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LONG-TERM MECHANICAL PROPERTIES AND DURABILITY OF A BIOLOGICAL COATING MORTAR USING *RHODOBACTER CAPSULATUS* PN7 IMMOBILIZED IN VERMICULITE

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Abstract

This study evaluates the influence of vermiculite immobilizing the indigenous purple nonsulfur bacterium *Rhodobacter capsulatus* PN7 on the long-term mechanical performance and selected durability-related properties of a cementitious coating mortar intended for sewer concrete structures. PN7 produces extracellular polymeric substances (EPS)/glycocalyx, forms biofilms and oxidizes sulfide. Short-term tests confirmed that the strain remained viable after 72 h of mortar hardening and retained sulfide-oxidizing activity. Three mortar systems were investigated: vermiculite without bacteria (V), vermiculite immobilizing the Korean bacterial strain (V-HQ), and vermiculite immobilizing the Vietnamese PN7 strain (V-VN). Vermiculite replaced 0, 10, 20, and 30 vol.% of the fine aggregate. Flexural and compressive strengths were measured at 28, 360, and 720 days under three curing/exposure conditions. Capillary water absorption and the total electrical charge passed were determined as transport-related indicators of water uptake and resistance to ionic/chloride ingress. V-vermiculite progressively reduced mortar strength as its content increased. In contrast, PN7-immobilized vermiculite mitigated part of the strength loss, with the effect becoming more evident at later ages. The relative performance of V-VN and V-HQ was not consistent across all mixtures and exposure conditions. The 30% V-VN mixture exhibited the lowest capillary water uptake, whereas 20% V-VN provided a more balanced combination of mechanical performance and durability. The results support the potential of PN7-immobilized vermiculite for biological coating applications; however, direct resistance to H_2S-induced or biogenic sulfuric-acid deterioration and long-term bacterial persistence require further verification.

Keywords: biological coating mortar; biomimetic coating; vermiculite; *Rhodobacter capsulatus* PN7; EPS/glycocalyx; compressive strength; capillary water absorption; chloride-ion penetration.

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1. Introduction

Reinforced-concrete sewerage systems are indispensable components of urban infrastructure, yet their internal surfaces are exposed to highly aggressive conditions governed by coupled chemical and microbiological processes. A characteristic deterioration mechanism is microbiologically influenced corrosion associated with sulfur compounds. Under anaerobic wastewater conditions, sulfate-reducing bacteria convert sulfate to sulfide. Hydrogen sulfide (H_2S) can then partition into the gas phase and diffuse toward concrete surfaces above the wastewater level. On moist, oxygenated surfaces, H_2S is further oxidized—primarily by sulfur-oxidizing microorganisms—to sulfuric acid (H_2SO_4) [1, 2]. Sulfuric acid reacts with calcium-bearing hydration products in the cement matrix, destabilizing the matrix and promoting the formation of gypsum and ettringite. Their accumulation can cause expansion, cracking, softening, and spalling of the concrete cover. As the damaged zone develops, mechanical resistance decreases and aggressive species gain easier access to the reinforcing steel, shortening structural service life [3, 4]. This form of corrosion is a major deterioration mechanism in concrete sewer systems. Similar damage has been observed in wastewater collection and drainage structures in Vietnam, including cracking, cover spalling, and reinforcement corrosion after periods of service.

Conventional mitigation strategies focus on controlling H_2S, increasing the chemical resistance of concrete, or applying protective surface coatings. Their effectiveness is limited by the coupled influence of the sewer atmosphere, wastewater chemistry, H_2S generation, and microbial activity. A passive barrier may therefore be unable to address the full deterioration process. Biomimetic coating systems in which bacteria are immobilized in porous carriers offer a different strategy: the coating provides a physical barrier while the microorganisms retain metabolic functions that can reduce corrosion-related species [4, 5]. The performance of such a biological coating depends on the bacterial strain, the type and content of carrier, bacterial survival within the cementitious matrix, and the effect of the carrier on mortar structure and mechanical behavior [6]. Biological activity, long-term mechanical response, and transport properties must thus be evaluated together when selecting a carrier content for sewer applications [7–9].

Purple nonsulfur bacteria (PNSB) are particularly attractive because they can produce extracellular polymeric substances (EPS)/glycocalyx and develop biofilms [6, 10]. These products can form a protective layer at the material surface and restrict contact with and ingress of corrosive agents [4, 10]. Previous studies have incorporated *Rhodobacter capsulatus* immobilized in vermiculite into biological coating mortars and reported sustained bacterial activity within cement-based matrices together with improved resistance to sulfuric-acid attack. At the same time, vermiculite is a highly porous, low-density carrier and can alter the pore structure and mechanical properties of the mortar. The content of bacteria-bearing vermiculite must therefore be optimized with respect to microbial activity, long-term strength, and transport resistance [11, 12].

Carrier characteristics and base-mortar composition are as important as bacterial activity. Vermiculite combines a porous layered structure, high moisture-retention capacity, and cation-exchange capability, features that favor cell immobilization and help sustain bacterial activity. Partial replacement of fine aggregate by vermiculite, however, can increase porosity, lower density, and reduce strength. The practical design problem is therefore to provide enough carrier to support viable and active cells without excessively compromising the mechanical performance or resistance of the coating to fluid and ion ingress.

Accordingly, this study examines a biological coating mortar for up to 720 days using the indigenous PNSB strain *Rhodobacter capsulatus* PN7, isolated in Vietnam and immobilized in vermi-

culite. Three systems – natural vermiculite (V), vermiculite immobilizing the Korean reference strain (V-HQ), and vermiculite immobilizing PN7 (V-VN) – are compared in terms of flexural strength, compressive strength, capillary water absorption, and total electrical charge passed as an electrical indication of resistance to chloride-ion penetration. The aim is to clarify the coupled effects of carrier content, bacterial immobilization, long-term mechanical performance, and transport behavior and, on that basis, identify a composition with potential for protective coating applications on sewer concrete. The electrical charge-passed test is used as a supplementary transport indicator and does not directly simulate H_2S-induced or biogenic sulfuric-acid deterioration.

2. Materials and Methods

2.1. Materials

Nghi Son PC40 cement was used as the binder; its physical and mechanical properties complied with TCVN 2682:2020 [13]. VINNAPAS 5010N redispersible polymer powder supplied by Wacker was added to improve adhesion, flexural performance, deformation resistance, and abrasion resistance [14]. The fine aggregate had a particle size of approximately 0.315 mm. Vermiculite was selected as the porous carrier. This naturally occurring Al-Fe-Mg silicate has a pH of approximately 7.0-7.5, a negatively charged surface, and a high specific surface area, giving it strong ion-adsorption and moisture-retention capacity [15]. Its layered, porous structure provides favorable sites for bacterial immobilization and helps maintain cell activity. Selected physical properties of the cement, sand, and vermiculite are listed in Table 1.

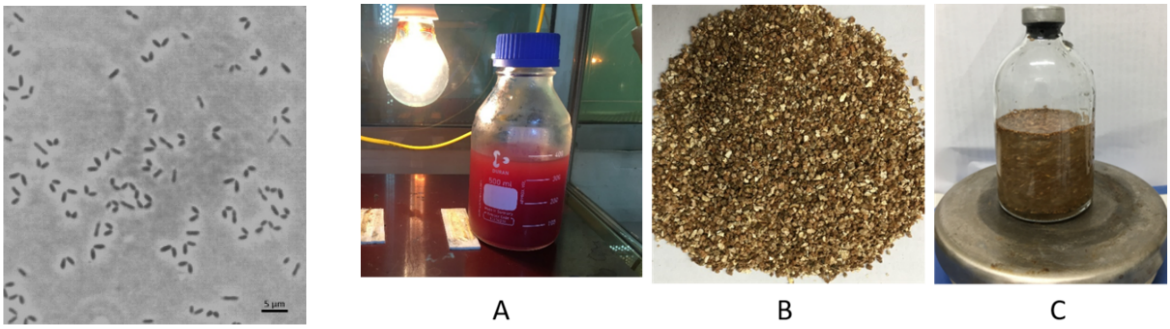
Table 1. Physical properties of cement, vermiculite, and fine aggregate

Property	Unit	Cement	Vermiculite	Sand
Specific gravity		3.1	0.41	2.638
Apparent particle density	g/cm^3		0.34	2.600
Blaine specific surface area	m^2/g	0.387	5.93	0.021
Soundness	mm	0.2		
pH	–		7.0–7.5	
Water absorption	%		301	
3-day compressive strength	MPa	33.5		
28-day compressive strength	MPa	54.6		
Mean particle size	μm	17.3		
Particle-size range	mm		0.14–2.5	0.14–0.315

2.2. Bacterial strain and immobilization in vermiculite

The bacterial strain used in this study was the indigenous PNSB *Rhodobacter capsulatus* PN7, isolated from wastewater in Hanoi and deposited at the Vietnam Type Culture Collection under accession number VTCC 60055. PN7 was enriched in an anaerobic/microaerobic mineral medium and characterized by 16S rDNA sequencing. The sequence showed approximately 99% similarity to *Rhodobacter capsulatus*; the isolate is referred to throughout this study by its established strain designation, *Rhodobacter capsulatus* PN7. Physiological tests showed that PN7 oxidizes sulfide, produces EPS/glycocalyx, and forms biofilms under both aerobic and anaerobic conditions. The sulfide oxidation rate reached 12.40 mg/L/day in medium supplemented with 2 mM sulfide, while the maximum EPS/glycocalyx production was 0.67 g/L with glucose as the carbon source. These traits motivated the selection of PN7 for the biological coating sample.

Vermiculite was used as the bacterial carrier because its high specific surface area, moisture-retention capacity, and porous layered structure reported in the literature favor cell attachment and help sustain activity [15]. PN7 was immobilized on vermiculite by adsorption, a simple procedure that minimizes stress on the cells during immobilization. After immobilization, the viable-cell density on the carrier reached approximately 9.3×10^8 MPN/g and the immobilized cells retained the ability to grow in PNSB medium. When exposed to a simulated sewer condition containing 4 mg/L H_2S, bacterial growth decreased but the immobilized cells remained active, indicating that vermiculite can support PN7 survival under adverse conditions.



(a) Cell morphology of PN7

(b) Immobilization of PN7 on vermiculite: A – PN7 culture; B – vermiculite carrier; C – bacterial immobilization on vermiculite

Figure 1. Cell morphology and immobilization of *Rhodobacter capsulatus* PN7 on vermiculite

Survival of PN7 after incorporation into the cement mortar was quantified by the most probable number (MPN) method. After 72 h of hardening, the viable-cell density in the hardened coating mortar was approximately 8.6×10^4 MPN/g, comparable to that of the Korean *Rhodobacter capsulatus* strain. These short-term results support the use of the vermiculite-PN7 system in the coating-mortar mixtures evaluated below. Bacterial viability and metabolic activity were not monitored at 360 or 720 days; therefore, the long-term mechanical differences are interpreted as the response of mortars containing PN7-immobilized vermiculite rather than as direct evidence of sustained bacterial activity throughout the full exposure period.

2.3. Selection of biological coating mortar mixtures

Three mortar systems were prepared with vermiculite as the carrier: (i) vermiculite without bacteria (V); (ii) vermiculite immobilizing the Korean reference strain (V-HQ); and (iii) vermiculite immobilizing the Vietnamese PN7 strain (V-VN). For each system, vermiculite replaced 0, 10, 20, or 30 vol.% of the fine aggregate so that the effects of carrier content and bacterial immobilization could be compared on a consistent volumetric basis.

The mortar mix proportions were determined using the absolute volume method based on the selected material ratios, which are presented in detail in Table 2. The polymer content was expressed as a percentage by mass of the binder (B); the vermiculite content was determined as a percentage of the sand volume; and the sand-to-binder ratio (S/B) and water-to-binder ratio (W/B) were determined by mass. The absolute volume method was used to calculate the quantities of materials required to produce 1 m^3 of mortar. Based on the reference mortar mix meeting the required strength, mortar mixtures in which a portion of the sand was replaced with vermiculite or bacteria-immobilized vermiculite were selected for further evaluation of the mechanical properties and durability of the coating mortar in the subsequent sections.

Table 2. Mix proportions of mortars containing natural and bacteria-immobilized vermiculite

No.	Mix proportions of mortars						Unit weight (kg/m^3)				
	S/B, by mass	W/B, by mass	Polymer, wt.% of binder	(V), % by vol.	(V-HQ), % by vol.	(V-VN), % by vol.	Cement	Vermi-culite	Sand	Polymer	Water
1	1.8	0.4	5	0	0	0	672	0.0	1210	33.6	269
2	1.8	0.4	5	10	10	10	672	18.5	1089	33.6	269
3	1.8	0.4	5	20	20	20	672	37.0	968	33.6	269
4	1.8	0.4	5	30	30	30	672	55.5	847	33.6	269

2.4. Curing/exposure conditions and test methods

To reproduce representative service conditions for a coating mortar in a wastewater sewer, specimens were subjected to three curing/exposure environments: MT1, positioned above the wastewater level and exposed to the gaseous headspace; MT2, fully immersed in wastewater; and MT3, immersed in clean water and served as the control. The wastewater used for MT1 and MT2 was laboratory-prepared wastewater from the original experimental programme. MT1 and MT2 specimens were placed in the same sealed vessel so that the two conditions differed primarily by gaseous versus submerged exposure: MT2 specimens remained fully submerged, whereas MT1 specimens were positioned above the liquid surface without direct contact with wastewater. The vessel was kept sealed during the exposure interval and the wastewater was renewed every month during exposure. The wastewater characteristics were as follows: COD concentration, 350-400 mg/L; organic loading rate (ORL), 1-1.2 kg COD/m^3·day; ammonium concentration, 45-50 mg/L; phosphorus concentration, 10 mg/L; and trace elements required for aerobic granular sludge cultivation. The sample arrangement for MT1 and MT2 is shown in Fig. 2.

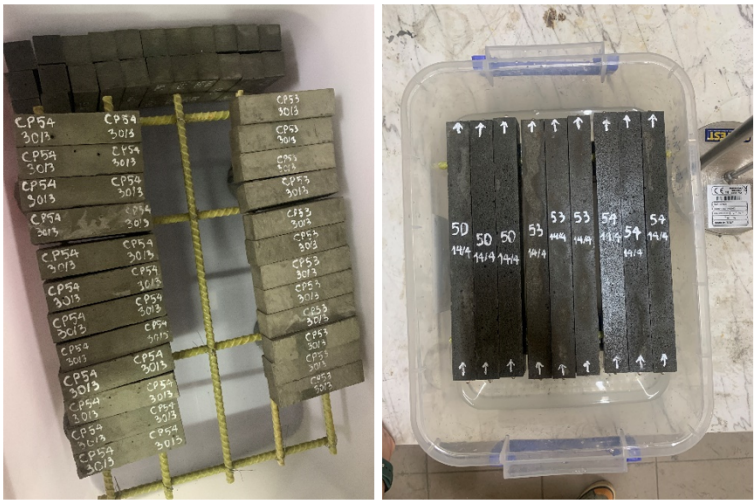


Figure 2. Sample arrangement under MT1 and MT2 exposure conditions

Flexural and compressive strengths were determined in accordance with TCVN 3121-11:2022 [16]. Prismatic samples measuring 40 × 40 × 160 mm were demolded after 24 h of initial curing and transferred to MT1, MT2, or MT3. Strength was measured at 28, 360, and 720 days to characterize long-term mechanical development under the three exposure conditions.

The capillary water absorption coefficient of mortars was evaluated based on ASTM C1585-2020 [17]. Cylindrical specimens (100 mm in diameter × 200 mm high) were exposed under MT1 for 28 days and then cut into 100 × 50 mm discs. The circumferential surface was sealed with epoxy to suppress lateral moisture exchange, leaving the bottom face in contact with water. Specimen mass was recorded at prescribed times from 60 s to 9 days. V and V-VN mortars containing 0, 20, and 30 vol.% vermiculite were tested. The reported mass-based capillary water absorption was calculated from the measured mass gain normalized by the exposed area and the square root of elapsed time, and is expressed in $kg/m^2 \cdot h^{0.5}$.

The electrical indication of resistance to chloride-ion penetration was assessed in accordance with TCVN 9337:2012; the principle is equivalent to that of ASTM C1202. Cylindrical discs measuring 100 mm in diameter × 50 mm high were tested for the V and V-VN systems containing 0, 10, 20, and 30 vol.% vermiculite. After casting, specimens were maintained under MT1 and the total electrical charge passed was determined at 28 and 365 days. This measurement is used here as a supplementary indicator of ionic transport resistance; it is not a direct measurement of chloride diffusion and does not simulate H_2S-induced or biogenic sulfuric-acid deterioration.

3. Results and Discussion

3.1. Mechanical properties of biological coating mortars

a. Flexural strength

Figs. 3–5 present the flexural strengths of the V, V-HQ, and V-VN samples, respectively.

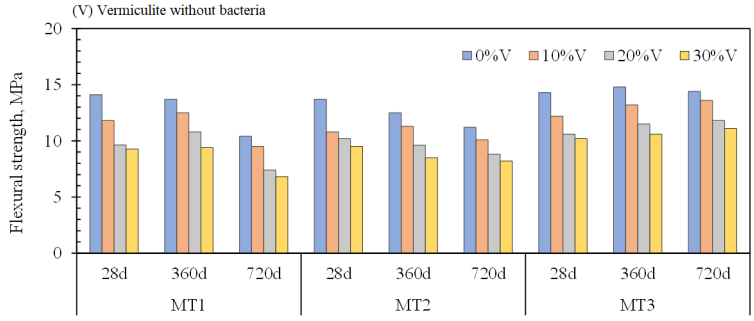


Figure 3. Flexural strength of V-samples under MT1, MT2, and MT3

Flexural strength varied with vermiculite type and content (0–30%), exposure duration (28, 360, and 720 days), and environment (MT1-MT3). In the V-samples (Fig. 3), strength generally decreased as the vermiculite content increased. Under MT1 at 28 days, for example, flexural strength fell from 14.1 MPa without vermiculite to 11.8 MPa at 10% vermiculite, a reduction of about 16.3%. At 30% vermiculite, the reduction reached approximately 34% relative to the control. The same tendency was observed under MT2 and MT3. The reduction in flexural strength at high vermiculite contents is consistent with the low particle density and very high water absorption and high specific surface area measured for the vermiculite used in this study (Table 1), together with its porous layered structure reported in the literature [15], rather than with a directly measured porosity value.

The V-HQ samples (Fig. 4) generally developed higher flexural strengths than their V-counterparts, most noticeably at 10–20% vermiculite and at later ages. Under MT3, the V-HQ sample with 10% vermiculite reached 13.4 MPa at 720 days, 1.8 MPa (15.5%) above the corresponding V-sample (11.6 MPa). The benefit diminished at 30% vermiculite, indicating that bacterial immobilization did not fully compensate for the adverse mechanical effect of a high porous-carrier content. Thus,

bacteria-immobilized vermiculite can partly mitigate vermiculite-induced strength loss under some test conditions, but the magnitude of the effect depends on carrier content, age, and exposure condition.

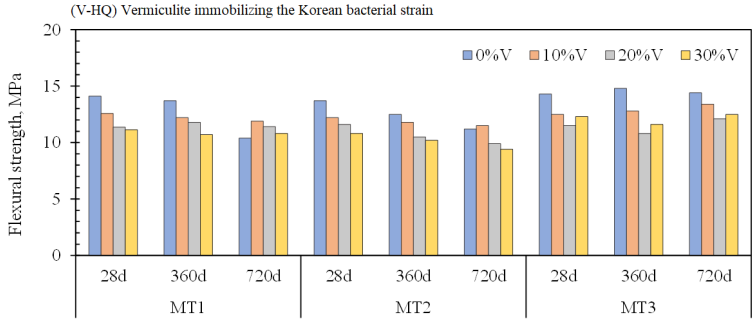


Figure 4. Flexural strength of V-HQ samples under MT1, MT2, and MT3

V-VN showed a similar pattern (Fig. 5). Its flexural strength was generally slightly lower than that of V-HQ but remained above that of the bacteria-free V series. Under MT2, V-VN with 10% vermiculite reached 10.8 MPa at 720 days, approximately 6.9% above the corresponding V sample (10.1 MPa). Under MT3, V-VN with 20% vermiculite reached 12.1 MPa at 720 days, about 2.5% above the V-HQ sample at the same content (11.8 MPa). These results show that the mortar containing PN7-immobilized vermiculite can retain flexural strength relative to natural vermiculite under some conditions. The differences between V-VN and V-HQ are small and not systematic, however, and cannot be attributed to sustained long-term microbial activity or microstructural changes without complementary evidence.

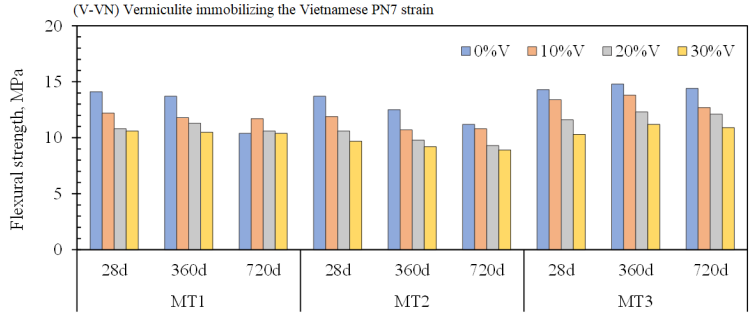


Figure 5. Flexural strength of V-VN samples under MT1, MT2, and MT3

Taken together, V-vermiculite reduced flexural strength, especially at the highest content, whereas the strength loss was less pronounced when the carrier contained immobilized bacteria. The benefit was clearest at 10–20% vermiculite and at later ages. No consistent superiority of V-VN over V-HQ was observed; the response remained dependent on carrier content, age, bacterial strain, and exposure environment.

b. Compressive strength

Figs. 6–8 show the compressive-strength development of V, V-HQ, and V-VN, respectively, for vermiculite contents of 0, 10, 20, and 30% under MT1, MT2, and MT3 at 28, 360, and 720 days. The test matrix therefore captures the combined effects of carrier type, content, exposure duration, and environment on long-term compressive behavior.

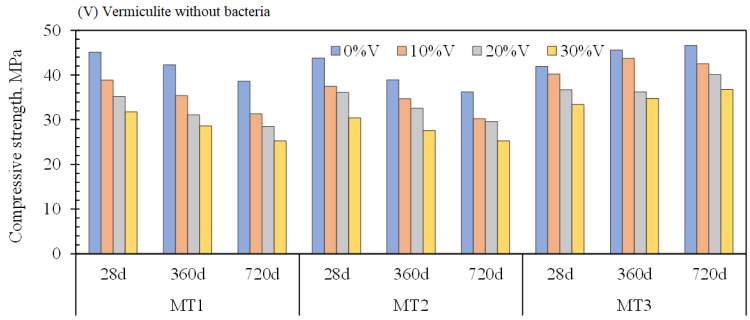


Figure 6. Compressive strength of V samples under MT1, MT2, and MT3

In the V series (Fig. 6), compressive strength generally decreased as vermiculite content increased in all three environments. Under MT1 at 28 days, strength declined from 44.5 MPa for the sample without vermiculite to 31.7 MPa at 30% vermiculite, a reduction of approximately 28.8%. Similar reductions persisted at 360 and 720 days and under MT2 and MT3, although their magnitude differed among environments. MT3 samples generally retained higher strengths at later ages; the sample containing 30% vermiculite, for example, reached 36.8 MPa at 720 days. Exposure condition therefore had a pronounced influence on long-term strength development, particularly when the porous-carrier content was high.

For V-HQ (Fig. 7), the Korean strain-immobilized vermiculite generally resulted in better compressive-strength retention than vermiculite (V), particularly at later ages. Under MT1, the mixture containing 10% vermiculite reached 36.4 MPa at 720 days, approximately 16.3% higher than the corresponding V-sample (31.3 MPa). Under MT3, V-HQ mortars with 10% and 30% vermiculite reached 44.6 and 34.7 MPa, respectively, at 720 days. The bacterial carrier thus partly offset the mechanical penalty associated with vermiculite, although the magnitude of the effect varied with content and exposure environment.

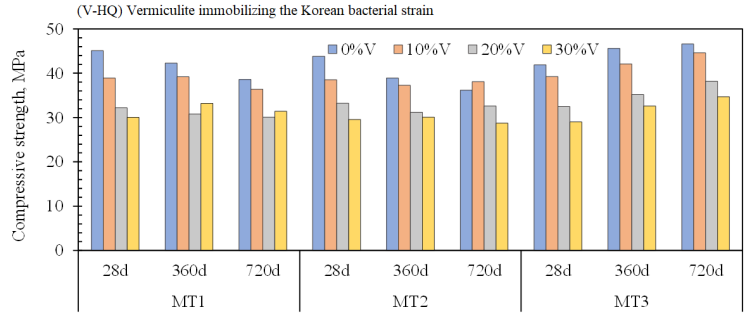


Figure 7. Compressive strength of V-HQ samples under MT1, MT2, and MT3

The V-VN samples (Fig. 8) exhibited the same general behavior. Under MT1, V-VN with 10% vermiculite reached 39.7 MPa at 720 days, 26.8% above the corresponding V sample (31.3 MPa). Under MT3, the 20% V-VN sample reached 35.2 MPa at 720 days, which was 7.9% lower than the corresponding V-HQ value (38.2 MPa). The data therefore do not support a general claim that V-VN consistently outperforms V-HQ. Both bacteria-bearing systems, however, retained compressive strength better than the corresponding natural-vermiculite mortar under several test conditions.

Comparison among the three environments shows that MT3 was generally the most favorable for long-term compressive-strength development, especially at 720 days. Strengths under MT1 and

MT2 were lower, with MT1 often showing the larger reduction. At 30% vermiculite and 720 days, the difference between MT1 and MT3 reached about 35.8%. This environmental sensitivity indicates that the effectiveness of a biological mortar cannot be judged from carrier content alone; the service exposure must be considered simultaneously.

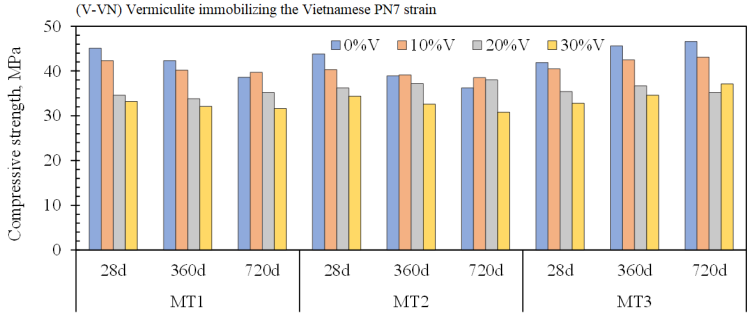


Figure 8. Compressive strength of V-VN samples under MT1, MT2, and MT3

Overall, high contents of natural vermiculite reduced compressive strength, whereas bacteria-bearing vermiculite partly mitigated this effect. Within the investigated range, 20% V-VN at S/B = 1.8 and W/B = 0.4 was selected as a practical compromise between carrier content and long-term strength retention across the three exposure environments. This composition was therefore carried forward to the durability assessment.

Considering flexural and compressive strength together, the 20% sample maintained comparatively stable mechanical performance over time under MT1, MT2, and MT3 and continued to develop strength at 360 and 720 days, particularly under MT3. Relative to the 30% samples, the 20% content provided a better balance between mechanical-property retention and the amount of carrier available for bacterial immobilization.

Accordingly, 20% PN7-immobilized vermiculite was considered the best overall compromise within the present experimental range: it avoided the more pronounced strength loss associated with higher carrier content while retaining a substantial amount of bacterial carrier. The 30% V-VN mixture nevertheless provided the lowest capillary water uptake, and both 20% and 30% V-VN exhibited low charge-passed values. The 20% mixture was therefore selected as the preferred overall composition based on the combined mechanical and transport-related results, not as a universal optimum.

3.2. Durability of biological coating mortars

a. Capillary water absorption coefficient

Capillary water absorption coefficient results for the V and V-VN systems are presented in Figures 9–11. The test procedure is described in Section 2.4.



Figure 9. Capillary water absorption coefficient test setup

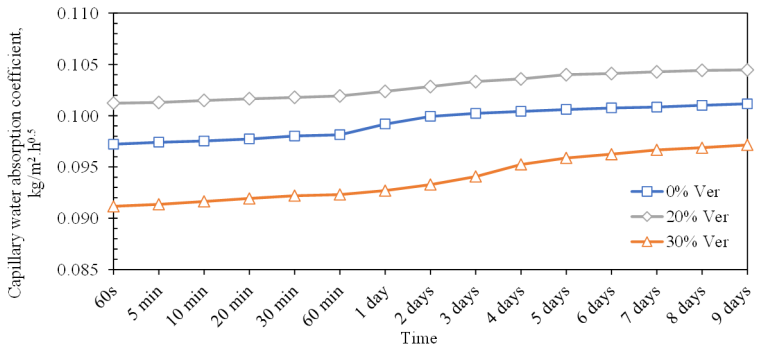


Figure 10. Capillary water absorption coefficient of samples containing vermiculite without bacteria

The results for samples containing V-vermiculite are presented in Fig. 10.
The corresponding results for PN7-immobilized samples are presented in Fig. 11.

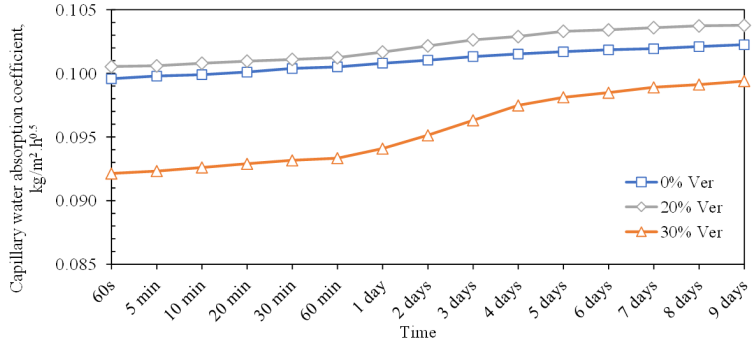


Figure 11. Capillary water absorption coefficient of samples containing PN7-immobilized vermiculite

Capillary water uptake increased continuously from 60 s to 9 days, reflecting progressive filling of the accessible pore network. The effect of vermiculite content differed markedly between V and V-VN samples. Throughout the test period, all measured coefficients remained below the target value of 0.5000 $kg/m^2 \cdot h^{0.5}$ specified for the coating mortar in the research protocol proposed by the Ministry of Science and Technology [18].

For the V-sample, the 20% vermiculite sample showed the lowest water uptake, with coefficients of 0.0912–0.0972 $kg/m^2 \cdot h^{0.5}$, below the 0% mixture (0.0972–0.1012 $kg/m^2 \cdot h^{0.5}$). Increasing vermiculite to 30% raised the coefficient to 0.1012–0.1045 $kg/m^2 \cdot h^{0.5}$, the highest range among the three samples. The effect of V-vermiculite was therefore non-monotonic: a 20% content was favorable, whereas 30% increased capillary uptake, plausibly because a larger volume of the highly porous, water-absorbing carrier introduced more accessible transport pathways.

The response of V-VN samples was different. The 0% sample gave 0.0996–0.1023 $kg/m^2 \cdot h^{0.5}$, only slightly above the corresponding V values. At 20% vermiculite, the coefficient increased to 0.1005–0.1038 $kg/m^2 \cdot h^{0.5}$, 6.8–10.3% above the V-sample at the same content. By contrast, the 30% vermiculite–PN7 sample produced the lowest values within the V-VN samples, 0.0921–0.0994 $kg/m^2 \cdot h^{0.5}$, which were 4.9–9.0% below those of the 30% vermiculite sample. Thus, the trend observed for V-vermiculite was reversed when the same carrier content contained immobilized PN7.

The ability of the vermiculite–PN7 system to restrict capillary uptake was strongly content-dependent. At 30%, the larger carrier surface area and moisture reservoir may provide a more favorable microenvironment for bacterial survival and activity. EPS/glycocalyx production and biofilm formation could, in turn, reduce the connectivity of water-conducting pores and pathways. This in-

terpretation is consistent with the barrier mechanism proposed for biological coating mortars, but it remains a working hypothesis in the present study because no direct microstructural or mineralogical evidence was obtained. SEM, XRD, and quantitative pore-structure analyses were outside the scope of the original long-term mechanical and transport programme and are required in future work to verify any proposed microstructural or mineralogical mechanism.

Among the mixtures tested, 30% PN7-immobilized vermiculite provided the greatest resistance to capillary water uptake, whereas 20% was optimal for V-vermiculite. The contrast confirms that PN7 performance is coupled to carrier content rather than determined simply by the presence of bacteria. This interaction should therefore be considered when selecting a biological coating-mortar composition for subsequent durability evaluation.

b. Electrical indication of resistance to chloride-ion penetration

The total electrical charge passed for the V and V-VN systems is summarized in Table 3; specimen preparation, exposure conditions, and test ages are described in Section 2.4. The test setup is shown in Fig. 12.



Figure 12. Chloride-ion penetration test setup

The measured charge-passed values varied strongly with vermiculite content, bacterial immobilization, and test age.

Table 3. Total electrical charge passed through vermiculite-containing mortar samples

No.	Vermiculite content, % by vol.	Total charge passed (C)			
		V-vermiculite		V-VN-vermiculite	
		28 days	365 days	28 days	365 days
1	0	237	244	237	244
2	10	125	362	244	352
3	20	85	328	13.9	114
4	30	56	743	19.9	118

The charge-passed response was strongly dependent on carrier content and age. For the bacteria-free V system, the 28-day charge decreased from 237 C at 0% vermiculite to 56 C at 30%, but by 365 days the values increased to 244–743 C, with the largest increase observed at 30% V. For V-VN, the 28-day values were 237, 244, 13.9, and 19.9 C at 0, 10, 20, and 30% vermiculite, respectively; the corresponding 365-day values were 244, 352, 114, and 118 C. Thus, the 20% and 30% V-VN mixtures retained substantially lower charge-passed values than their bacteria-free counterparts at 365 days (approximately 65.2% and 84.1% lower, respectively), whereas the difference at 10% was small.

The age-dependent increase, especially for the 30% V mixture, shows that the electrical transport response was not monotonic. These results are interpreted as an electrical indication of resistance to chloride-ion penetration; total charge passed is not a direct chloride diffusion coefficient and is not evidence of resistance to H_2S-induced or biogenic sulfuric-acid attack. The exceptionally low 28-day values for 20% and 30% V-VN should therefore be interpreted cautiously together with their longer-term evolution.

4. Conclusions

This study investigated the long-term mechanical performance and transport-related durability of biological coating mortars containing the indigenous PNSB strain *Rhodobacter capsulatus* PN7 immobilized in vermiculite. Based on the experimental results, the following conclusions can be drawn:

- The indigenous PNSB strain *Rhodobacter capsulatus* PN7 remained viable during the early hardening stage of the coating mortar, with a viable-cell density of approximately 8.6×10^4 MPN/g after 72 h, and retained sulfide-oxidizing activity of 12.40 mg/L/day, significantly higher than that of the Korean reference strain. These results confirm the feasibility of incorporating PN7 into cementitious coating mortar.

- Increasing natural vermiculite content generally reduced flexural and compressive strengths, whereas bacteria-bearing vermiculite partly mitigated this reduction under several exposure conditions. For example, at 720 days under MT1, the 10% V-VN mixture reached a compressive strength of 39.7 MPa, approximately 26.8% higher than the corresponding V mixture (31.3 MPa). Nevertheless, V-VN did not consistently outperform V-HQ, confirming that the mechanical response depended on carrier content, age, and exposure condition.

- The effect on capillary water absorption was strongly dependent on vermiculite content. The 30% V-VN mixture showed the lowest capillary water absorption coefficient within the PN7-bearing series, ranging from 0.0921 to 0.0994 $kg/m^2 \cdot h^{0.5}$, approximately 4.9–9.0% lower than the corresponding 30% natural-vermiculite mortar. Considering mechanical and transport-related performance together, 20% PN7-immobilized vermiculite provided the best overall compromise within the investigated range, whereas 30% was more favorable specifically for reducing capillary water uptake.

- At 365 days, the total electrical charge passed through the 20% and 30% V-VN mixtures was 114 and 118 C, respectively, which was approximately 65.2% and 84.1% lower than the corresponding natural-vermiculite mixtures (328 and 743 C). At 28 days, the corresponding values were 13.9 and 19.9 C, respectively. These results demonstrate a substantial reduction in electrical/ionic transport for the higher-content PN7-bearing mortars. However, total charge passed is an indirect transport indicator and should not be interpreted as a direct measure of chloride diffusion or resistance to H_2S-induced or biogenic sulfuric-acid attack.

Overall, the results demonstrate the potential of PN7-immobilized vermiculite for biological coating mortars intended for sewer-concrete applications. A PN7-bearing vermiculite content of 20% is recommended as the most balanced composition within the investigated range, while further studies involving direct biogenic sulfuric-acid exposure, long-term bacterial monitoring, and microstructural characterization are required to establish the long-term protective mechanism and field performance.

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Declaration of generative AI and AI-assisted technologies

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to improve the English language of the manuscript. The authors reviewed and edited the output and take full responsibility for the final content.

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