concrete with the age of concrete, bacteria density and nutrient concentration was evaluated using the sorptivity of the concrete. The result is presented in **Figure 2** and it can be seen that the rate of water absorption decreases as the concrete ages, with the 56 days concrete showing better sorptivity than the 7 days concrete.

Also, for each age of concrete, the rate of water absorption (sorptivity behavior) decreased as the bacteria cell density and nutrient concentration increased. This can be seen with the trendlines in **Figure 2**.

The reduction of pore size as a result of calcium carbonate deposition is the main reason for reduced water absorption through the interconnected pores. The reduction in sorptivity with additional calcium lactate (calcium source) and bacterial concentration is due to the additional calcium lactate acting as a catalyst for further deposition of calcium carbonate [13,14].

The filling of the pores by the products of cement hydration as well as the products of the pozzolanic reaction could also account for some reduction in the sorptivity, hence the reduced absorption by S0 with age even though it contains no bacteria and nutrients [15-18]. The statistical relationship between the sorptivity,

Table 3. Bonferroni and Holm significance test.

treatments pair	Bonferroni and Holm T-statistic	Bonferroni p-value	Bonferroni inference	Holm p-value	Holm inference
A vs B	5.6215	1.1346e-05	** p < 0.01	1.1346e-05	** p < 0.01
A vs C	5.6215	1.1346e-05	** p < 0.01	7.9425e-06	** p < 0.01
A vs D	5.6215	1.1346e-05	** p < 0.01	9.0771e-06	** p < 0.01
A vs E	5.6215	1.1346e-05	** p < 0.01	1.0212e-05	** p < 0.01
B vs C	0.0000	9.9999998	Insignificant	5.9999999	Insignificant
B vs D	0.0000	10.0000000	Insignificant	3.0000000	Insignificant
B vs E	0.0000	10.0000000	Insignificant	1.0000000	Insignificant
C vs D	0.0000	9.9999999	Insignificant	3.9999999	Insignificant
C vs E	0.0000	9.9999998	Insignificant	4.9999999	Insignificant
D vs E	0.0000	10.0000000	Insignificant	2.0000000	Insignificant

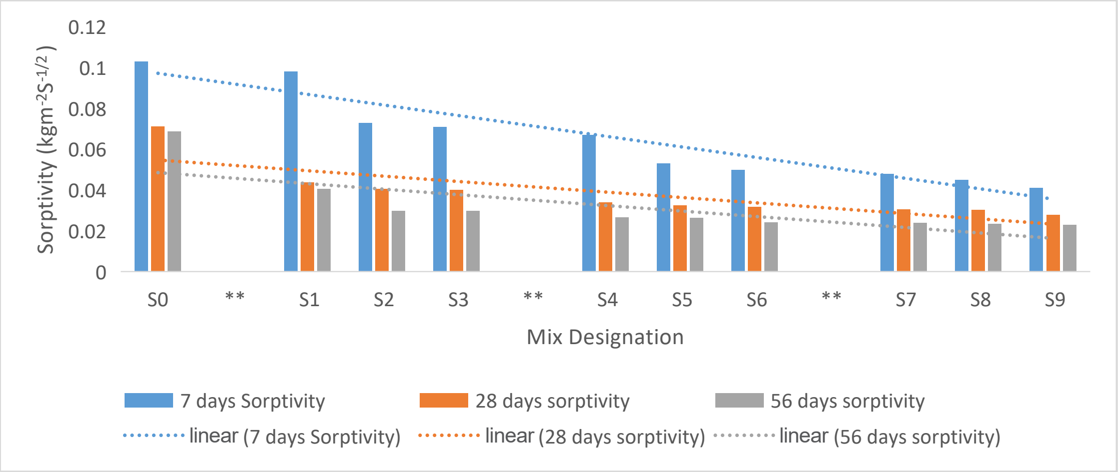

Figure 2. Variation of sorptivity with concrete properties.

age of concrete, bacteria cell density and calcium lactate concentration is evaluated using one-way ANOVA and the result is presented in **Table 4**.

The null hypothesis postulates that there is no significant relationship between the variables and within them, while the alternate hypothesis postulates a significant relationship. As can be seen from **Table 4**, the null hypothesis does not hold because the P-value is less than 0.05. The p-value of 5.64×10^{-7} shows that there is almost no probability of the null hypothesis being true, and it is thus discarded. This is further confirmed by the F value (12.64045) being far more than the critical value of 2.578739. The alternate hypothesis is thus accepted that there is a significant relationship between and within the variables under investigation. A post hoc test carried out to determine where the exact relationships lie using Scheffé's multiple comparisons is given in **Table 5**.

It can be seen that Scheffé's p-value of less than 0.01 holds true for the relationship between bacteria content and nutrient concentration as well as the age of the concrete. There however exists no significant relationship between the nutrient content and the sorptivity at different ages or within the sorptivity at different ages. It has been shown that the effect of nutrients is restricted to it being used up by the bacteria to deposit calcium calcite. This result agrees with the result of the experimental program presented earlier [19].

Table 4. Result of one-way ANOVA for sorptivity.

Summary						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Bacteria Conc.	10	1.13E+10	1.13E+09	1E+18		
Calcium lactate	10	10.5	1.05	0.525		
7 days	10	0.651	0.0651	0.000476		
28 days	10	0.383	0.0383	0.000161		
56 days	10	0.3171	0.03171	0.000196		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**Fcrit**
Between groups	1.01E+19	4	2.53E+18	12.64045	5.64E-07	2.578739
Within groups	9.01E+18	45	2E+17			
Total	1.91E+19	49				

Table 5. Scheffé's post hoc test on sorptivity.

treatments pair	Scheffé T-statistic	Scheffé p-value	Scheffé inference
A vs B	5.6215	6.5174e-05	** $p < 0.01$
A vs C	5.6215	6.5174e-05	** $p < 0.01$
A vs D	5.6215	6.5174e-05	** $p < 0.01$
A vs E	5.6215	6.5174e-05	** $p < 0.01$
B vs C	0.0000	1.0000000	Insignificant
B vs D	0.0000	1.0000000	Insignificant
B vs E	0.0000	1.0000000	Insignificant
C vs D	0.0000	1.0000000	Insignificant
C vs E	0.0000	1.0000000	Insignificant
D vs E	0.0000	1.0000000	Insignificant

3.3 Tensile strength relationships

The relationship between bacteria cell density, nutrient content and tensile strength at 7 and 28 days is plotted in **Figure 3**, As expected, the variation in tensile strength with curing age, bacteria concentration and nutrient content takes the same trend as the compressive strength. This is because of the positive correlation between compressive and tensile strengths [20]. The same explanation for this trend holds for tensile strength as earlier explained for compressive strength.

The result of the ANOVA test to determine the possibility of a significant relationship between the bacteria content, calcium lactate content, and the tensile strength at 7 and 28 days is given in **Table 6**, with the null hypothesis postulating that there is no significant correlation between the bacteria content, the nutrient content and the tensile strength at 7 and 28 days.

The F value (12.64045) is far greater than the F

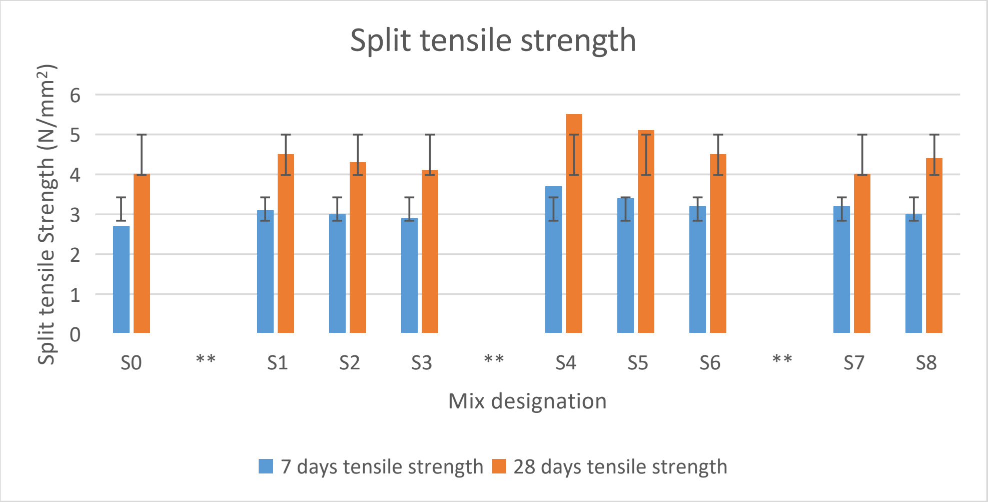

Figure 3. Tensile strength testing.

Table 6. ANOVA test on tensile strength.

Summary						
Groups	**Count**	**Sum**	**Average**	**Variance**		
Bacteria Conc.	10	1.13E+10	1.13E+09	1E+18		
Calcium lactate	10	10.5	1.05	0.525		
7 days	10	31.1	3.11	0.081		
28 days	10	44.41	4.441	0.25281		
ANOVA						
Source of Variation	**SS**	**df**	**MS**	**F**	**P-value**	**Fcrit**
Between groups	9.49E+18	3	3.16E+18	12.64045	8.51E-06	2.866266
Within groups	9.01E+18	36	2.5E+17			
Total	1.85E+19	39				

critical (2.866266) and the P-value (8.5×10^{-6}) is far less than 0.05, which means that the null hypothesis is rejected. It means that there is a significant relationship between the bacteria content, the nutrient content and the strength at 7 and 28 days.

To determine where the exact relations lie statistically, the Tukey HSD test is carried out on the result of the ANOVA test, using NIST Engineering Statistics Package. The result is presented in **Table 7**.

Table 7. Tukey HSD result for tensile strength.

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inference
A vs B	7.1107	0.0010053	** $p < 0.01$
A vs C	7.1107	0.0010053	** $p < 0.01$
A vs D	7.1107	0.0010053	** $p < 0.01$
B vs C	0.0000	0.8999947	Insignificant
B vs D	0.0000	0.8999947	Insignificant
C vs D	0.0000	0.8999947	Insignificant

It can be seen from **Table 7** that there is a significant relationship between bacteria density and the calcite content as well as the strength at 7 and 28 days, with a Tukey P-value of less than 0.01. There is however no significant relationship between the calcium lactate content and the strength at 7 and 28 days and within the strengths. This shows that the bacteria content affects the usage of the calcium lactate as well as the tensile strength development. This is in agreement with results presented earlier.

4. Conclusions

The incorporation of calcite precipitation bacteria into calcined clay and limestone powder blended self-compacting concrete positively impacted the strength and permeation properties of the concrete at all ages. The statistical evaluation using ANOVA and the post hoc tests using the Tukey HSD test and Scheffé, Bonferroni and Holm multiple comparisons show that there is a positive and significant relationship between the bacteria dosage and the nutrient content, as well as the age of concrete and the effect on the concrete properties. The use of *sporosarcina pasteurii* at different bacterial cell densities and calcium calcite concentrations is recommended as it has a positive impact on the strength and permeation properties of the ternary SCC.

Author Contributions

Engr Dr. K. Taku Designed the experiment and supervised the Laboratory work and proofreading and correction of the draft manuscript

Engr. T. Agber carried out some of the laboratory work to generate the data as well as wrote the draft Manuscript.

Engr Dr. B. H. S. Amartey supervised the experimentation, carried out the statistical design as well proofread and corrected the manuscript.

Conflict of Interest

There is no conflict of interest.

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