Bacteria-induced internal carbonation of reactive magnesia cement

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Abstract

With lower calcination temperature, reactive magnesia cement (RMC) can be a potential alternative to the Portland cement. However, RMC concrete requires accelerated carbonation curing from external sources which greatly hinder the wider applications of RMC. This study proposed a bacteria-based method for the strength gain of RMC through internal carbonation. *Sporosarcina pasteurii*, urea, and yeast extract were used as a carbonation agent for internal carbonation of RMC pastes. Results showed that the flowability of the fresh bio-RMC paste increased by 20% while the initial setting time remained unchanged. Besides serving as the CO_2 provider, urea can also function as superplasticizer to reduce the water demand of the bio-RMC pastes. The resulting bio-RMC pastes showed a continuous strength gain with time, demonstrating the feasibility of bacteria-induced internal carbonation of RMC. Microstructure analysis revealed abundant formation of hydrated magnesium carbonates in the bio-RMC pastes, which is responsible for the strength gain of the bio-RMC pastes.

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1. Introduction

Being the most widely used man-made material, Portland cement (PC) is globally produced at an amount exceeding 4 billion tonnes a year [1]. The production of PC leads to the release of large quantities of CO_2, taking up roughly 8% of global anthropogenic CO_2 emissions [2]. To reduce the emissions from cement production, the use of alternative binding materials has been proposed [3-5]. Reactive magnesia cement (RMC), which has lower calcination temperatures than PC, *i.e.,* 700-900°C vs. 1450°C [6], is one of the potential alternatives. Apart from the typical dry route, *i.e.,* calcination of magnesite, RMC can also be synthesized from magnesium-rich sources such as reject brine [7]. Moreover, the strength gain of RMC involves hydration and carbonation reactions, presenting a route for the permanent sequestration of CO_2 within the RMC matrix [8, 9].

During the strength gain process of RMC, MgO firstly hydrates to form brucite ($Mg(OH)_2$), which is porous and does not significantly contribute to strength development. Subsequently, brucite reacts with CO_2 to form a range of hydrated magnesium carbonates (HMCs), such as nesquehonite ($MgCO_3 \cdot 3H_2O$), hydromagnesite ($4MgCO_3 \cdot Mg(OH)_2 \cdot 4H_2O$), and dypingite ($4MgCO_3 \cdot Mg(OH)_2 \cdot 5H_2O$). Carbonation is the most crucial step for the strength gain of RMC formulations as HMCs are the primary sources of strength within these mixes [8-10]. However, the carbonation of RMC under ambient conditions is very limited due to the low atmospheric

CO_2 concentration, *i.e.,* 0.04% [11]. Therefore, accelerated carbonation is essential and generally realized by providing CO_2 at elevated concentrations, *i.e.,* 10% and higher. While accelerated carbonation allows RMC to gain initial strength, the high concentration of CO_2 provided externally can have adverse effects on long-term strength development [12]. Due to the formation of a dense impermeable layer on the sample surface, further diffusion of CO_2 from outside into the inner sections towards the sample core is hindered. As a result, a majority of hydrated phases, *e.g.,* brucite, remains uncarbonated within the sample, thereby limiting the overall strength gain of RMC [13, 14]. To alleviate this issue, methods have been developed to enhance CO_2 diffusion into the RMC matrix by introducing porosity via varying the particle size distribution of aggregates [15].

The requirement of accelerated carbonation curing limits the applications of RMC to precast elements only, because accelerated carbonation requires the use of enclosed chamber or incubator with controlled CO_2 concentration, relative humidity (RH) and temperature. It is also a challenge to carry out accelerated carbonation curing for large precast elements, such as bridge girders, due to space constraint, low production efficiency and high cost. All of the above greatly hinder the wider applications of RMC.

One potential solution to overcome this bottleneck is to provide an internal source of CO_2 within RMC matrix by using microorganisms. Accordingly, *Sporosarcina pasteurii* is a moderately alkaliphilic bacteria with a high urease-producing performance [16-18]. Urease is an enzyme that catalyses the hydrolysis of urea into CO_2 and NH_3. The hydrolysis rate of urea under room temperature and pressure is very slow. With the help of urease, the hydrolysis of urea is accelerated, as shown in Equation 1 [19].

$$(NH_2)_2CO\ (Urea) + H_2O \xrightarrow{urease} CO_2 + NH_3 \quad (1)$$

The external application of *Sporosarcina pasteurii* and urea on RMC samples for crack repairing has been reported in an earlier study [20], where the formation of HMCs was observed in the cracks. It is therefore plausible to incorporate bacteria directly into the RMC matrix to engage such CO_2 producing mechanism as an internal source of CO_2 for internal carbonation of RMC. Until now, the use of *Sporosarcina pasteurii* in cementitious materials is mainly studied in PC-based systems [21-23], whereas the application of this method for internal carbonation of RMC-based systems that purely rely on the carbonation process for strength gain, has not been previously investigated.

In line with this gap in the literature, this study proposes a bacteria-based method to accelerate and increase the carbonation degree of RMC. In order to provide CO_2 internally, *Sporosarcina pasteurii* cells, urea and yeast extract (YE) were mixed directly into RMC pastes together as a three-component carbonation agent. The RMC paste with this bacteria-based carbonation agent is referred to as the bio-RMC paste. Among the carbonation agent, urea is the CO_2 precursor and YE serves as the nutrient for bacteria which are responsible for releasing the enzyme (urease). With all the components needed for the carbonation process incorporated in the matrix, the enzyme released from the bacteria catalyses the hydrolysis of urea to generate CO_2, which is then used to accelerate the carbonation reaction. This proposed method presents two main advantages. First, changing the source of CO_2 from external to internal fully eliminates limitations associated with the diffusion of CO_2 within the sample depth. Second, this method eliminates the need for any special curing environment such as those provided by

incubators/carbonation chambers. This allows the RMC concrete to be cast in-situ and cured on site which greatly increase the potential applications of RMC.

In this paper, the concept of bacteria-induced internal carbonation of RMC for strength gain was demonstrated for the first time. Influence of the bacteria-based carbonation agent inclusion on the fresh properties of RMC was assessed. The hardened properties and microstructure of the resulting bio-RMC pastes were examined. To further optimize the performance of the bio-RMC pastes, the dosage of urea was increased to provide more CO_2 for carbonation. Meanwhile, water-to-RMC ratio was reduced due to urea can potentially work as a superplasticizer.

2. Materials and methodology

2.1 Materials

Sporosarcina pasteurii (DSM33) used in the current study was purchased from Leibniz Institute DSMZ. The liquid growth medium for the bacteria contained 1 L 0.13 M tris buffer, 20 g yeast extract, and 8.1 g NH_4Cl. Before use, all ingredients were autoclaved separately. The bacteria culture was aerobically incubated at 30℃ on an orbital shaker with a rotational speed of 200 rpm. After 20-24 hours of incubation, the bacteria culture obviously turned turbid, indicating the growth of cells. Serial dilutions and spread plate technique were used to carry out colony-forming unit (CFU) counting [24], and the bacteria culture was stored in 4℃ fridge

until use. Before using the bacteria culture for sample preparation and casting, the volume of the culture needed was calculated according to Equation 2.

$$V_{need} = (C_{need} \times W_{RMC}) / C_{bc} \quad (2)$$

where V_{need} is the volume of the culture needed for casting, C_{need} is the bacteria needed for 1 g RMC, W_{RMC} is the weight of RMC, and C_{bc} is the concentration of the batch culture. The bacteria cells were then harvested by centrifuging the culture at 5000 rpm for 10 minutes. The supernatant was removed, and the cell pellets were kept and used for preparing the bio-RMC pastes, as described in Section 2.2.1.

The RMC used in this study was from International Scientific Pte. Ltd. The chemical compositions and physical properties of RMC, as provided by the supplier, are shown in Table 1. The reactivity of the RMC is 12 seconds, which is obtained by the time needed for the neutralization of 100 ml 0.13 M citric acid monohydrate by 5 g of RMC.

Table 1 Chemical composition and physical properties of RMC

Chemical composition (%)						Physical properties	
MgO	CaO	SiO_2	Fe_2O_3	Al_2O_3	LOI	Bulk density (kg/m^3)	Surface area (m^2/g)
97.75	0.85	1.13	0.12	0.16	3.8	650	45

2.2 Methodology

2.2.1 Mix design and sample preparation

The mix design in the current study is shown in Table 2. To study the influences of carbonation agent on the fresh properties of RMC paste, individual component (i.e., bacteria, YE, urea) or their combination was added into the RMC paste (mixes 1-8). To reveal the effects of bacteria-based carbonation agent on the hardened properties of the resulting bio-RMC pastes, mixes 8-10 were prepared together with the control mix (mix 1). The name code for each mix strictly follows their mix design. In the code, the number behinds W denotes the water-to-RMC ratio, the number behinds U denotes the weight percentage of urea with respect to RMC, Y denotes the addition of 1.5 wt.% YE with respect to RMC, and B denotes the use of 1.5×10^8 CFU bacteria per gram of RMC.

Table 2 RMC mix designs in the current study

S/N	Name	RMC	Water	Urea	YE	Bacteria (CFU/g RMC)
1	W0.5 (control)	1	0.5	-	-	-
2	W0.5-B	1	0.5	-	-	1.5×10^8
3	W0.5-Y	1	0.5	-	0.015	-
4	W0.5-U5	1	0.5	0.05	-	-
5	W0.5-U10	1	0.5	0.10	-	-
6	W0.5-U15	1	0.5	0.15	-	-
7	W0.5-U5-Y	1	0.5	0.05	-	-
8	W0.5-U5-Y-B	1	0.5	0.05	0.015	1.5×10^8
9	W0.45-U10-Y-B	1	0.45	0.10	0.015	1.5×10^8
10	W0.43-U15-Y-B	1	0.43	0.15	0.015	1.5×10^8

To prepare the fresh mix, half of the water was used to dissolve urea, and another half was used to dissolve YE and bacteria cell. Suspending bacteria with YE instead of urea was to prevent the consumption of urea before casting. To prepare the bio-RMC pastes, the two solutions were mixed and quickly stirred first for 20 seconds. After which, the solution was added into the

RMC powder and mixed in a Kenwood KVL6100B mixer for 5-10 minutes until a homogenous state was achieved.

Flowability and setting time of pastes 1-8 were evaluated first to study the influence of urea, YE, and B and their combination on the fresh properties of the resulting pastes. Fresh pastes 1, 7-10 were cast into 50 mm cube moulds and all mixes were cured at the same laboratory air conditions (28±2°C, 80±5% RH) to study the hardened properties of the resulting pastes. After initial hardening for two days, samples were demoulded and cured in the same condition until further tests.

2.2.2 Tests

Flow table model 63-L0040/A from Controls Group was used for the flowability test in accordance with ASTM C1437 [25]. Accordingly, flowability is defined as the increased percentage of the diameter of the paste, as shown in Equation 3.

$$\text{Flowability} = (D_{\text{after}} - D_{\text{before}}) / D_{\text{before}} \times 100\% \quad (3)$$

where D_{before} is the original inside base diameter and D_{after} is the diameter of the paste after the test, which takes the average of four readings along the four lines scribed in the tabletop.

An automatic Vicat apparatus was used for the measurement of setting time in accordance with ASTM 191 [26]. After mixing, the fresh paste was placed on the apparatus for 7 hours before

the penetration test. The penetrating interval was 30 minutes over a duration of 18.5 hours. The penetration depth was automatically recorded to monitor the setting process of the sample.

The compression test was carried out on cube samples at ages of 7, 14 and 28 days according to ASTM C109/C109M-13 [27]. A Toni Technik Baustoffprüfsysteme machine was used to determine the maximum load capacity of the sample under a constant loading rate of 55 kN/min. Each set of test contains at least three cubes and the average of the three tests were reported.

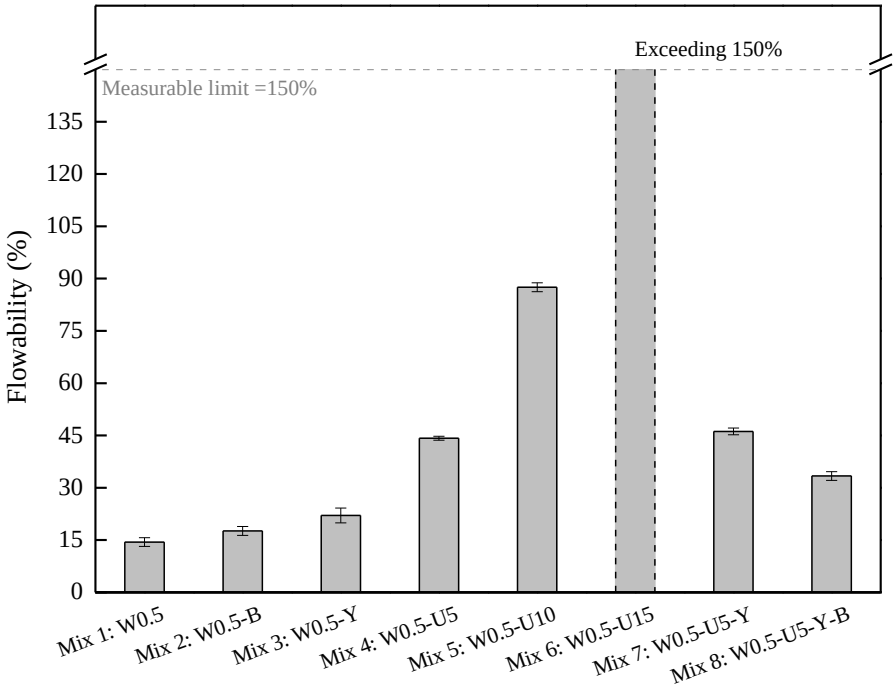
Fractured samples from the compression test were collected and stored in isopropanol for 7 days to stop hydration, followed by vacuum drying for 1 day. After the treatment, fragments were collected and coated with platinum using an auto fine coater (JEOL JFC1600) under 20 mA current for 40 seconds. Then the coated sample were studied through JEOL JSM-7600 F equipment under SEI mode at 5.0 kV voltage. For the XRD scan, fragments were first crushed using a mortar and then sieved through a 75 μm mesh. The XRD scan was conducted on Panalytical Xpert Pro using Cu Ka radiation (40 kV, 30 mA) with a scanning rate of 0.017° 2θ/step from 5° to 80° 2θ.

3. Results and discussion

3.1 Flowability

Figure 1 compares the flowability of RMC pastes with the inclusion of individual component of carbonation agent (i.e., bacteria, YE, urea) and their combination. The diameter of the flow

206 table is 254 mm and the inner diameter of the flow mould is 101.6 mm. According to Equation
207 3, the maximum measurable flowability is 150%. As can be seen, the inclusion of individual
208 component of carbonation agent results in increased flowability of RMC paste. Among the
209 three components, urea addition leads to the most significant improvement of the flowability.
210 While both bacteria and YE brought minor increases less than 8%, the addition of 5% urea
211 results in a triply increase on the flowability of RMC paste from 14.4% to 44.2%. Furthermore,
212 at 10% urea inclusion, the flowability can reach 87.5%. And with 15% urea addition, the
213 flowability of the RMC paste was beyond the measurable limit of the flow table (i.e., 150%).
214 This may be attributed to the interaction between urea and water molecules in the solution,
215 where some water molecules are found "occupied" by urea. The occupied water molecule is
216 either strongly immobilized by one urea molecule through two hydrogen bonds or shared by
217 two urea molecules [28, 29]. When urea solution was applied to RMC, these occupied water
218 molecules were not able to immediately accessible by cement as shown in Figure 2. As a result,
219 they were able to flow freely in the fresh paste and act as "lubricant" to enhance the flowability
220 of MgO paste. In fact, similar results have also been observed in PC and geopolymer mixes
221 [30, 31].



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223 **Figure 1** Flowability of RMC pastes (mix 1 to mix 8)

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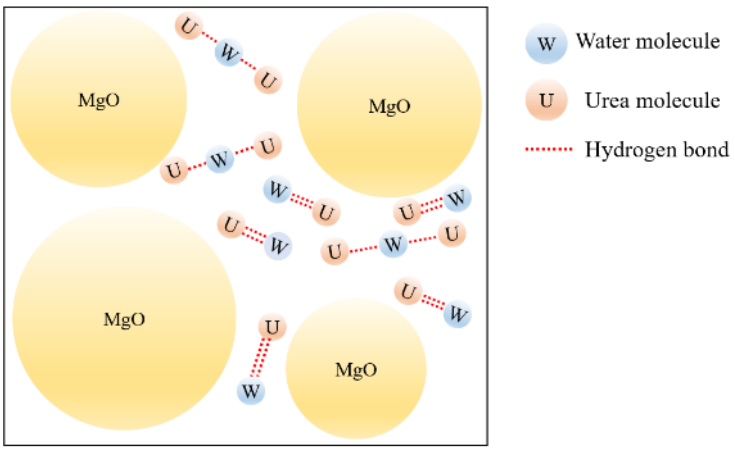

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226 **Figure 2** Schematic illustration of strongly immobilized water molecules with urea in the

227 paste

228 With the inclusion of the complete carbonation agent set (W0.5-U5-Y-B), the flowability

229 increased by 19% when compared to the control mix (W0.5). This shows the inclusion of

bacteria-based carbonation agent in RMC paste is beneficial in terms of flowability. It is worth noting that the flowability enhancement by adding the complete set of carbonation agent is lower than that by including only the organic compounds (i.e., urea and YE). Comparing mix 8 (W0.5-U5-Y-B) with mix 7 (W0.5-U5-Y), the further addition of bacteria has decreased the flowability more than 10%. This may be attributed to the metabolic activity of bacteria which catalyses the hydrolysis of urea during sample preparation and testing.

3.2 Initial setting time

The initial setting time of RMC pastes 1-8 is shown in Figure 3. As can be seen, the inclusion of individual component of carbonation agent results in increased initial setting time of fresh RMC paste. Among the three components, urea addition leads to the most significant delay of the initial setting time. While the addition of bacteria did not influence the setting and YE has delayed the setting by 3 hours, inclusion of 5% urea in RMC paste has delay the initial setting time from 7.5 hours to 15.5 hours. When the addition of urea increased to 15%, the initial setting is further delayed to 24.5 hours. The significant delay of setting may be attributed to the abrupt and drastic temperature reduction when urea dissolves in water [32]. The dissolution of urea in water is an endothermic process [33]. Therefore, the inclusion of urea during mixing and setting reduced the temperature of the pastes, prolonging the setting time. From the result of mix 7 (W0.5-U5-Y) and control mix (W0.5), it is known that the addition of organic compounds (i.e., urea and YE) delays the initial setting by 11 hours. However, the addition of the complete set of carbonation agent (W0.5-U5-Y-B) results in comparable initial setting time around 7.5 hours to the control RMC mix (W0.5). This is again believed to be attributed to the

metabolic activity of bacteria which catalyses the hydrolysis of urea during setting where urea and water were continuously consumed through the hydrolysis of urea. Furthermore, CO_2 generated from the hydrolysis of urea leads to carbonation of brucite and formation of HMCs resulting in comparable setting time to the control mix. This highlights the inclusion of bacteria-based carbonation agent in RMC paste does not delay the initial setting time of the RMC mix.

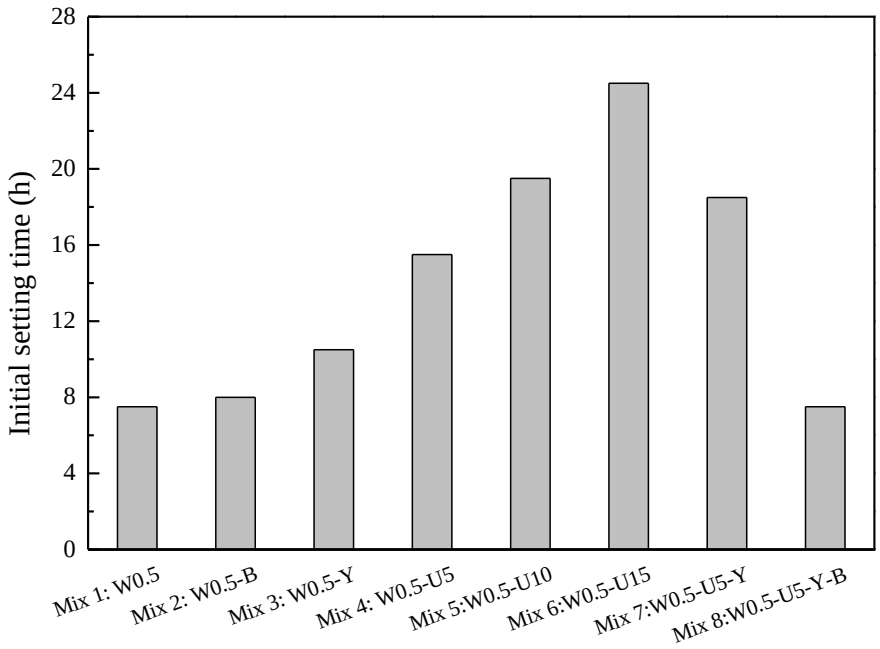


Figure 3 Initial setting time of RMC pastes (mix 1 to mix 8)

3.3 Compressive strength

The compressive strength development of the control RMC (W0.5), nutritious RMC (W0.5-U5-Y) and the bio-RMC (W0.5-U5-Y-B) pastes are shown in Figure 4. RMC pastes demonstrated low strengths that remained stable at around 0.8 MPa throughout the entire 28

days of curing. These constantly low strengths were due to the low CO_2 content, *i.e.,* 0.04%, in air and were in line with the findings of previous studies [1, 5]. After the addition of urea and YE, although the compressive strength of group W0.5-U5-Y has a minor increase than group W0.5, the samples still present low strength around 1 MPa over the entire 28 days curing. An alternative scenario was observed in bio-RMC samples, whose strength went up from 1 MPa at 7 days to 2.4 MPa at 28 days. Since all three groups of samples (W0.5, W0.5-U5-Y, and W0.5-U5-Y-B) were cured under the same conditions thus underwent same external carbonation from air, the steady strength development of bio-RMC pastes was an indication of the role of bacteria-based carbonation agent in the internal carbonation process and associated mechanical performance, resulting in strength that were three times that of the control RMC sample, and more than twice of the nutritious RMC samples.

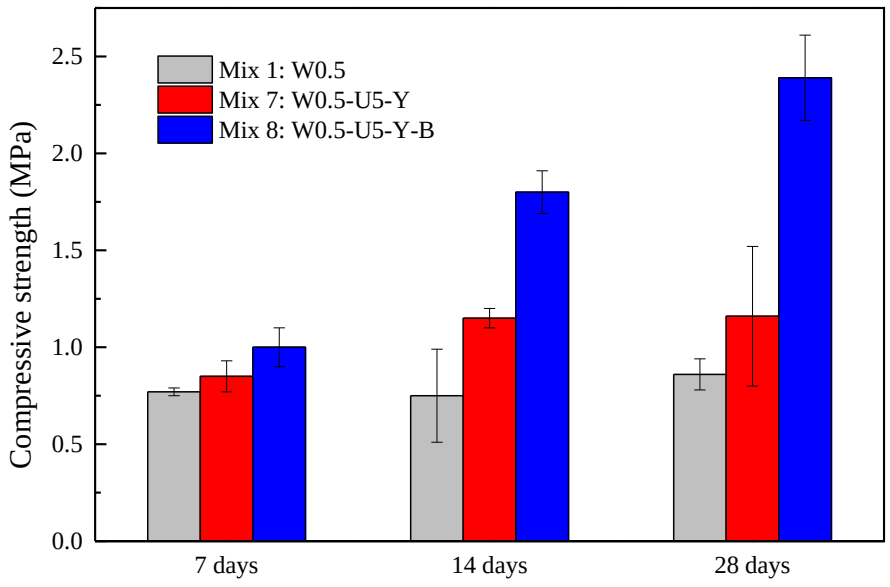


Figure 4 Compressive strength of control RMC pastes (mix 1), nutritious RMC paste (mix 7), and bio-RMC paste (mix 8) at different ages

Although PC pastes present a relatively harsh environment for bacteria due to their high alkalinity that can inactivate bacteria [34], the lower pH of RMC pastes than those of PC pastes could present an advantage for bacterial activity. Previous studies [5] have reported the pH of the pore solution of RMC pastes without carbonation to range between 10.2 and 10.5. Accordingly, *Sporosarcina pasteurii* was found to retain around 40% of its precipitation ability at a pH of 10 [35]. Therefore, the lower pH environment presented by RMC could explain the higher strengths of bio-RMC samples in line with the survival of some bacteria, for which the presence of YE in the same environment acted as a nutrient. The urease released by bacteria was also reported to retain ~80% of its activity at a pH of 10 [36]. Therefore, even if the bacteria were not active, the enzyme still functioned in the system. However, the effectiveness of the bacteria-based carbonation agent decreased over time, as revealed by the lower increasing rate in strength from 14 to 28 days (0.04 MPa/day), when compared to the initial increase from 7 to 14 days (0.11 MPa/day). This reduction in strength development might be associated with the number of viable bacteria cells, which decreased with time, leading to a reduction in the urea decomposition rate. Another reason for the decline of carbonation efficiency was linked with the consumption of urea and water over time, limiting their further access by bacteria.

3.4 Microstructure

The XRD patterns of RMC powder, control RMC (W0.5), nutritious RMC (W0.5-U5-Y) and bio-RMC (W0.5-U5-Y-B) pastes at 28 days are shown in Figure 5. Along with the main peaks of periclase (at 43.0° 2θ), peaks of undecomposed magnesite (at 32.6° 2θ) and brucite (at 38.0° 2θ) were observed in the original RMC powder. The existence of magnesite was associated

with the incomplete decomposition of the original material during the decomposition process, whereas the presence of brucite could be due to the partial hydration of RMC during storage since Singapore has an average daily humidity of ~81% [37].

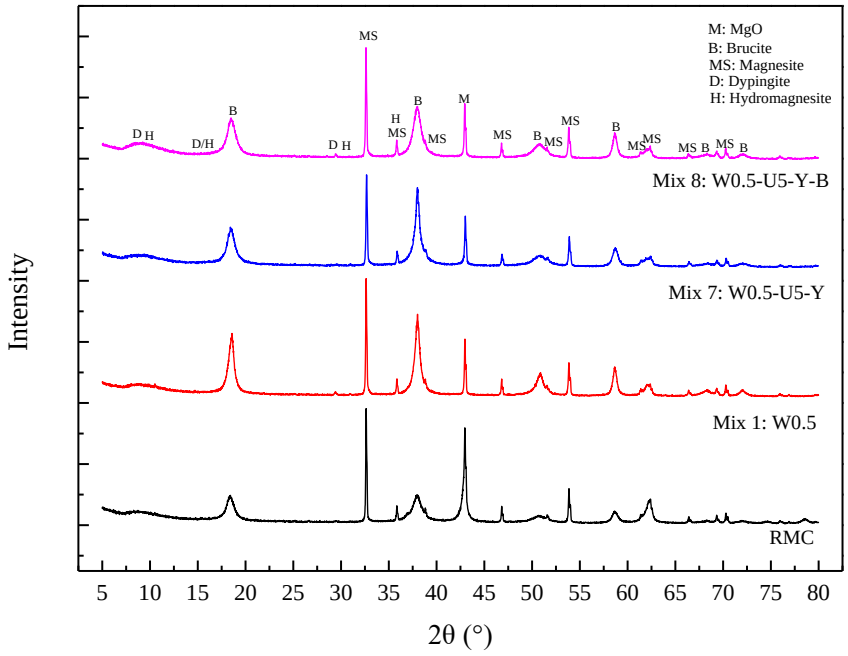


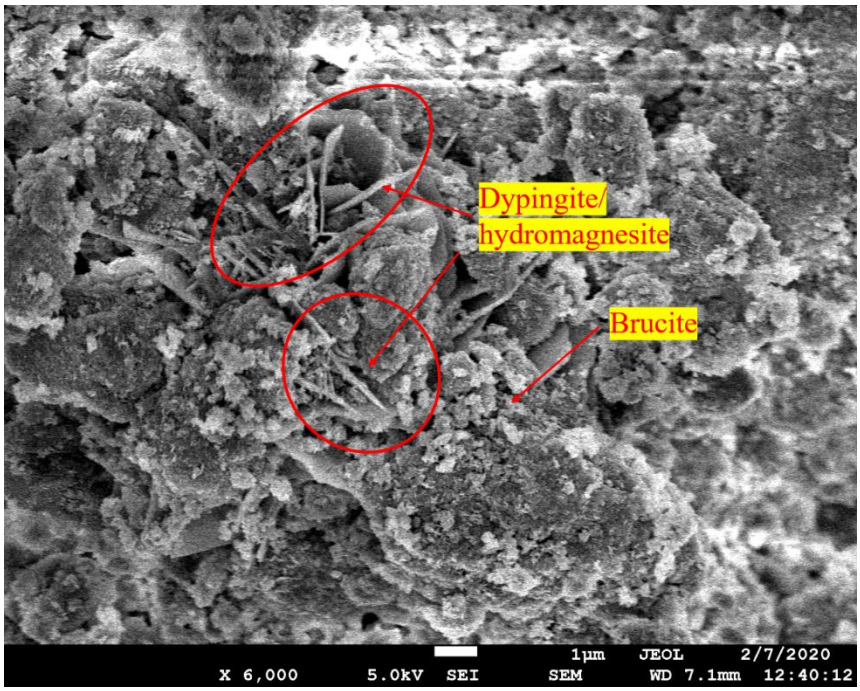
Figure 5 XRD patterns of RMC powder, control RMC paste (mix 1), nutritious RMC paste (mix 7), and bio-RMC paste (mix 8) at 28 days

Accordingly, these peaks were also reflected in the control RMC (W0.5), nutritious RMC (W0.5-U5-Y) and bio-RMC (W0.5-U5-Y-B) pastes. The main carbonate phases observed within these samples were dypingite and hydromagnesite, with main peaks at 8.3° and 15.5° 2θ, respectively. While the intensities of the magnesite and periclase peaks present within each sample were comparable, the bio-RMC sample revealed a brucite peak with a lower intensity than that observed in the control and nutritious RMC sample. This lower brucite peak could be

an indication that more brucite in the bio-RMC sample has been carbonated due to the introduction of carbonation agent for internal carbonation.

The microstructures of the control RMC (W0.5), nutritious RMC (W0.5-U5-Y) and bio-RMC (W0.5-U5-Y-B) pastes at 28 days are shown in Figures 6(a), (b) and (c), respectively. In line with the XRD results, all samples revealed the presence of carbonation products resembling the rosette-like morphology of dypingite/hydromagnesite. These phases were surrounded by brucite and unreacted MgO particles, explaining the relatively low strength performance demonstrated by both samples.

The main difference observed amongst the three groups of samples was the more widespread and denser formation of carbonate phases within the bio-RMC sample, in comparison to the sparse distribution of a few crystals of carbonates in the RMC sample and nutritious RMC sample, as indicated on Figure 6(a) to (c). The higher degree of formation of dypingite/hydromagnesite within the bio-RMC sample was in line with the strength and XRD results, indicating the active role the carbonate agent played in the internal carbonation of RMC samples. Furthermore, the presence of bacteria-like particles could also be observed in the bio-paste, as shown in Figure 6(d). The validity of this observation could be verified via a comparison of this particle with the actual morphology of the bacteria shown in Figure 6(e).



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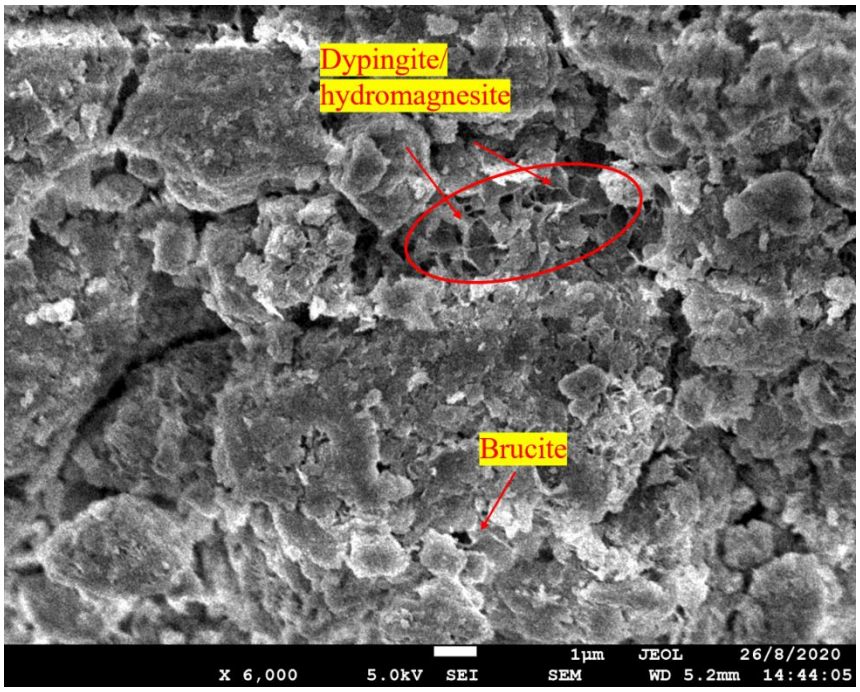
341 **(a)**



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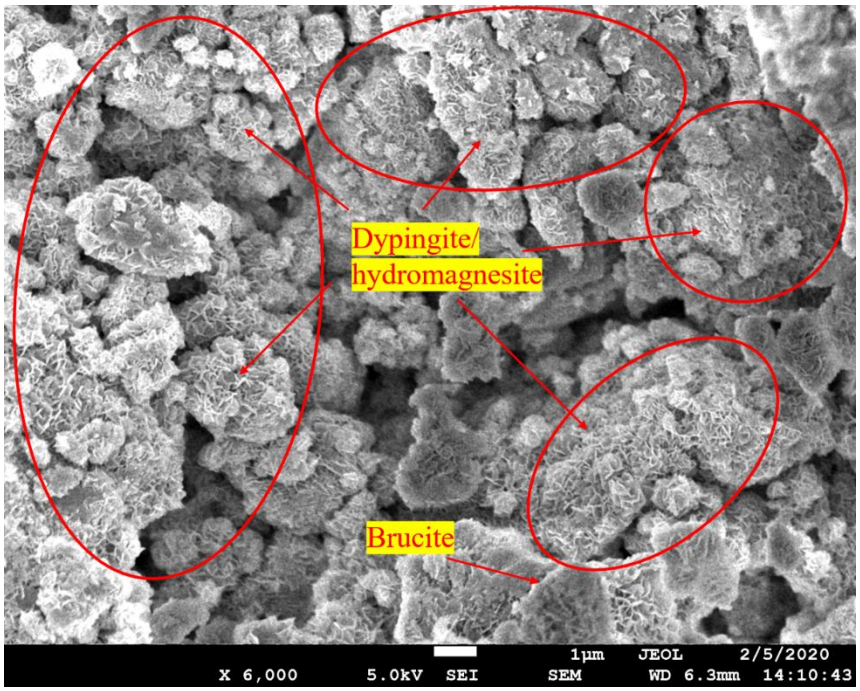
343 **(b)**



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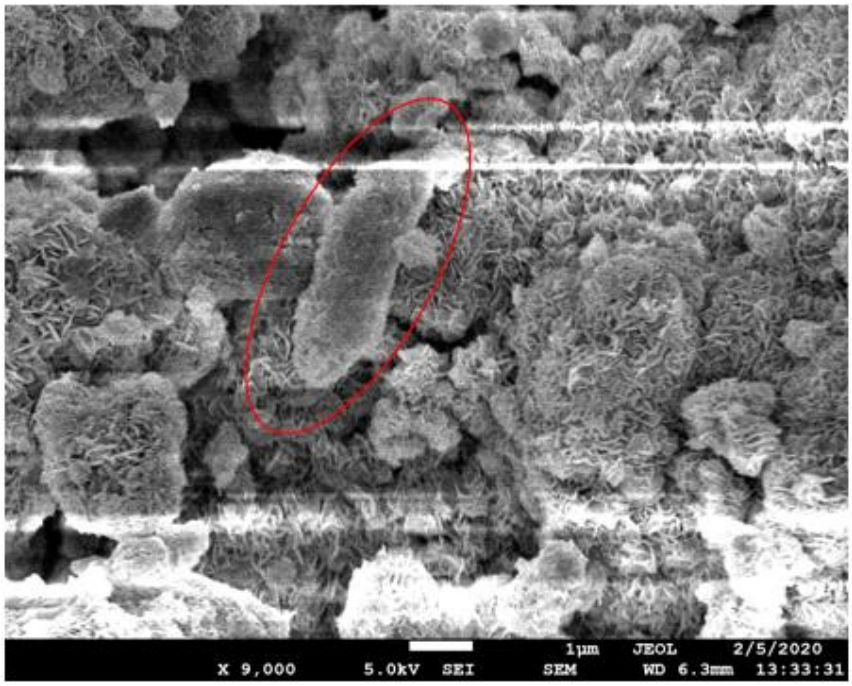
345 **(c)**



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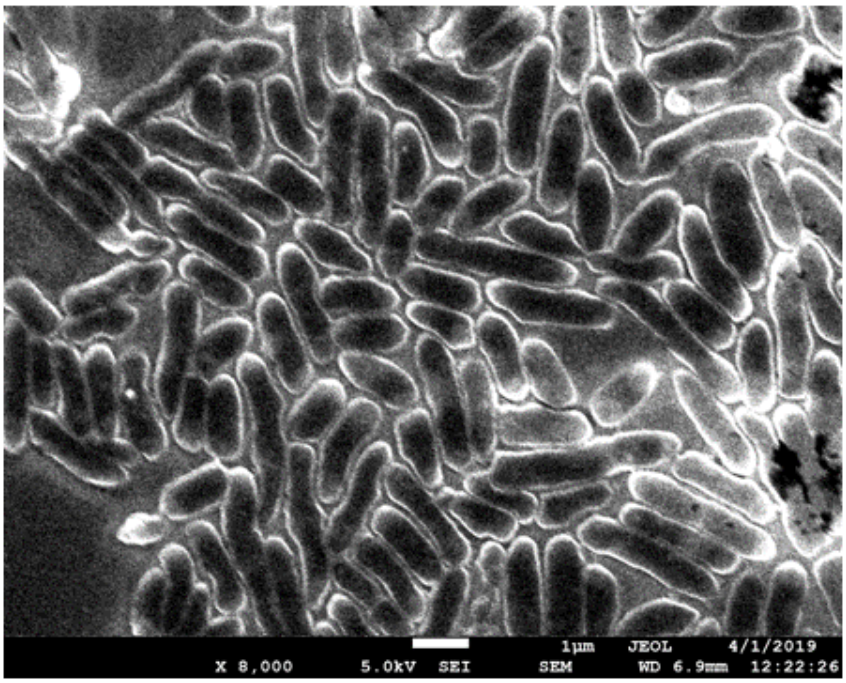
347 **(d)**



(e)

Figure 6 SEM image of (a) the control RMC paste (mix 1) at 28 days, (b) the nutritious RMC paste (mix 7), (c) the bio-RMC paste (mix 8) at 28 days, (d) bacteria-like phase in bio-RMC paste (mix 8), and (e) morphology of the bacteria used in this study

3.5 Discussion and outlook

In the above feasibility study, although the comparison of bio-RMC pastes with bacteria-free pastes shows the active role of carbonation agent, the compressive strength of bio-RMC paste still needs further improvement to be used as structure components. The low strength of above bio-RMC paste (W0.5-U5-Y-B) is associate with the low dosage of urea (i.e., 5%). Urea is the direct source of CO_2 in this study, the amount of urea determines the amount of CO_2 that potentially available to carbonation, which determines the final strength of RMC. In order to improve the performance of bio-RMC, the dosage of urea should be increased. However, above

results have revealed that increasing urea significantly brought up the flowability and prolonged the initial setting of the paste. As a result, water-to-RMC ratio should be adjusted accordingly to prevent that fresh pastes cannot be properly casted or hardened when urea dosage is elevated. It is worth noting that elevated urea dosage will lead to higher NH_3 emission, which can be oxidized to form nitrous oxide (N_2O) and may arouse environmental impact concern. However, the oxidation reaction of ammonia to N_2O requires the presence of catalyser such as Pt, Mn-Bi-O/α-Al_2O_3, and manganese-bismuth oxide [38-42]. In some cases, temperature over 100°C is also required [41, 42]. Therefore, the ammonia released from the internal carbonation can hardly convert to N_2O under ambient conditions.

Based on above discussion, increasing urea dosage while reducing the water-to-RMC ratio is adopted to improve the strength of bio-RMC paste. Through this method, urea not only serve as a CO_2 provider, but also work as a superplasticizer in RMC. Mix 9 (W0.45-U10-Y-B) with w/RMC = 0.45 and 10% urea and mix 10 (W0.43-U15-Y-B) with w/RMC = 0.43 and 15% urea were prepared to evaluate the performance of the resulting bio-RMC pastes. Figure 7 compares the compressive strength of the three bio-RMC pastes (mixes 8-10) at different ages. As can be seen, both mixes 9 and 10 have shown higher strength than mix 8 with w/RMC = 0.5 and 5% urea. The results indicate urea not only can serve as CO_2 provider for internal carbonation of brucite but also can function as water reducer to lower water demand. Both help to reduce porosity and to enhance strength of RMC pastes.

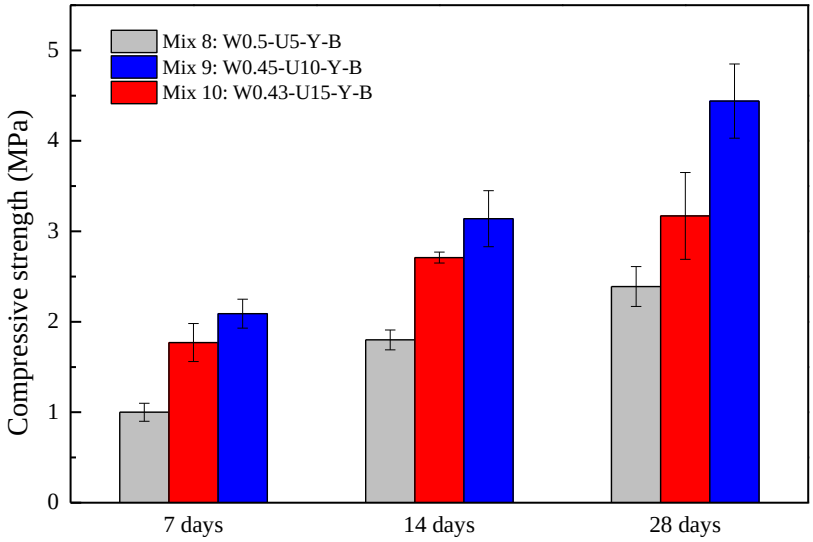


Figure 7 Compressive strength of bio-RMC pastes (mixes 8-10) at different ages

Specifically, mix 9 (W0.45-U10-Y-B) presented the highest compressive strength in the current study while the w/RMC ratio of mix 9 is higher than that of mix 10. This may be understood by the role and function of water in the bio-RMC paste system. Water is necessary for the hydration of MgO and the hydrolysis of urea. It is plausible mix 10 with the lowest w/RMC ratio and the highest amount of urea did not have sufficient water for MgO hydration and urea hydrolysis. While lower w/RMC ratio in mix 10 reduced the initial porosity in the system, insufficient water led to low degree of hydration and carbonation resulting in lower strength when compared to a more balanced system of mix 9. Further studies are necessary to further optimize the performance of the bio-RMC pastes.

4 Conclusions

This study proposed a bacteria-based method for the strength gain of RMC through internal carbonation for the first time. *Sporosarcina pasteurii*, urea, and YE were used as a bacteria-based carbonation agent and mixed directly into RMC pastes. The inclusion of bacteria-based carbonation agent resulted in a 20% improvement in the paste flowability while no noticeable change in the initial setting time. Urea not only can serve as the CO_2 provider but also can potentially function as a superplasticizer to reduce the water demand in the bio-RMC pastes. The resulting bio-RMC pastes showed a continuous strength gain with time which demonstrated the feasibility of bacteria-induced internal carbonation of RMC. Microstructure analysis revealed abundant formation of dypingite/hydromagnesite with less brucite in the bio-RMC pastes, which is responsible for the strength gain of the bio-RMC pastes due to bacteria-induced internal carbonation.

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