Hindawi
Advances in Materials Science and Engineering
Volume 2022, Article ID 3387928, 15 pages
https://doi.org/10.1155/2022/3387928

Research Article

Effects of *Lysinibacillus sphaericus* on Physicomechanical and Chemical Performance of OPC Blended with Natural Tuff and Pulverized Fly Ash

Kelvin Mwangi Wanjiku,[1] **Jackson Wachira Muthengia**,[1] **Joanne Ogunah**,[1] **Daniel Karanja Mutitu**,[3] **John Kinuthia**,[4] **Romano Mwirichia**,[2] **Joseph Karanja Thiong'o**,[5] **Munyao Onesmus Mulwa**,[5] **Muriithi Genson**,[1] **Peter Waithaka**,[6] **and David Musyoki Munyao**[1]

[1]*Department of Physical Sciences, University of Embu, Embu, Kenya*
[2]*Department of Biological Sciences, University of Embu, Embu, Kenya*
[3]*Department of Physical Sciences, Machakos University, Machakos, Kenya*
[4]*School of Engineering, University of South Wales, Newport, UK*
[5]*Department of Chemistry, Kenyatta University, Nairobi, Kenya*
[6]*Department of Physical and Biological Sciences, Murang'a University of Science and Technology, Murang'a, Kenya*

Correspondence should be addressed to Kelvin Mwangi Wanjiku; 1314@student.embuni.ac.ke

Received 26 February 2022; Revised 7 May 2022; Accepted 16 May 2022; Published 3 June 2022

Academic Editor: Robert Černý

This paper reports study findings on the use of *Lysinibacillus sphaericus* (at 1.0×10^7 cells/ml concentration) to enhance and improve the physicomechanical and chemical properties of blended Ordinary Portland Cement (OPC). Blending was done separately with pulverized fly ash (PFA) and natural pozzolana (volcanic tuff) at substitution levels of 10%, 20%, 30%, 40%, 50%, 60%, and 70%. Mortar prisms of dimensions 40 mm by 40 mm by 160 mm were prepared and cured for the 2nd, 7th, 28th, 56th, and 90th days using L*ysinibacillus sphaericus* solution as mixing water and curing media. Commercial OPC and PPC mortar prisms cast and cured using distilled water were used as controls. Results showed that prisms treated with bacteria exhibited the highest performance on compressive strength development. Further microstructure analysis of blended cement incorporated with L*ysinibacillus sphaericus* bacteria showed significant amounts of reacted secondary cementitious materials compared to samples without bacteria. Bacteria presence was also found to reduce water demand during mixing and setting times and exhibited low porosity in relation to samples without bacteria. These results showed that the presence of alkaliphilic bacteria in the blended cement resulted in synergistic effect in enhancing the physicochemical and mechanical properties.

1. Introduction

The need to reduce carbon (IV) oxide emissions, a major greenhouse gas, from Ordinary Portland Cement (OPC) production has led to innovative ways of producing cementitious materials [1–4]. Environmental friendly pozzolanic materials have been used to substitute a fraction of OPC due to their richness in amorphous silicates and aluminates [5, 6]. The silicates and aluminates react with lime produced through the hydration of OPC. This reaction further produces secondary hydration products such as calcium silicate hydrate (C-S-H) [7–10]. Such secondary hydration products enhance the physicochemical properties of blended cement and concrete [2, 11].

One of the challenges of making substituted or blended cement is the low level of substitution (usually 25 %–30%) of supplementary cementitious materials (SCMs) by the mass of the cementitious binder [12]. Beyond 30% substitution,

the resultant blended cement exhibits poor physicochemical properties below most standard requirements [13, 14]. The properties include a lower degree of hydration reaction, poor compressive strength development, and highly porous cement with poor durability in the aggressive environment [9, 10, 15].

Various improvements have been made to enhance the performance of highly substituted cement. They include high-temperature curing, use of alkali activating chemicals, and applying a mixture of blended SCMs [8, 16]. However, such improvements are associated with major shortcomings, such as increased unhydrated clinkers and early ettringite production from certain activators (Na_2SO_4 and K_2SO_4) [13, 14]. Further, the activators also suppress the additional output of portlandite (Ca $(OH)_2$), which is the main precursor to the formation of secondary hydration products such as C-S-H. Also, microstructure analysis of highly blended cement shows significant amounts of unreacted SCM attributed to slow pozzolanic reaction [13, 14, 17].

Ureolytic bacteria solution has been used to improve the physicochemical properties of OPC and PPC. Incorporating such bacteria has been attributed to the formation of more C-S-H and calcium carbonate ($CaCO_3$) precipitation, increase in strength development, and reduction of permeation properties [18–21]. To significantly lower the cost of the mortar/concrete in real application of bacteria solution as mixing water, nutrients and cells not necessarily of high purity grade as those in laboratory scale have been used in other researches [22]. Such inexpensive nutrient source and medium ingredients that have been applied include lactose mother liquor and corn steep liquor [22, 23]. To further reduce cost and make bacteria solution application in mortar/concrete, nonaxenic ureolytic spores have been produced and applied which are more economical than pure bacteria culture [24].

Existing research shows there has been little focus on incorporating these beneficial bacteria in blended cements made by over 30% substitution level. This paper finding reports on the use of L*ysinibacillus sphaericus* bacteria to enhance the performance of highly substituted (over 30%) cement in regard to physicochemical and mechanical properties improvements. From the findings of this study, combining the use of beneficial bacteria with blended cement made of PFA and natural pozzolana (NP) resulted in synergistic effect by improving the physicomechanical and chemical performance of OPC blended with PFA and NP. The increase in the properties was only recorded in samples treated with bacteria unlike those treated with distilled water. OPC was substituted with volcanic tuff and PFA from 0% to 70% separately where positive results were obtained.

2. Material and Methods

2.1. Materials. Ordinary Portland Cement (OPC) and Portland Pozzolana Cement (PPC) conforming to [25] were obtained from Savannah Cement Limited in Athi River town, Kenya. Standard sand used conformed to ISO 679: 2009 and EN 196-1. The sand was sourced from Xiamen ISO Standard Sand Company Limited in China. Pulverized Fly Ash (PFA) was obtained from Savannah Cement Limited, Kenya. Natural pozzolana was obtained from local deposits of volcanic origin in Nguruga, Kajiado County, Kenya. The natural pozzolana was crushed and dried in an oven to constant weight for 24 hours at 105°C to eliminate free water. The dried material was cooled and pulverized to achieve the desired fineness. The chemical analysis of the test materials used is given in Table 1 as obtained from the X-ray fluorescence analysis.

Actively growing L*ysinibacillus sphaericus* bacteria spores (DSM 28) were sourced from Leibniz Institute DSMZ, Deutsche Sammlung von Germany. Analytical grade reagents used during the preparation of bacteria medium were obtained from Kobian Scientific Kenya Limited.

2.2. Methods

2.2.1. Growth of Lysinibacillus sphaericus. *Lysinibacillus sphaericus* bacteria solution was prepared using the respective microbial spores in the University of Embu microbiology laboratory. The bacteria cultures were grown following BS EN (1999) protocol in an alkaline medium. The media consisted of nutrient broth weighing 16.0 g, calcium acetate (3.95 g), $NaHCO_3$ (42.0 g), Na_2HCO_3 (5.30 g), yeast extract (0.1 g), NH_4Cl (0.2 g), $CaCl_2$ (0.225 g), $MgCl_2.6H_2O$ (0.2 g), $MnSO_4.2H_2O$ (0.01 g), and citric acid (5.16 g) in a 1000.0 ml conical flask. The salts, more so chloride salts, were added in traces where the amounts were insignificant to promote any appreciable effect in corrosion of the reinforcement or impair the properties of the cement or the concrete/mortar made from the cement. The additives were also regulated by the Kenya standards of cement where the total quantity of additives did not exceed 1.0% by mass of the cement (KS EAS 18-1: 2017).

The medium was autoclaved for 15 minutes at 121°C. Carbonate salts were added to the media after cooling by filter sterilization. Sterilized water was added to the stock to make up to 1 liter. 100.0 ml of the bacteria spores was inoculated to the stock and properly sealed using cotton wool. The bacteria solution was kept for 24 hours at a temperature of 25°C in a Stuart BJPX-200B orbital laboratory incubator shaking at 150 rpm. The bacteria growth was monitored until an optical density (OD) of 1.0 (an equivalent of 1.0×10^7 cells/mL) was obtained. A 600 nm UV/VIS Spectrometer was used to record OD and evaluate microscopic organisms' development. This solution concentration was kept constant and used as mixing and curing water.

2.2.2. Normal Consistency and Setting Time. Normal consistency and setting times for OPC, PPC, and blended cement samples were determined following KS EAS 148-3: 2017. To establish a standard consistency value, 500.0 g of cement sample was weighed using analytical balance and transferred to a mixing bowl to make a crater. Using a burette, a trial volume of water was added to the crater. The cement and water were introduced into an automatic mix machine (model number: JB/T10391-2008) and

Table 1: Results of chemical analysis of the test materials used.

Oxide (%)	OPC	PPC	PFA	Volcanic tuff
SiO_2	20.312	33.510	64.207	63.20
Cao	64.35	42.801	0.810	1.740
Al_2O_3	5.731	7.240	22.800	17.21
Fe_2O_3	3.524	5.400	6.701	8.161
Na_2O	0.194	0.080	0.010	0.230
K_2O	0.402	1.030	0.031	1.104
SO_3	2.316	1.811	—	—
MgO	1.204	0.780	0.180	0.300
LOI	1.519	2.300	2.242	4.821
Residue (45 μm)	4.200	3.231	2.814	3.001

homogeneously mixed. The resultant fresh paste was directly transferred into a setting time mold previously lightly greased. Vicat apparatus with needle-like pointer was used to determine normal consistency. The plunger of the Vicat apparatus was observed to rest at 6 ± 1 mm as per the standard requirements. This was in agreement with the standard requirements. This method was done for each type of cement. To test the effect of bacteria solution in normal consistency and setting times, distilled water was replaced with *Lysinibacillus sphaericus* solution. The same procedures for setting time and normal consistency were followed. . The difference occurred only in the needle application for setting time, which was different from normal consistency. The Vicat needles for the initial and final setting time were applied appropriately as per the standards.

2.2.3. Mortar Preparation and Curing. Mortar preparation was done following KS EAS 18-1:2017. The test mortars were prepared from commercial OPC and PPC as controls. The laboratory simulated cement was made from blended OPC with PFA and volcanic tuff at 10%, 20%, 30%, 40%, and 50%, respectively. The 10% NP blended OPC was labeled as 10% NP. The same analogy was used to label the OPC blended with NP at varied levels as 20% NP, 30% NP, 40% NP, 50% NP, 60% NP, and 70% NP. 0.5 water-to-cement ratio (w/c) or microbial solution was used as mixing water to prepare the mortar.

For the bacteria solution as mixing water, it was presumed that 0.5 w/c would make a consistent paste. This ratio was workable with volcanic tuff, but the workability with PFA was poor and watery. Therefore, a lower ratio (0.4) was used for PFAC to make it workable. In this study, two (2) w/c ratios, i.e., 0.4 and 0.5, were used. Molds of dimensions 40 mm by 40 mm by 160 mm were used for mortar prism preparation as defined in Kenyan standards (KS EAS 18-1: 2017). Mortar molding was done in the Physical Laboratory of Savannah Cement Limited, Kenya. In each cement, two categories were prepared, i.e., with and without bacteria solution as mixing and curing water labeled as PFAC and PFAC-B and NP and NP-B where 'B' referred to mortars with bacteria. In all experiments, the prepared prims after casting were cured in a 95% relative humidity environment with a temperature maintained at $20^{\circ}C \pm 2^{\circ}C$ for 24 hours. After 24 hours, demolding was done, and the prisms cured in the respective curing media were kept at room temperature after the 2nd, 7th, 28th, 56th, and 90th days. The curing media consisted of distilled water for the control specimens and bacteria solution for the test samples. Table 2 shows the mixture proportions of cements.

2.2.4. Compressive Strength Determination. For each sample preparation, 1350.0 g of standard sand was placed in an automatic sand dispenser. 450.0 g cement was put into the bowl of an automatic mixer containing 225.0 ml of distilled water (or *Lysinibacillus sphaericus* solution for bacteria samples). The automatic mixing machine was started and run for four (4) minutes. After mixing, the mortar bowl was removed and placed on a jolting table. The mortar was placed half-filled in greased prisms and leveled, and 60 jolts were applied for compaction. The mortar was then filled, and the other 60 jolts were taken for compaction. A straight edge blade was used to saw off excess material and level the mortar prisms. The prisms were then kept in a cabinet maintained at $25 \pm 1^{\circ}C$ with relative humidity of not less than 90% for 24 hours. After 24 hours, the molds were demolded, labeled, and submerged in the respective curing tanks maintained at $25 \pm 1^{\circ}C$ and relative humidity of not less than 90% for separate curing days.

Compressive strength development reported in MPa was determined on the 2nd, 7th, 28th, 56th, and 90th day of curing in triplicate for all samples with and without bacteria. An automatic compression testing machine (model YAW-300) from Savannah Cement Limited, Kenya, was used. Kenyan standards for compressive strength testing were used for the test (KS EAS 18-1: 2017). The test prism was centrally placed on the compressive machine plate longitudinally such that its face overhung by about 10 mm. The load was increased smoothly at the rate of 2400 ± 200 N/s over the entire prism until fracture.

2.2.5. Scanning Electron Microscopy Analysis (SEM). The cement samples at the 28th day of curing from both experiments (with and without bacteria) were prepared for SEM analysis as described by Scrivener et al. [26]. After 28 days of curing, the samples were oven-dried for 24 hours at $105^{\circ}C$. Isopropanol was used to eliminate any microbial contamination. Waterproof resin ERL-4206 was used to impregnate the hardened cement mortar for SEM analysis. SEM analysis of the cement samples was determined using Zeiss Ultra Plug FEG-SEM.

2.2.6. Determination of Porosity. Porosity of all samples with and without bacteria was determined at the 28th day of curing. The test samples were dried at $105^{\circ}C$ for 24 hours in a ventilated oven to remove the water of hydration prior to the analysis. Achal et al. 2013 method was followed to determine sorptivity coefficient, k, using equation

$$\frac{Q}{A} = kt^{1/2}. \quad (1)$$

Q is the measure of water ingested, A is the cross-area of the sample in contact with water, and t is the time of

Table 2: Mixture proportions of cements.

Components of mortars					
Binder	CEMI ($kg{\cdot}m^{-3}$)	PFA ($kg{\cdot}m^{-3}$)	NP ($kg{\cdot}m^{-3}$)	Sand ($kg{\cdot}m^{-3}$)	Mixing water ($1\ m^{-3}$)
OPC	450	—	—	1350	225
PPC	450	—	—	1350	225
10% PFAC	405	45	—	1350	225
20% PFAC	360	90	—	1350	225
30% PFAC	315	135	—	1350	225
40% PFAC	270	180	—	1350	225
50% PFAC	225	225	—	1350	225
60% PFAC	180	270	—	1350	225
70% PFAC	135	315	—	1350	225
10% NP	405	—	45	1350	225
20% NP	360	—	90	1350	225
30% NP	315	—	135	1350	225
40% NP	270	—	180	1350	225
50% NP	225	—	225	1350	225
60% NP	180	—	270	1350	225
70% NP	135	—	315	1350	225

exposure. Equation of a straight line was plotted to obtain k value. The % water sorption gain at each interval was obtained using equation

$$\%\text{Hsgain} = \frac{(W_{\text{is}}) - (W_{BS})}{(W_{BS})} \times 100, \tag{2}$$

where % H_s gain is the calculated % water sorption gain of the mortar, W_{is} is the weight of the mortar prism at i^{th} interval of immersion into water where i in this study ranged from 15 minutes to 168 hours, and W_{Bs} was the weight of the prism before immersion into water.

3. Results and Discussion

3.1. Effect of Lysinibacillus sphaericus Bacteria on Normal Consistency. Table 3 shows the normal consistency for mortars prepared and cured with *Lysinibacillus sphaericus* bacteria compared to those prepared and cured in distilled water.

As seen from the results in Table 3 OPC paste exhibited a low water demand compared to PPC paste when distilled water was used as mixing water. Mutitu et al. [20]; Kanta & Btech [27]; and Sahoo et al. [29] made similar observation. High water demand in PPC was attributed to porosity nature of natural pozzolana (NP) present in PPC [29]. The micropores in NP particles enhance water uptake, thus increasing water demand in PPC mixtures.

However, substituting distilled water with *Lysinibacillus sphaericus* solution as mixing water reduced the water demand required to obtain a standard consistency, i.e., from 28.0 to 27.7 for OPC and 35.01 to 32.5 for PPC (Table 3). Elsewhere, use of *Bacillus licheniformis* was found to reduce water demand (from 30.5% to 29.0%) in concrete made from OPC [30]. On contrast, results using degrading bacteria (*Thiobacillus intermedius*) increased normal consistency from 28.0 to 28.4 [31].

Reduction in water demand was attributed to the presence of L*ysinibacillus sphaericus* bacteria and its feeds, e.g., calcium chloride ($CaCl_2$). Presence of $CaCl_2$ salt in cement has been found to reduce water demand [29]. In addition, calcium chloride accelerates the pozzolanic reaction in PPC [9]. Further, according to [32, 33], bacteria presence serves as nucleation sites for hydration lowering hydration energy [32, 33]. With the reduction in a hydration energy barrier, hydration reaction was hypothesized to proceed with less water needed. This research found that $CaCl_2$ salts and bacteria present accelerated hydration mechanisms where, usually, water presence initiates the hydration reaction. It was therefore concluded that, in the presence of bacteria and $CaCl_2$ salts, less amount of water was needed to cause the cement hydration process. However, the amount of $CaCl_2$ salts was introduced in trace not to cause any appreciable effect on steel corrosion used in cement reinforcement.

Substituting OPC with PFA resulted in decrease in normal consistency (Table 3). As the substitution dosage increased, the normal consistency (NC) values decreased further. Elmrabet *et al.* noted a similar observation [34]. The authors attributed this effect to a high fineness of PFA particles. These particles acts as a filler and ball-bearing, enhancing workability. In addition, the particles facilitate the movement of adjacent particles with ease, requiring less water to achieve the needed consistency and flowability [35, 36]. The same observation has also been attributed to the glassy and spherical nature of fly ash particles [37]. Further, PFA particles with spherical nature exhibit less resistance to the free movement of Vicat's plunger. Therefore, the higher the PFA substitution, the less the water needed to enhance workability and consistency.

Substituting distilled water with L*ysinibacillus sphaericus* bacteria as mixing water for OPC substituted with PFA resulted in decrease in NC further (Table 3). Paper [30] showed similar findings. The authors noted that use of *Bacillus licheniformis* solution reduced OPC normal consistency from 30.5% to 29.0%. Similar findings were obtained by [20, 28]. The authors attributed the reduction in water demand to bacteria feed ($CaCl_2$). However, it was

Table 3: Normal consistency (NC) for all categories of test samples.

Sample	NC (%) with distilled water	NC (%) with *Lysinibacillus sphaericus*	Sample	NC (%) with distilled water	NC (%) with *Lysinibacillus sphaericus*
OPC	28.0	27.7	PPC	35.0	32.5
10% PFAC	28.8	28.0	10% NP	30.5	29.2
20% PFAC	27.8	27.6	20% NP	31.6	30.6
30% PFAC	27.0	26.7	30% NP	32.0	30.8
40% PFAC	26.8	26.4	40% NP	33.5	32.2
50% PFAC	26.6	26.0	50% NP	35.8	32.8
60% PFAC	26.0	25.5	60% NP	36.6	33.6
70% PFAC	25.5	25.0	70% NP	36.8	33.8

maintained that the salts were in very little quantities that may not significantly affect other physicochemical properties of cement.

Substituting OPC with natural pozzolana (NP) of volcanic tuff origin resulted in increase in water demand when distilled water was used as mixing water. The higher the substitution, the higher the increase in water demand (Table 3). Similar observations were reported by Senhadji *et al.*, 2012 [11]. The increase in water demand was attributed to the irregular shape and higher microporous characteristics of natural pozzolana [29].

Substituting distilled water with L*ysinibacillus sphaericus* solution as mixing water for OPC blended with NP significantly reduced water demand (Table 3). This observation was attributed to bacteria presence and its feed which included $CaCl_2$. In this study, beneficial bacteria were found to behave more of water reducing admixtures. *Lysinibacillus sphaericus* can thus be used to reduce water demand in cement. Reduction in water demand is crucial in reducing bleeding for mortar and concrete with natural pozzolana due to its high water demand [29].

3.2. Effect of Lysinibacillus sphaericus on Setting Time. Table 4 shows setting time (in minutes) of cement paste prepared with *Lysinibacillus sphaericus* bacteria solution compared to distilled water. Initial and final setting times were abbreviated as IST and FST, respectively.

As observed, OPC paste exhibited shorter initial and final setting time than PPC when distilled water was used as mixing water (Table 4). Paper [9] recorded similar observations. In OPC, the high content of tricalcium sulphate (C_3S) and tricalcium aluminate (C_3A) phases in comparison to PPC was attributed to this effect. Upon interaction with water, C_3S and C_3A phase reacts rapidly and exothermically. The high temperatures in the reaction raise the setting temperature responsible for OPC rapid setting and hardening [29]. In PPC, the pozzolanic materials replace part of C_3S and C_3A responsible for accelerated setting time as well as reducing setting temperatures. Temperature reduction in PPC has been found to retard PPC setting time [11, 29, 39]. The authors also observed that high water demand in pozzolanic cement lowers the cohesiveness of the cement paste, thus prolonging the setting time. Similar observations were made in this study.

Incorporating *Lysinibacillus sphaericus* solution as mixing water accelerated the setting time for OPC and PPC (Table 4). The reduction was more significant with PPC compared to OPC. Similar observations were made elsewhere using *Lysinibacillus sphaericus, Bacillus cohnii,* and *Bacillus megaterium* [21, 28]. The authors attributed this effect to bacteria feed such as chloride salts used during the preparation of the bacteria medium. Chloride salt ($CaCl_2$) acts as a chemical accelerator in cement to achieve rapid setting time and hydration [6, 9, 40].

On the contrary to findings elsewhere, incorporating cement degrading bacteria (*Thiobacillus intermedius*) in OPC paste was found to retard the initial setting time from 100 to 130 and the final setting time from 180 to 230 [31]. The authors attributed this to different feed such as sulphate salts used to prepare bacteria media. Sulphate ions are adsorbed on the Al-rich surface, forming C_3A sulphate. The formed C_3A sulphate product retards the hydration process and time by inducing the formation of a diffusion barrier around C_3A phase which is responsible for the accelerated setting time [29].

Blending OPC with PFA using distilled water as mixing water significantly retarded the setting time (Table 4). Similar observations were made elsewhere [37]. Reduction of C_3A as a result of pozzolanic substitution was attributed to delayed setting time [29, 41, 42]. Instead, highly reactive C_3A was substituted with less reactive silicate. The retardation degree is proportional to sulphate content in PFA [29].

Adsorption of SiO_4^{2-} on C_3A surface reduces the hydration of C_3A responsible for accelerated setting time. Pulverized fly ash (PFA), with high content of SiO_2, also undergoes an endothermic reaction in the presence of water to form a saturated solution of monosilicic acid. The reaction lowers the temperature of the blended cement, which results in low hydration process [34]. As a result, the cement setting time is delayed and prolonged. According to Mehta and Siddique [2], high temperature is crucial to enhance the hydration of pozzolanic blended cement [2]. Moreover, PFA substitution reduces the amount of reactive C_3S and C_3A responsible for accelerated setting time by replacing it with less reactive pozzolanic materials, thus delaying setting time [9]. This explained why the PFA effect was less dominant at low substitution than high substitution.

Use of *Lysinibacillus sphaericus* solution as mixing water for OPC blended with PFA significantly accelerated the setting time (Table 4). This effect was attributed to bacteria incorporation with salts. Chloride salts act as chemical

Table 4: Setting times for cement samples prepared separately with *Lysinibacillus sphaericus* and distilled water.

Sample	IST with distilled water (mins)	FST with distilled water (mins)	IST with bacteria (mins)	FST with bacteria (mins)
OPC	100	180	90	140
PPC	180	240	160	215
10% PFAC	110	210	90	150
10% NP	115	215	90	155
20% PFAC	140	235	125	205
20% NP	150	250	120	235
30% PFAC	175	255	160	225
30% NP	195	280	120	245
40% PFAC	235	270	210	240
40% NP	260	290	235	255
50% PFAC	275	295	240	255
50% NP	315	345	275	335
60% PFAC	295	370	265	335
60% NP	380	465	320	410
70% PFAC	305	390	265	345
70% NP	355	445	315	405

accelerators to achieve rapid hardening by accelerating the setting time [9]. Further, bacteria presence serves as nucleation sites that fasten the hydration process. By acting as nucleation sites and the salt as chemical activator, bacteria presence was said to lower the hydration energy, accelerating the setting during cement hydration [32, 33].

Blending OPC with natural pozzolana (NP) exhibited a similar trend to PFA. An increase in setting time as the substitution level increased was observed (Table 4). However, NP substitution resulted in relatively higher initial and final setting time values compared to PFA. Other authors recorded similar observations [11, 29]. This was attributed to a higher amount of water used to obtain a standard consistency with NP. In addition, the substitution of C_3A and C_3S responsible for rapid setting with unreactive pozzolanic materials also played a significant role in setting time delay. Paper [11] showed that the use of NP delayed the setting time by lowering hydration temperature [11]. High temperature is crucial in accelerating the setting time of pozzolanic blended cement [2].

Use of *Lysinibacillus sphaericus* as mixing water in OPC blended with NP accelerated setting time (Table 4). This effect was attributed to bacteria incorporation with its feed. Bacteria act as nucleation sites for hydration while the salts act as chemical activators for hydration reaction [9]. In this study, bacteria enhanced the hydration reactivity of blended cement by accelerating the setting time. Rapid setting time was also attributed to water reduction by the bacteria as recorded in the normal consistency results (Table 3). At the same substitution level between the two pozzolanas used, NP recorded higher setting time than PFA. This was attributed to the high amount of silicates in PFA than NP. This showed that NP could achieve early strength and fast setting compared to PFA. It was also maintained that introducing salts such as $CaCl_2$ was done in trace quantity not to cause any appreciable effect on steel corrosion used in cement reinforcement.

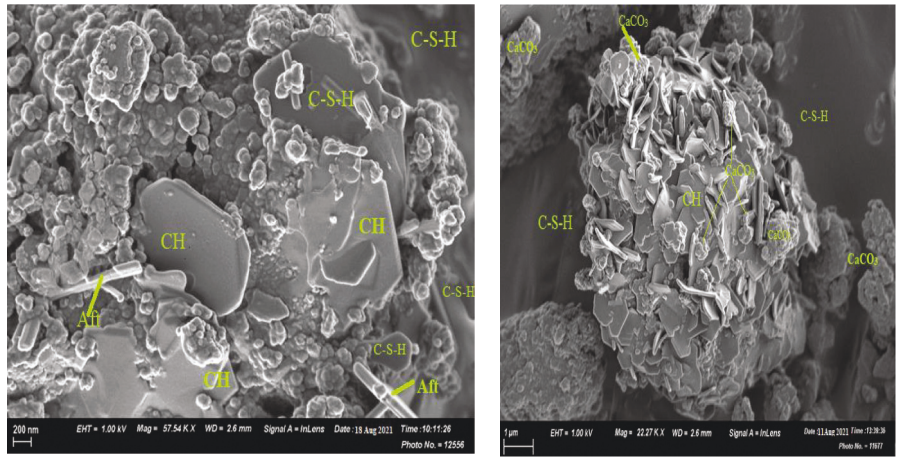
3.3. Microstructure Analysis

3.3.1. OPC Morphology. Visual inspection of cement samples (after 28th day of curing) was done using scanning electron microscopy (SEM) to understand bacteria's effect on the formation of hydration products. The scans obtained for OPC samples cured in water and bacteria solution are as shown in Figure 1 respectively.

As observed in Figure 1(a), CH and C-S-H dominated as the major hydration products compared to Figure 1(b). This was attributed to the normal hydration product of OPC when cured in water [31]. However, CH plates were reduced in Figure 1(b) cured in *Lysinibacillus sphaericus* solution. This reduction was attributed to calcium (Ca^{2+}) consumption to form $CaCO_3$ induced by bacteria precipitation. Additionally, the formation of $CaCO_3$ in the outer surface of the cement sample could have masked some CH, reducing its visibility. Significant comparison of the SEM micrographs in this study is related to other works [20, 21, 43, 44].

Needle-like ettringite observed was attributed to sulphate content from gypsum added to control OPC setting time and workability [43]. Ettringite (Aft) formation was more dominant and visible for samples cured with distilled water (Figure 1(a)) compared to samples cured using *Lysinibacillus sphaericus* solution (Figure 1(b)). The reduction with bacteria incorporation was attributed to CO_2 produced as a result of bacteria feed. According to [29], the presence of CO_2 (from carbonate salts) in cement paste results in the conversion of Aft to monosulphate [29]. Therefore, presence of bacteria feed consisting of carbonate salts may have produced CO_2 that led to the transformation of Aft, reducing its morphology and identification.

Contrary findings using degrading bacteria (*Acidithiobacillus*) were recorded elsewhere regarding ettringite formation [31, 45]. The authors found that the use of degrading bacteria induced more ettringite formation. The authors attributed this to sulphate salts used in bacteria



(a) (b)

Figure 1: OPC cured with distilled water (a) and OPC cured with *Lysinibacillus sphaericus* solution (b).

media preparation. Ettringite formation in cement is known to cause adverse effects by drastically reducing cement durability. This is through the production of expansive products that causes spalling, exposing the concrete/mortar to more aggressive environment [6, 46]. In this work, *Lysinibacillus sphaericus* enhanced formation of less ettringite which is severe in cement.

Hydrated C-S-H morphology was also more dominant in OPC cured in *Lysinibacillus sphaericus* solution than OPC cured in distilled water. The rate of nucleation, precipitation, and microstructure of cement depends on various factors such as Ca^{2+} from unhydrated particles [47, 48]. *Lysinibacillus sphaericus* surfaces may have created extra nucleation sites while Ca^{2+} from bacteria feed ($CaCl_2$) increased C-S-H precipitation. The filler effect of bacteria and its feed also have been found to enhance pozzolanic and clinker reactions [26, 49].

Precipitation of $CaCO_3$ was prevalent for the samples with *Lysinibacillus sphaericus* (Figure 1(b)) in comparison to samples prepared and cured using distilled water (Figure 1(a)). Other authors made a similar observation [19, 21, 28]. The authors attributed $CaCO_3$ precipitation to the ureolytic nature of *Lysinibacillus sphaericus*. The negatively charged bacterial cell surface acts as a nucleation site for Ca^{2+} precipitation inducing $CaCO_3$ deposition [50]. In this work, calcite precipitation was more attributed to the presence of beneficial alkaliphilic *Lysinibacillus sphaericus* bacteria and calcium lactate [51]. These two factors catalyze the calcite production rate, unlike without bacteria and calcium salts.

Microbial induced $CaCO_3$ and C-S-H have been found to enhance the microstructure of OPC mortars treated with *Bacillus* species elsewhere by densifying its morphology [19]. Bioprecipitates are usually deposited in mortar pores, acting as a sealant, thus improving the cement microstructure. This results in enhanced compressive strength and low porosity. Paper [44] found that OPC mortars cured with *Lysinibacillus sphaericus* bacteria recorded the lowest apparent diffusion coefficient compared to OPC samples cured in distilled water. The authors attributed this to microbial precipitation that reduced permeation properties significantly [44].

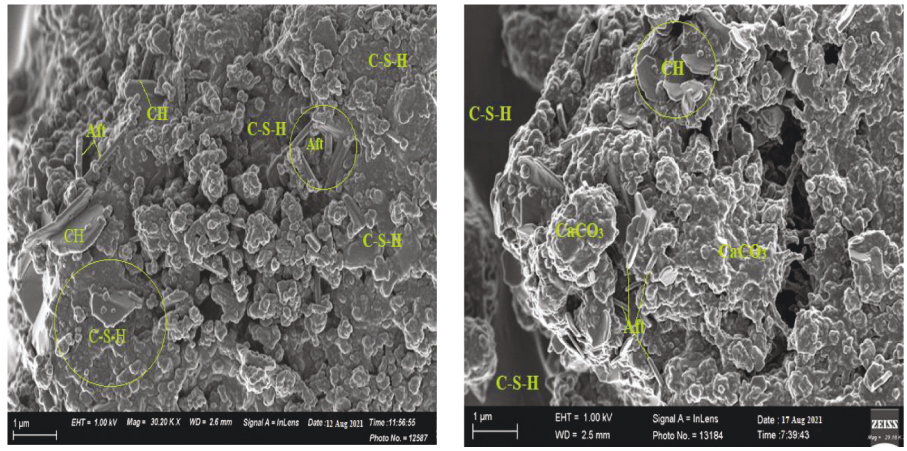
3.3.2. PPC Morphology. Figure 2 shows the microstructure of PPC samples made as a result of blending OPC with pozzolanic material of volcanic tuff origin. Figure 2 shows SEM scans for PPC samples cured in water and bacteria, respectively.

In the two images, C-S-H, CH, $CaCO_3$, and Aft were observed as the main hydration products after 28 days of curing. CH was found to dominate for the PPC sample treated with distilled water. However, C-S-H and $CaCO_3$ dominated as major hydration products for samples treated with *Lysinibacillus sphaericus*. Other authors made similar micrographs using ureolytic *Bacillus cohnii* bacteria [21].

High rate of secondary C-S-H production was attributed to the pozzolanic reaction in PPC through hydrolysis of lime [29]. Pozzolanic reaction consumes CH forming more C-S-H. Combined with CSH derived from the hydration of clinker silicates, both CSH products increased their production significantly compared to OPC cement which only had CSH from hydration of clinker silicates. Furthermore, an increase in inner CSH was attributed to the added filler effect of bacteria with natural pozzolana. With filler effect, [26] observed that the hydration kinetics of PPC is accelerated, forming more CSH [26].

Morphology of PPC samples with *Lysinibacillus sphaericus* bacteria was denser (Figure 2(b)) with calcite more pronounced than samples cured in distilled water (Figure 2(a)). A similar observation was made by [21]. The precipitation was attributed to bacteria and calcium salts [21, 43, 51]. According to the authors, bacteria acted as nucleation sites to induce calcite precipitation while bacteria feed provided Ca^{2+} ions. Precipitated calcite within the mortar pores was attributed to the improved physicochemical properties of PPC. Elsewhere, the ureolytic bacteria strain of AKKR5 was found to increase compressive strength and reduced the permeation properties of OPC blended with baghouse filter dust [50].

Ettringite morphology was more pronounced in PPC (Figure 2) in comparison to OPC (Figure 1). In PPC, needle-like hydrated products of Aft were observed for all samples cured in distilled water and *Lysinibacillus sphaericus* bacteria. However, the effects were more prevalent for samples



(a) (b)

Figure 2: PPC cured with distilled water (a) and PPC cured with L*ysinibacillus sphaericus* bacteria solution (b).

cured in distilled water than those of *Lysinibacillus sphaericus*. According to [29], PPC undergoes hydration forming Aft rapidly in comparison to OPC due to the high sulphate content from pozzolanic materials (Table 1). However, Aft morphology was reduced in samples treated with *Lysinibacillus sphaericus* solution. This was attributed to ureolytic bacteria through CO_2 production that led to ettringite transformation.

In this work, *Lysinibacillus sphaericus* was found to densify cement structure which is known to enhance cement mortar's durability. Calcite precipitate helps to densify the cement matrix by sealing any cracks and poles in the dry paste [52]. Moreover, *Lysinibacillus sphaericus* bacteria consumed ettringite through its conversion to form less harmful monosilicate compound in cement. This enhances the resistance of aggressive ion transportation in the cement matrix, which are deleterious to cement structure and durability [15].

3.4. Compressive Strength

3.4.1. OPC and PPC. Figure 3 shows the compressive strength development for OPC and PPC mortars at the 2nd, 7th, 28th, 56th, and 90th days. Mortars cured in distilled water were labeled as OPC and PPC, while those cured in *Lysinibacillus sphaericus* solution were labeled as OPC-B and PPC-B.

As observed, OPC mortars prepared and cured using *Lysinibacillus sphaericus* recorded significantly higher compressive strength compared to OPC samples prepared and cured using distilled water. This observation was recorded across the curing days and increased with curing days. The great impact of *Lysinibacillus sphaericus* bacteria on strength development was achieved with 90 days of curing (OPC-57.85, OPC-B- 62.56, and PPC-39.9, and PPC-B 47.2). Similar results were observed by [21], using ureolytic *Bacillus cohnii* bacteria [21]. The gain in compressive strength was attributed to microbial induced calcite precipitation known to densify dry cement matrix [23, 44, 53].

In contrast, [31] noted different observations using degrading bacteria (*Starkeya novella* and *Thiobacillus intermedius*) [31]. S*tarkeya novella* and *Thiobacillus intermedius* are neutrophilic sulfur-oxidizing bacteria (NSOB). NSOB predominantly thrive in a moist concrete environment with a pore solution of pH around 9 to 9.5 [54]. Sulphate salts produced by NSOB bacteria react with the cement matrix creating a cement matrix with extra pores. This effect was found to lower compressive development. Same observations in OPC were recorded when using PPC cement. However, with alkaliphilic bacteria, instead of creating attack sites, the bacteria induce beneficial precipitation enhancing compressive strength.

Strength increments attributed to *Lysinibacillus sphaericus* activity for OPC were recorded as 1.54%, 8.99%, 5.73%, 6.14%, and 8.14% for the 2nd, 7th, 28th, 56th, and 90th day of curing, respectively. At day 2, the gain in strength development was the lowest (1.54%), while on the 7th day of curing, microbial activity was at a peak stage, recording the highest gain in compressive strength development (8.99%). From the 28th day, gain in strength development started decreasing and later increased from the 56th to 90th day of curing. Paper [21] also found that ureolytic *Bacillus cohnii* activity was at a peak between the 14th to 28th days of curing [21]. Paper [30] observed a similar trend with *Bacillus licheniformis* between the 3rd and 28th days [30]. This trend was attributed to microbial activity represented as lag phase, exponential phase, stationary phase, and death phase in the bacterial growth curve [53, 55, 56].

According to [53, 55, 56], microbial activity is observed to be insignificant during the lag phase. At this phase, the bacteria try to adapt to a new environment in fresh cement/concrete paste. Therefore, before 2 days of curing in this research, the low gain in strength was attributed to normal cement hydration process and not *Lysinibacillus sphaericus* activity. After the 2nd day of curing, the bacteria were said to be well acclimatized with the activity at exponential growth (log phase) [57]. The stage was responsible for high microbial activity leading to the highest increase in

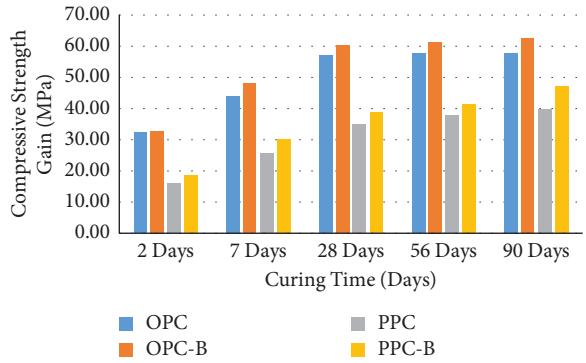


Figure 3: Compressive strength development for OPC and PPC (w/c = 0.5).

compressive strength development up to around 28 days of curing [55].

After the 28th day of curing, bacteria activity reduced significantly, as reflected by a gain in compressive strength (Figure 3). The bacteria were found to enter the stationary phase caused by nutrient depletion. Nutrients are responsible for microbial activity within the cement matrix [53]. Without the nutrient supplement, the bacteria would go into hibernation (death phase). This phase was predominant from the 56th day of curing, where the strength gain started picking slowly from 6.14% to 8.14% from the 28th day of curing (5.73%). The increase in compressive strength from the 56th day of curing onwards was attributed to Portland cement's normal cement hydration process [6, 10].

Similar trends in OPC treated with *Lysinibacillus sphaericus* bacteria were observed in PPC samples prepared and cured with the same bacteria. The gain in PPC compressive strength attributed to bacteria was 17.11%, 17.96%, 11.19%, 12.63%, and 17.47% for the 2nd, 7th, 28th, 56th, and 90th day of curing, respectively. However, after the 56th day of curing, compressive strength gain was much higher than that of OPC (6.14% and 8.14%). The great improvement of PPC was attributed to the pozzolanic reaction, which is prevalent in late curing [6, 9, 10].

As observed with PPC samples incorporated with *Lysinibacillus sphaericus*, compressive strength was significantly increased at 2 days of curing. This was despite the formation of microbial activity such as calcite precipitation. The gain was attributed to bacteria feed containing chloride salts ($CaCl_2$). The addition of $CaCl_2$ has been used to speed up the hydration reaction of Portland pozzolana cement [9, 30, 39]. The presence of *Lysinibacillus sphaericus* bacteria feed may also have increased the filler effect [26, 58]. The filler effect in pozzolanic material increases the clinker reaction that enhances strength development [26, 59].

At early curing (2nd, 7th, and 28th days), OPC samples developed higher compressive strength compared to PPC samples for samples treated with *Lysinibacillus sphaericus* and distilled water. However, at late curing (from 56th to 90th days) PPC samples recorded higher gain in compressive strength in comparison to OPC samples with bacteria solution and distilled water. Similar observations were observed by [43]. At early curing, the gain in OPC was attributed to the high content of C_3S and C_3A that reacts almost to depletion in the first 28 days of curing [21]. However, the gain in compressive strength for PPC was attributed to the late pozzolanic reaction between lime produced from clinker hydration and added pozzolanic materials to give secondary C-S-H [29].

On the 28th day of curing, all mortar samples met the strength requirement of 32.5 MPa (PPC) and 42.5 MPa (OPC) as prescribed by Kenya standards of testing cement, KS EAS 18:1- 2017 (KS EAS 18-1: 2017).

3.4.2. OPC Substituted with Pulverized Fuel Ash. Figure 4 shows the compressive strength development for OPC blended with PFA mortars at the 2nd, 7th, 28th, 56th, and 90th days. Mortars prepared and cured in distilled water were labeled as PFAC, while those prepared and cured in *Lysinibacillus sphaericus* solution were labeled as PFACB.

Across all mortars substituted with PFA from 10% to 70%, there was a reduction of compressive strength gain as substitution increased. This observation was made for all samples cured with distilled water and *Lysinibacillus sphaericus* solution. A similar trend was observed by [17] for concrete samples prepared and cured with distilled water [17]. Paper [34] also observed that an increase in the amount of fly ash in cement substitution resulted in a reduction in strength development at all curing ages [34]. The authors attributed this observation to an increase in SiO_2 due to PFA addition, which contains higher silicates content (Table 1). Elsewhere, a similar observation of SiO_2 was found and attributed to PFA properties of retarding the hydration of C_3A [29].

High contents of silicate are adsorbed onto the Al-rich surface (produced by nonstoichiometric dissolution), forming C_3A sulphate products [29]. This formed product retards the hydration process of C_3A. Further, in the presence of saturated aqueous solution, SiO_2 reacts endothermically to give monosilicic acid. This process reduces the heat evolution, which is critical in the hydration of pozzolanic cement at any age, thus reducing compressive strength development [34].

Use of *Lysinibacillus sphaericus* solution during mortar preparation and curing resulted in a significant increase in compressive strength compared to samples treated with distilled water. The increase was significantly observed between the 2nd, 7th, and 28th days of curing. After 28th day of curing bacteria activity resulted in an insignificant rise in compressive development. A similar observation using Sporosarcina *pasteurii* bacteria increased the compressive strength of concrete mortars [57]. This observation was attributed to microbial activities such as calcite precipitation deposited in the pores present in blended cement, thus densifying the cement matrix. At 40% substitution, both samples treated with L*ysinibacillus sphaericus* and distilled water as mixing and curing water met the strength of 32.5 MPa as prescribed by Kenyan standards for commercial PPC. However, samples with *Lysinibacillus sphaericus* bacteria recorded higher compressive strength in comparison to that treated with distilled water, i.e., 34.47 MPa and 36.68 MPa, respectively.

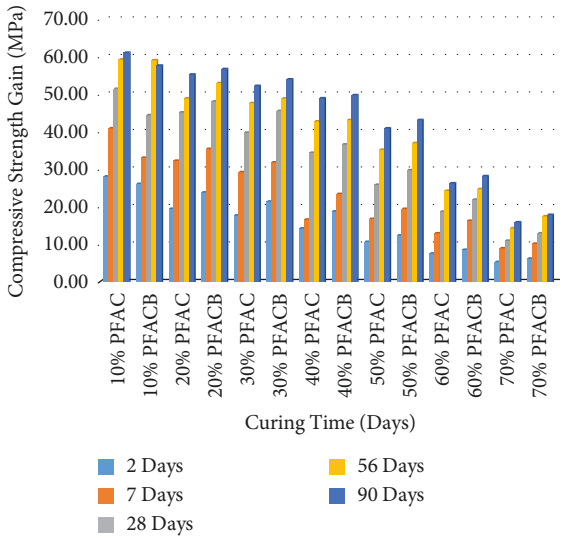


Figure 4: Compressive strength development for OPC substituted with PFA (w/c = 0.5).

The presence of *Lysinibacillus sphaericus* bacteria was also found to significantly accelerate early strength development at 20% to 30% development when used as a curing solution. However, contradicting findings were found by [34] when using distilled water as a mixing and curing solution [34]. At 2nd, 7th, and 28th days of curing, the authors found that OPC substituted with PFA from 15% to 45% resulted in a decrease in early strength gain. The authors attributed this observation to high SiO_2 content. However, ureolytic bacteria in this research enhanced early strength development. The improvement was attributed to bacteria feeds such as chloride salts ($CaCl_2$) found to accelerate the reaction of pozzolanic cement [9].

Elsewhere, the use of calcium sources from calcium-based products such as $CaCl_2$ and blast furnace slag enhanced microstructure and mechanical properties of OPC blended with PFA [2]. This effect is due to the filler effects of such materials that enhance the reaction of clinker. In this research, bacteria presence and other feeds were also said to have contributed to the filler effect. Ureolytic bacteria used were attributed to increased clinker reactivity in pozzolanic cement by creating more nucleation sites for hydration reaction to occur [26, 33, 59–62].

As the curing days increased, there was a gradual increase in compressive strength across all substituted mortars. The increase was attributed to the additional hydration products from pozzolanic reactions such as CASH and secondary CSH [2, 63, 64]. In addition, C-S-H and C-A-H enhance the binder's microstructure, making it denser [65]. A great impact on strength development was observed at 90 days of curing at 10% substitution at 61.1 MPa and 57.6 MPa for samples with and without *Lysinibacillus sphaericus* bacteria. PFA particles may also have an increased rate of hydration by creating heterogeneous nucleation sites for hydration products deposition. The sites reduce the energy barrier for hydration, and thus the extent of cement hydration is enhanced accordingly [32].

In this work, when *Lysinibacillus sphaericus* bacteria were used as mixing water, the workability increased significantly at the recommended w/c of 0.5 for cement mortars, making it impossible to place and cast. This called for a reduction of w/c to 0.4 to allow the placement and compaction of the mortar in the mold.

Figure 5 shows compressive development for mortars prepared and cured using *Lysinibacillus sphaericus* solution at w/c as 0.4.

A significant and observable increase in compressive strength was observed with a 0.4 w/c ratio compared to a 0.5 w/c ratio. A similar trend was observed by [66]. The authors noted that when using 0.3 and 0.5 water-to-cement ratio, the lowest ratio (0.3) produced less porous C-S-H than 0.5 ratio, which exhibited a denser cement matrix with high strength development. The increase was exhibited at 10% to 60% PFA substitution. In this study, the use of ureolytic *Lysinibacillus sphaericus* solution as mixing and curing water was found to reduce the w/c ratio which enhanced strength development at all curing ages.

A high w/c ratio has been confirmed to result in a poor microstructure that is more porous and poor in strength properties of cement [29]. With use of excess water and high w/c ratio, the excess water evaporates after hydration leaving unfilled pores within the dry paste. Further, high water content leads to increased bleeding of concrete materials [67]. In this study, *Lysinibacillus sphaericus* bacteria were found to reduce water demand and consequently led to increased compressive strength and microstructure densification that would be poor in a high w/c ratio. Ureolytic *Lysinibacillus sphaericus* bacteria enhanced densification through its beneficial precipitation. With a 0.4 w/c ratio, a great impact on strength development of 70.33 MPa was observed at 90 days of curing at 10% substitution compared to that of 61.1 MPa which was achieved with a 0.5 w/c ratio at 10% substitution level at 90 days of curing.

3.4.3. OPC Substituted with Natural Pozzolana. Figure 6 shows compressive strength development for OPC-NP mortars at the 2nd, 7th, 28th, 56th, and 90th days. Mortars prepared and cured in distilled water were labeled as NP, while those prepared and cured in *Lysinibacillus sphaericus* solution were labeled as NPB.

Across the mortars prepared and cured with *Lysinibacillus sphaericus* solution and distilled water, compressive strength decreased as substitution of natural pozzolana (NP) increased. Similar trend was observed by [11]. However, the compressive strength increased as the curing age increased. Greatest gain was achieved at 10% substitution for mortar samples treated with *Lysinibacillus sphaericus* solution, i.e., 68.89 MPa. For samples prepared and cured in distilled water, 10% substitution gave the highest strength gain, i.e., 65.95 MPa. However, at the same substitution level, *Lysinibacillus sphaericus* solution gave the highest strength development as compared to distilled water.

Reduction in strength development as substitution increase was attributed to reduction of phases such as C_3A and C_3S phases by being substituted by pozzolanic material [29].

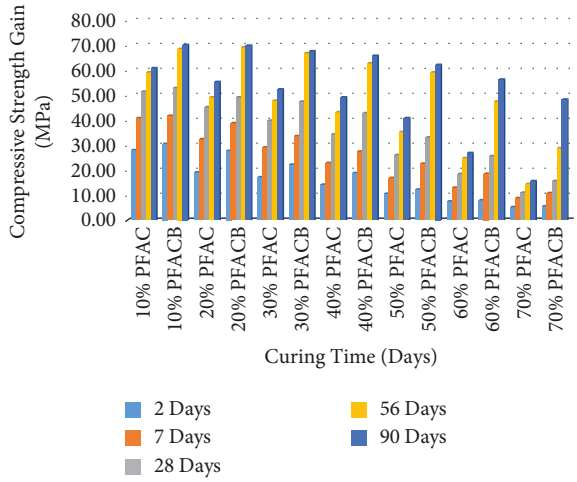


Figure 5: Compressive strength of OPC blended with PFA (w/c = 0.4).

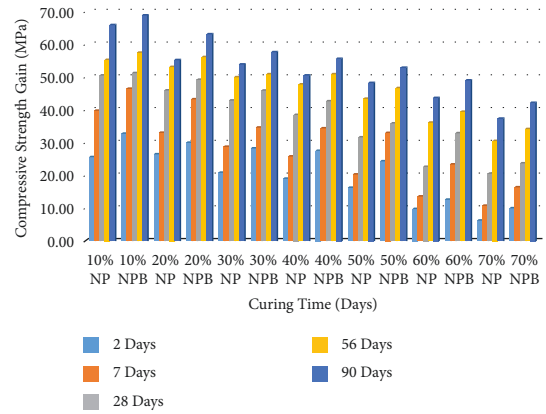


Figure 6: Compressive strength of OPC blended with NP (w/c = 0.5).

As pozzolanic substitution increased, silicate (SiO_2) also increased which is less reactive than C_3A and C_3S phases. Further, SiO_2 in pozzolanic materials reacts endothermically with water reducing hydration temperature which is crucial in pozzolanic cement hydration. High temperature raises pozzolanic cement's hydration process and vice versa [2, 34]. Increased strength development as curing day increase was attributed to pozzolanic reaction [68, 69].

Use of *Lysinibacillus sphaericus* solution resulted in an increase in strength development across all mortars in all curing days, more so at an early age (Figure 6). This was attributed to *Lysinibacillus sphaericus* bacteria that were found to enhance microstructure of blended cement through microbial precipitation [19, 21, 44, 70–72]. This was unlike when distilled water was used as mixing and curing water. Microbial beneficial precipitation has been found to enhance the microstructure of cement matrix by densifying concrete/mortar samples. The densification process is accompanied by an increase in durability process such as compressive strength [73, 74].

At 40% substitution, with distilled water used as mixing and curing water, the samples met the strength requirement of 32.5 MPa (PPC), i.e., 38.46 MPa as prescribed by Kenya standards of testing cement, KS EAS 18:1-2017 at 28 days of curing (KS EAS 18-1: 2017). However, at 50% substitution, the samples achieved a compressive strength of 36.08 MPa when *Lysinibacillus sphaericus* solution was used as mixing and curing solution which was above that of commercial PPC at 28 days of curing. However, the same samples at 50% substitution failed to meet the strength of 32.5 MPa as per the standards when bacteria solution was substituted with distilled water. Instead, they achieved a strength of 31.73 MPa which was below that of 32.5 MPa.

3.4.4. Sorptivity. Figure 7 shows sorptivity of OPC and PPC mortars prepared and cured using distilled water and *Lysinibacillus sphaericus* solution.

As observed in Figure 7, OPC and PPC samples incorporated with *Lysinibacillus sphaericus* bacteria recorded lowest cumulative water absorptions in comparison to the samples prepared and cured using distilled water. Paper [44] recorded similar observation [28]. According to the authors, they attributed high porosity to high amounts of non-hydrated cement particles that undergo delayed or secondary hydration as curing continues.

For samples treated with *Lysinibacillus sphaericus* solution, low sorptivity was attributed to microbial calcite deposition [28]. Similar observations were recorded by [19, 43]. Calcite precipitation helps to densify the cement matrix sealing available pores thus reducing ingress of excess water or aggressive ions into the mortar matrix/concrete [20].

As obtained from the experimental data, cumulative water absorption increased gradually with time depicting a curve. The curve became constant after 120 hours of exposure to distilled water and the experiment was stopped. A linear trend line was drawn on the curve for OPC and PPC to obtain sorptivity coefficient (K) as shown below (Table 5). The same procedure was followed to obtain K value for OPC substituted with PFA and NP samples prepared and cured with *Lysinibacillus sphaericus* solution. The obtained water sorptivity coefficient for all the samples was summarized in Table 5 below.

In all the cement samples, there was a positive decrease in sorptivity coefficient value (K) when *Lysinibacillus sphaericus* bacteria were used apart from OPC substituted with 60% and 70% PFA. According to [27, 28], cumulative water absorption in cement should be low for a better cement durability [27, 28]. The positive decrease was attributed to microbial calcite precipitation that helped to densify the cement matrix. Similar findings were recorded by other authors [19, 21, 28]. The authors found that use of alkaliphilic bacteria (*Bacillus cohnii* and *Bacillus megaterium*) resulted in beneficial precipitation that densified the cement matrix by sealing micropores present in cement.

In this research, microbial precipitation was attributed to enhance cement samples densification further up to 50%. However, from 60% onwards, the bacteria effect was insignificant due to high porosity caused by higher substitution, thus negative decrease in sorptivity coefficient value. According to [29], high PFA in cement results in high amount

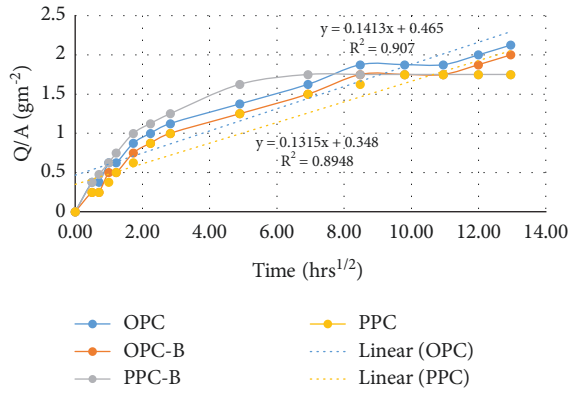


Figure 7: Cumulative water absorption (Q/A) for control cement samples.

Table 5: Sorptivity coefficient (k) of OPC and PPC samples derived from the graphical illustration.

Cement sample	(k) without bacteria	(k) with bacteria	K value decrease (%)
OPC	0.1413	0.1392	0.21
PPC	0.1315	0.1142	1.73
10% PFAC	0.1555	0.123	3.25
20% PFAC	0.1718	0.1256	4.62
30% PFAC	0.1981	0.1415	5.66
40% PFAC	0.296	0.2265	6.95
50% PFAC	0.2683	0.2136	5.47
60% PFAC	0.1909	0.2484	−5.75
70% PFAC	0.2563	0.3143	−5.80
10% NP	0.2402	0.1471	9.31
20% NP	0.2134	0.1761	3.73
30% NP	0.1942	0.189	0.52
40% NP	0.2316	0.2281	0.35
50% NP	0.2352	0.2117	2.35
60% NP	0.2233	0.1805	4.28
70% NP	0.2445	0.1993	4.52

of unnecessary water causing breeding in cement materials [29]. After cement undergoes hydration, excess water evaporates leaving unfilled pores within cement samples making them more porous relative to those with less water or PFA content. Use of *Lysinibacillus sphaericus* bacteria instead of distilled water was also found to increase water content in substituted cement making the paste less plastic and watery. The combined effects were attributed to cause high porosity such that bacteria activity could not overrun them to cause positive reduction in sorptivity values.

However, use of degrading bacteria such as *Acidithiobacillus thiooxidans* and *Starkeya novella* has been found to exhibit contrasting results. Paper [43] found that use of degrading bacteria (*Acidithiobacillus thiooxidans* and *Starkeya novella*) increased porosity in cement samples. The authors attributed the observations to deleterious effect of such bacteria unlike beneficial bacteria such as *Lysinibacillus sphaericus* and *Bacillus megaterium* [44].

Pozzolanic reaction was also attributed to micropores sealing and densification for OPC blended with PFA [29, 34, 75]. Paper [36] found that substitution up to 55% of PFA reduced water uptake in concrete samples [36]. Paper [34] also observed that substitution of OPC with 35% PFA resulted in samples with enhanced physicochemical properties [34]. The authors used distilled water in samples preparations (w/c 0.5) and curing. The author also attributed this effect to pozzolanic reaction.

Lysinibacillus sphaericus microbial activity was also observed to reduce water uptake for OPC substituted with NP. This was attributed to microbial induced calcite precipitation as a result of *Lysinibacillus sphaericus* bacteria presence. As the substitution increased with presence of alkaliphilic bacteria, there was reduction in water uptake. A highest reduction in water uptake was observed at 10% and decreased gradually as substitution increased up to 40%. Similar observation was achieved elsewhere [11]. However, the author used distilled water for mortar preparation and curing only. They attributed reduction of permeation properties up to 25% due to refined pore structure for blended cement. At high dosage, permeation was found to increase. Pozzolanic reaction has been attributed to refining of the pores structures [69, 76].

At low substitution, the increase in bacteria activity to reduce water uptake was attributed to pores densification; thus its presence was more pronounced. This was in contrast to higher substitution where higher porosity overrun microbial activity; thus its effect in reducing water uptake was less pronounced. At higher substitution, unreacted pozzolanic

material causes higher porosity. The higher the NP substitution in OPC, the higher the permeation properties, more so at low curing days [29]. The authors attributed this to late reaction of natural pozzolana with lime produced in clinker hydration to form secondary CSH which is denser than CH.

Sorptivity results above were seen to well correlate with compressive strength in a linear fashion. The higher the compressive strength, the lower the k value [28]. From these results, it is apparent that the transport mechanisms in cement mortar are highly affected by pozzolanic substitution and bacteria precipitation. The calcite layer has the ability to enhance the resistance of cementitious materials against degradation [77].

4. Conclusion

At same substitution level, pulverized fly ash resulted in a higher gain in compressive strength compared to volcanic tuff. This was observed for both mortars prepared and cured with distilled water and *Lysinibacillus sphaericus* bacteria solution. Also, pulverized fly ash resulted in higher setting time and normal consistency compared to volcanic tuff at the same substitution. However, when *Lysinibacillus sphaericus* bacteria were incorporated, the bacteria resulted in reduction in water demand used during mortar preparation. This was well observed with PFA where for NP, the reduction was not workable due to porosity nature of NP that demands more mixing water. The reduction in water used for PFA mortar preparation resulted in increase in compressive strength gain. Pulverized fly ash resulted in low porosity at the same substitution compared to volcanic tuff. For both pozzolana, *Lysinibacillus sphaericus* bacteria resulted in microstructure densification which considerably reduced the porosity of hardened cement paste. This observation was validated with sorptivity test where all mortars cured using *Lysinibacillus sphaericus* bacteria resulted in low sorptivity coefficient value compared to samples treated with distilled water.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

[1] L. A. Ghani, A. Nora, and N. Z. Mahmood, "The Use of MFA and LCA in the Agriculture Waste Management System in Kuala Terengganu," *Malaysian Construction Research Journal*, vol. 5, no. 3, 2018.

[2] A. Mehta and R. Siddique, "Properties of low-calcium fly ash based geopolymer concrete incorporating OPC as partial replacement of fly ash," *Construction and Building Materials*, vol. 150, pp. 792–807, 2017.

[3] S. Surya, S. Aparna, S. G. Sertse, and S. Muhammad, "Microstructural study of Portland cement partially replaced with fly ash, ground granulated blast furnace slag and silica fume as determined by pozzolanic activity," *Construction and Building Materials*, vol. 238, Article ID 117561, 2020.

[4] J. Thomas, N. N. Thaickavil, and T. N. Syamala, *Supplementary Cement Replacement Materials for Sustainable Concrete*, Springer, Singapore, 2019.

[5] H. C. Law, L. M. Gan, and H. L. Gan, "Experimental Study on the Mechanical Properties of Biomass Briquettes from Different Agricultural Residues Combination," *Renewable and Non-renewable Energy Resources and Power Generation*, vol. 225, Article ID 04026, 2018.

[6] J. M. Wachira, J. K. Thiong'o, J. M. Marangu, and L. G. Murithi, "Physicochemical performance of portland-rice husk ash-calcined clay-dried acetylene lime sludge cement in sulphate and chloride media," *Advances in Materials Science and Engineering*, vol. 2019, Article ID 5618743, 2019.

[7] F. N. Musyimi, J. M. Wachira, J. Karanja, and J. M. Marangu, "Performance of Ground Clay Brick Mortars in Simulated Chloride and Sulphate Media," *Journal of Engineering*, vol. 2019, Article ID 6430868, 2019.

[8] N. F. Musyimi, J. Karanja, M. J. Wachira, and M. O. Mulwa, "Pozzolanicity and compressive strength performance of kibwezi bricks based cement," vol. 9, no. 2, pp. 28–32, 2016.

[9] M. J. Mwiti, T. J. Karanja, and W. J. Muthengia, "Properties of Activated Blended Cement Containing High Content of Calcined clay," *Heliyon*, vol. 4, Article ID e00742, 2018.

[10] J. M. Wachira, "Effects of chlorides on corrosion of simulated reinforced blended cement mortars," *International Journal of Corrosion*, vol. 2019, no. 4, Article ID 2123547, 2019.

[11] Y. Senhadji, G. Escadeillas, H. Khelafi, M. Mouli, and A. S. Benosman, "Evaluation of natural pozzolan for use as supplementary cementitious material," *European Journal of Environmental and Civil Engineering*, vol. 16, no. 1, pp. 77–96, 2012.

[12] P. Dinakar, M. Kartik Reddy, and M. Sharma, "Behaviour of self compacting concrete using Portland pozzolana cement with different levels of fly ash," *Materials & Design*, vol. 46, pp. 609–616, 2013.

[13] R. Mukharjee, "An overview of the properties of sustainable concrete using fly ash as replacement for cement Rajib Kumar Majhi and Amar Nath Nayak Bibhuti Bhusan Mukharjee," *International Journal of Sustainable Materials and Structural Systems*, vol. 4, no. 1, pp. 47–90, 2020.

[14] G. Xu and X. Shi, "Resources Conservation & Recycling Characteristics and applications of fl y ash as a sustainable construction material: a state-of-the-art review," *Resources, Conservation and Recycling*, vol. 136, no. April, pp. 95–109, 2018.

[15] J. M. Wachira and J. M. Marangu, "Chloride diffusivity in blended cement made from selected industrial and agro-wastes," *Advances in Materials Science and Engineering*, vol. 2019, Article ID 2814320, 2019.

[16] F. Matalkah, L. Xu, W. Wu, and P. Soroushian, "Mechanochemical synthesis of one-part alkali aluminosilicate hydraulic cement," *Materials and Structures*, vol. 50, no. 1, 2017.

[17] G. K. Kate and P. B. Murnal, "Effect of Addition of Fly Ash on Shrinkage Characteristics in Effect of Addition of Fly Ash on Shrinkage Characteristics in High Strength concrete," *International Journal of Advanced Technology in Civil Engineering*, vol. 2, no. 1, 2013.

[18] D. K. Mutitu, J. M. Wachira, R. Mwirichia, J. K. Thiong'o, O. M. Munyao, and G. Muriithi, "Influence of Lysinibacillus sphaericus on compressive strength and water sorptivity in microbial cement mortar," *Heliyon*, vol. 5, no. 11, Article ID e02881, 2019.

[19] O. Mulwa, J. Karanja, and J. Muthengia, "Use of Bacillus species bacteria in protecting the concrete structures from sulphate attack- A review," *Journal of Chemical Reviews*, vol. 1, no. 4, pp. 187–299, 2019.

[20] K. D. Mutitu, M. O. Munyao, M. J. Wachira, K. J. Thiong, and M. J. Marangu, "Effects of biocementation on some properties of cement-based materials incorporating Bacillus Species bacteria – a review," *Journal of Sustainable Cement-Based Materials*, vol. 0, no. 0, pp. 1–17, 2019b.

[21] N. R. Wangui, J. Karanja, and J. M. Wachira, "Effect of Bacillus Cohnii on Some Physicomechanical and Microstructural Properties of Ordinary Portland Cement," *Journal of Chemistry*, vol. 2020, Article ID 7816079, 2020.

[22] T. Zhu and M. Dittrich, "Carbonate precipitation through microbial activities in natural environment, and their potential in biotechnology: a review," *Frontiers in Bioengineering and Biotechnology*, vol. 4, no. JAN, pp. 1–21, 2016.

[23] A. Rajasekar, C. K. S. Moy, and S. Wilkinson, "MICP and advances towards eco-friendly and economical applications," *IOP Conference Series: Earth and Environmental Science*, vol. 78, no. 1, 2017.

[24] F. B. Da Silva, N. De Belie, N. Boon, and W. Verstraete, "Production of non-axenic ureolytic spores for self-healing concrete applications," *Construction and Building Materials*, vol. 93, pp. 1034–1041, 2015.

[25] KS EAS 18-1, "KENYA STANDARD Cement Part 1 Composition Specification and Conformity Criteria for Common Cements," *European standard EN*, 2000.

[26] B. Lothenbach, K. Scrivener, and R. D. Hooton, "Supplementary cementitious materials," *Cement and Concrete Research*, vol. 41, no. 12, pp. 1244–1256, 2011.

[27] K. Kanta and S. Btech, "Improvement of the mechanical properties of mortar and concrete using ureolytic bacteria," *ICE Virtual Library*, vol. 171, 2016.

[28] K. K. Sahoo, A. K. Sathyan, C. Kumari, P. Sarkar, and R. Davis, "Investigation of cement mortar incorporating Bacillus sphaericus," vol. 5411, no. July, 2016.

[29] K.-Y. Show, J.-H. Tay, and D.-J. Lee, "Cement replacement materials," in *Sustainable Sludge Management*Salmon Tower Building New York City, 2018.

[30] H. Thiyagarajan, S. Maheswaran, M. Mapa et al., "Investigation of bacterial activity on compressive strength of cement mortar in different curing media," *Journal of Advanced Concrete Technology*, vol. 14, no. 4, pp. 125–133, 2016.

[31] O. M. Munyao, J. K. Thiong'o, J. W. Muthengia et al., "Study on the effect of Thiobacillus intermedius bacteria on the physico-mechanical properties of mortars of ordinary portland cement," *Heliyon*, vol. 6, no. 1, Article ID e03232, 2020.

[32] Y. Sun and H. Lee, "Research on properties evolution of ultrafine fly ash and cement composite," *Construction and Building Materials*, vol. 261, Article ID 119935, 2020.

[33] S. L. Williams, M. J. Kirisits, and R. D. Ferron, "Influence of concrete-related environmental stressors on biomineralizing bacteria used in self-healing concrete," *Construction and Building Materials*, vol. 139, pp. 611–618, 2017.

[34] R. Elmrabet, A. El Harfi, and M. S. El Youbi, "Study of properties of fly ash cements," *Materials Today Proceedings*, vol. 13, pp. 850–856, 2019.

[35] T. Hemalatha and A. Ramaswamy, "A review on fly ash characteristics - towards promoting high volume utilization in developing sustainable concrete," *Journal of Cleaner Production*, vol. 147, pp. 546–559, 2017.

[36] M. M. Hossain, M. R. Karim, M. Hasan, M. K. Hossain, and M. F. M. Zain, "Durability of mortar and concrete made up of pozzolans as a partial replacement of cement: a review," *Construction and Building Materials*, vol. 116, pp. 128–140, 2016.

[37] G. Mallikarjuna Rao and T. D. Gunneswara Rao, "Final setting time and compressive strength of fly ash and GGBS-based geopolymer paste and mortar," *Arabian Journal for Science and Engineering*, vol. 40, no. 11, pp. 3067–3074, 2015.

[38] K. D. Mutitu, M. O. Munyao, M. J. Wachira, R. Mwirichia, and K. J. Thiong'o, "Effects of biocementation on some properties of cement-based materials incorporating Bacillus Species bacteria – a review," *Journal of Sustainable Cement-Based Materials*, vol. 8, no. 5, pp. 309–325, 2019a.

[39] J. M. Marangu, J. K. Thiong'o, and J. M. Wachira, "Chloride ingress in chemically activated calcined clay-based cement," *Journal of Chemistry*, vol. 2018, pp. 1–8, Article ID 1595230, 2018.

[40] A. Poursaee, *Corrosion of Steel in Concrete Structures*, Elsevier, Amsterdam, Netherlands, 2016.

[41] M. Kumar Tiwari, S. Bajpai, and U. Kumar Dewangan, "Fly ash utilization: a brief review in Indian context," *International Research Journal of Engineering and Technology*, vol. 3, pp. 949–956, 2016.

[42] K. Phoolphagar, M. Saini, and M. T. Student, "To study effects on the properties of concrete after partially," vol. xii, no. x, pp. 179–185, 2019.

[43] O. M. Munyao, J. Karanja, J. M. Wachira, D. K. Mutitu, G. Murithi, and R. Mwirichia, "Chloride Ingress in Cement Mortars Exposed to Acidithiobacillus Thiooxidans Bacteria," *Advances in Materials Science and Engineering*, vol. 2020, Article ID 4191806, 2020.

[44] D. K. Mutitu, J. M. Wachira, R. Mwirichia, J. K. Thiong'o, O. M. Munyao, and M. Genson, "Biocementation influence on flexural strength and chloride ingress by lysinibacillus sphaericus and Bacillus megaterium in mortar structures," *Journal of Chemistry*, vol. 2020, pp. 1–13, Article ID 1472923, 2020.

[45] O. M. Munyao, J. Karanja, J. M. Wachira, D. K. Mutitu, and R. Mwirichia, "Influence of Starkeya Novella on Mechanical and Microstructural Properties of Cement Mortars," *Journal of Chemistry*, vol. 2020, Article ID 8212396, 2020.

[46] H. N. Atahan and K. M. Arslan, "Improved durability of cement mortars exposed to external sulfate attack: the role of nano & micro additives," *Sustainable Cities and Society*, vol. 22, pp. 40–48, 2016.

[47] S. Krishnan, S. K. Kanaujia, S. Mithia, and S. Bishnoi, "Hydration kinetics and mechanisms of carbonates from stone wastes in ternary blends with calcined clay," *Construction and Building Materials*, vol. 164, pp. 265–274, 2018.

[48] K. Kupwade-patil, A. F. Al-aibani, M. F. Abdulsalam et al., "Microstructure of cement paste with natural pozzolanic volcanic ash and Portland cement at different stages of curing," *Construction and Building Materials*, vol. 113, pp. 423–441, 2016.

[49] S. Nie, S. Hu, F. Wang, C. Hu, X. Li, and Y. Zhu, "Pozzolanic reaction of lightweight fine aggregate and its influence on the hydration of cement," *Construction and Building Materials*, vol. 153, pp. 165–173, 2017.

[50] R. Siddique, V. Nanda, K., E. H. Kunal, M. Iqbal Khan, M. Singh, and A. Rajor, "Influence of bacteria on compressive strength and permeation properties of concrete made with cement baghouse filter dust," *Construction and Building Materials*, vol. 106, pp. 461–469, 2016.

[51] W. Khaliq and M. B. Ehsan, "Crack healing in concrete using various bio influenced self-healing techniques," *Construction and Building Materials*, vol. 102, pp. 349–357, 2016.

[52] T. Ikumi and I. Segura, "Numerical assessment of external sulfate attack in concrete structures. A review," *Cement and Concrete Research*, vol. 121, no. April, pp. 91–105, 2019.

[53] H. Kim, H. Son, J. Seo, and H. K. Lee, "Impact of bio-carrier immobilized with marine bacteria on self-healing performance of," 2020.

[54] C. Grengg, F. Mittermayr, N. Ukrainczyk, G. Koraimann, S. Kienesberger, and M. Dietzel, "Advances in concrete materials for sewer systems affected by microbial induced concrete corrosion: a review," *Water Research*, vol. 134, pp. 341–352, 2018.

[55] M. Wu, X. Hu, Q. Zhang, D. Xue, and Y. Zhao, "Growth environment optimization for inducing bacterial mineralization and its application in concrete healing," *Construction and Building Materials*, vol. 209, pp. 631–643, 2019.

[56] J. Xu and X. Wang, "Self-healing of concrete cracks by use of bacteria-containing low alkali cementitious material," *Construction and Building Materials*, vol. 167, pp. 1–14, 2018.

[57] N. Chahal, R. Siddique, and A. Rajor, "Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete," *Construction and Building Materials*, vol. 28, no. 1, pp. 351–356, 2012.

[58] L. H. J. Martin, F. Winnefeld, E. Tschopp, C. J. Müller, and B. Lothenbach, "Influence of fly ash on the hydration of calcium sulfoaluminate cement," *Cement and Concrete Research*, vol. 95, pp. 152–163, 2017.

[59] W. K. W. Lee and J. S. J. Van Deventer, "The effect of ionic contaminants on the early-age properties of alkali-activated fly ash-based cements," *Cement and Concrete Research*, vol. 32, no. 4, pp. 577–584, 2002.

[60] R. Siddique and N. K. Chahal, "Effect of ureolytic bacteria on concrete properties," *Construction and Building Materials*, vol. 25, no. 10, pp. 3791–3801, 2011.

[61] J. S. J. van Deventer, J. L. Provis, P. Duxson, and G. C. Lukey, "Reaction mechanisms in the geopolymeric conversion of inorganic waste to useful products," *Journal of Hazardous Materials*, vol. 139, no. 3, pp. 506–513, 2007.

[62] P. G. Vekilov, "Principles of Crystal Nucleation and Growth," *Reviews in Mineralogy and Geochemistry*, vol. 54, no. 1, 2014.

[63] A. Mehta and R. Siddique, "Sustainable geopolymer concrete using ground granulated blast furnace slag and rice husk ash: strength and permeability properties," *Journal of Cleaner Production*, vol. 205, pp. 49–57, 2018.

[64] V. Srivastava, A. Imam, P. K. Mehta, and M. K. Tripathi, "Supplementary Cementitious Materials in Construction -An Attempt to Reduce CO2 Emission Supplementary Cementitious Materials in Construction - an Attempt to Reduce CO 2 Emission," *Journal of Environmental Nanotechnology*, vol. 7, no. 2, 2018.

[65] Y. K. Cho, S. H. Jung, and Y. C. Choi, "Effects of chemical composition of fly ash on compressive strength of fly ash cement mortar," *Construction and Building Materials*, vol. 204, pp. 255–264, 2019.

[66] Q. Zeng and K. Li, "Reaction and microstructure of cement–fly-ash system," *Materials and structures*, vol. 48, no. 6, 2014.

[67] Z. Giergiczny, "Fly ash and slag," *Cement and Concrete Research*, vol. 124, Article ID 105826, 2019.

[68] S. Kramar and V. Ducman, "Evaluation of ash pozzolanic activity by means of the strength activity index test, frattini test and DTA/TG analysis," *Tehnicki vjesnik - Technical Gazette*, vol. 25, no. 6, pp. 1746–1752, 2018.

[69] J. M. Marangu, J. K. Thiong'o, and J. M. Wachira, "Review of carbonation resistance in hydrated cement based materials," *Journal of Chemistry*, vol. 2019, pp. 1–6, Article ID 8489671, 2019.

[70] M. Rahman, R. N. Hora, I. Ahenkorah, and S. Beecham, "State-of-the-Art Review of Microbial-Induced Calcite Precipitation and its Sustainability in Engineering Applications," *sustainability-logo*, vol. 12, no. 15, 2020.

[71] S. Ruan, J. Qiu, Y. Weng et al., "The use of microbial induced carbonate precipitation in healing cracks within reactive magnesia cement-based blends," *Cement and Concrete Research*, vol. 115, no. June, pp. 176–188, 2019.

[72] C. Sheng, T. Li, J. Cheng, Z. Hao, Z. Hao, and L. Bin, "Factors affecting the performance of microbial - induced carbonate precipitation (MICP) treated soil a review," *Environmental Earth Sciences*, vol. 79, 2020.

[73] S. Mondal and A. Ghosh, "Review on microbial induced calcite precipitation mechanisms leading to bacterial selection for microbial concrete," *Construction and Building Materials*, vol. 225, pp. 67–75, 2019.

[74] D. Mujah, M. A. Shahin, L. Cheng, D. Mujah, M. A. Shahin, and L. Cheng, "State-of-the-Art Review of Biocementation by Microbially Induced Calcite Precipitation (MICP) for Soil Stabilization," *Geomicrobiology Journal*, vol. 34, no. 6, pp. 524–537, 2017.

[75] J. R. Manoj Kumar, "Effect of partial replacement of cement with fly ash and rice husk ash on strength and durability of," *Xii*, vol. 19, no. i, pp. 1–8, 2019.

[76] J. Shao, J. Gao, Y. Zhao, and X. Chen, "Study on the pozzolanic reaction of clay brick powder in blended cement pastes," *Construction and Building Materials*, vol. 213, pp. 209–215, 2019.

[77] S. Bhaskar, K. M. Anwar Hossain, M. Lachemi, G. Wolfaardt, and M. Otini Kroukamp, "Effect of self-healing on strength and durability of zeolite-immobilized bacterial cementitious mortar composites," *Cement and Concrete Composites*, vol. 82, pp. 23–33, 2017.

[78] ASTM, *Standard Specification for Coal Fly Ash and Raw or Calcined Natural Pozzolana for Use in concrete (C618)*, American Society for Testing and Materials (ASTM) International, West Conshohocken, PA, 2003.

[79] H. Rong, G. Wei, G. Ma et al., "Influence of bacterial concentration on crack self-healing of cement- based materials," *Construction and Building Materials*, vol. 244, Article ID 118372, 2020.

[80] J. M. Wachira, R. Wangui Ngari, J. K. Thiong'o, and J. M. Marangu, "Effect of sulphate and chloride ingress on selected cements mortar prisms immersed in seawater and leather industry effluent," *Advances in Civil Engineering*, vol. 2019, Article ID 8191689, 2019.