

Microbial Technique to Treat Recycled Aggregates from Construction Waste for its Effective reutilization in Concrete

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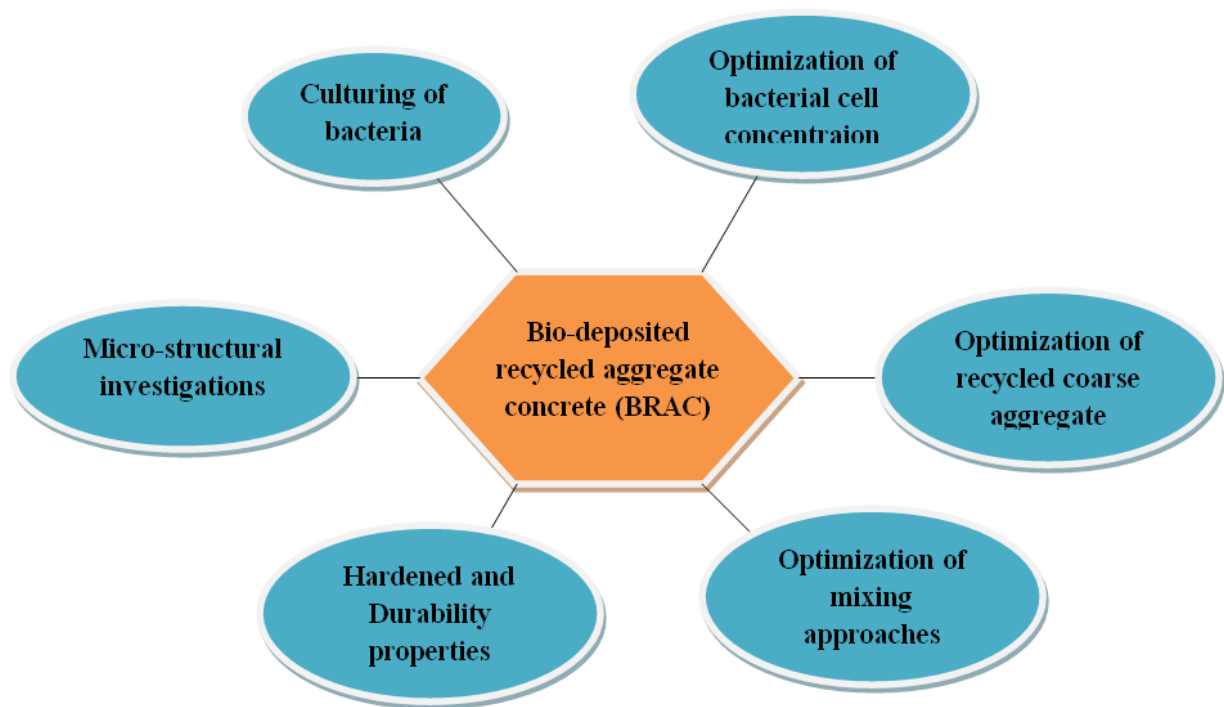
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Graphical Abstract



1 **ABSTRACT**

2 Excessive consumption of natural resources for concrete production results in the diminution
3 of conventional resources, leading to the scarcity of construction materials. Perhaps, the dumping of
4 construction wastes increases disposal problems. Nevertheless, the requirement for aggregates in the
5 construction sector increases. The recycling of construction waste to produce recycled coarse
6 aggregate (RCA) as a suitable alternative to natural coarse aggregates (NCA) conserves natural
7 resources and promotes sustainability in construction. However, the quality of recycled coarse
8 aggregate was inferior compared to natural coarse aggregates due to the adherence of mortar. This
9 paper investigates the sustainable use of *Bacillus subtilis* with different concentrations to enhance the
10 quality of RCA. The concrete mixes manufactured with optimized bio-deposited recycled coarse
11 aggregate (BRCA) were tested for their mechanical and durability properties. It could be observed
12 that the strength of bio-deposited recycled aggregate concrete (BRAC) was enhanced by 12.63%
13 relative to normal aggregate concrete (NAC), and the durability properties such as water absorption,
14 chloride penetration and carbonation of BRAC were reduced by 18.53%, 16.52% and 20% relative
15 to recycled aggregate concrete (RAC). Microstructural studies through SEM and XRD revealed the
16 deposition of CaCO_3 on the micro-pores of RCA, and that improves the properties of the concrete.
17 **Keywords:** *Bacillus subtilis*, Recycled aggregate concrete, Bio-deposition, CaCO_3 , Sand enveloped
18 mixing approach.

19 1. Introduction

20 Construction & Demolition (C&D) wastes resulting from the retrofitting of concrete
21 structures were generated in huge quantities annually and dumped in landfills. Subsequently, almost
22 800 billion tons of natural resources are utilized by industrialists as ingredient to manufacture
23 concrete in 2017 and which they anticipated would rise the generation of demolition waste to 270
24 billion tonnes in next 10 years (Schandl *et al.* 2017). The concrete production rose to 4.4 billion
25 tonnes in 2022 and anticipated to increase over 5.5 billion tons in 2050. Almost one-third of such
26 manufacturing ends up as C&D waste. The central pollution control board (CPCB) of India estimated
27 that around 65 million tons of municipal wastes are generated yearly, of 62% comprises of recyclables
28 such as wood, concrete, glass etc. Among them, only 75% were collected and out of which 20 to 30%
29 were being processed (TERI, 2022). European Aggregate Association (EAA) estimated the
30 requirement of 4000 tonnes of aggregates for constructing a residential villa and 3000 tonnes for
31 laying one kilometre of a road. From the statistical data, it is observed that the use of conventional
32 resources as construction material and the generation of C&D wastes were increasing simultaneously.
33 The former will cause an imbalance in the ecology by diminution of natural resources, while the latter
34 will affect the integrity of the environment. Thus C&D waste has to be used as an appropriate
35 alternative for NCA in the concrete. However, the smearance of cement mortar on RCA affects the
36 concrete properties, hence restraining its use in the concrete. Suitable treatments for RCA will tend
37 to enhance the quality of RCA by eradicating cement mortar or coating it with an impermeable
38 medium.

39 Several research have been done with the RCA as an effective replacement to NCA in
40 concrete. (Puthussery *et al.* 2017) reported that beyond 25% substitution of RCA, the strength of RAC
41 was reduced ensuing from the poor quality of the RCA, adhered mortar, and void packing nature of
42 the RCA. (Andal *et al.* 2016) utilized both preserved and unpreserved RCA in the study and observed
43 replacement beyond 30% shown negative influence on the concrete properties, however the preserved
44 RCA performed better compared to unpreserved RCA. (Lavado *et al.* 2020) found that for the RAC

45 mix with lower w/c ratio (0.35 to 0.4), addition of superplasticizers shown higher slump loss whereas
46 at 0.5 w/c ratio, slump loss was only 6% at first 30 minutes and gradually increased to 63% at 90
47 minutes. (Arunkumar *et al.* 2023) observed that optimizing polypropylene aggregate to 15% with
48 recycled water increased the strength equivalent to the control concrete and replacement beyond 15%
49 reduced its strength. (Naveen Arasu *et al.* 2023) used recycled nano-material and observed that the
50 strength was compressive enhanced by 32% and tensile strength was enhanced by 24% and 0.04%
51 replacement. (Mavroulidou *et al.* 2010) investigated the possible utilization of rubber tyre as
52 aggregates in concrete and observed that the strength was nearly 95% of control concrete when used
53 as coarse aggregate and 89% when used as fine aggregate at 28 days. The lower specific gravity of
54 rubber tyre reduces the strength of the concrete.

55 (Mi *et al.* 2020) reported that RCA collected from the source concrete with higher strength
56 shown improved strength and carbonation resistance with ratio ranging from 0.6 to 1.1 for the former
57 and 11 to 8 for the latter. (Mwasha *et al.* 2018) optimized the particle packing of pre-soaked RCA
58 and observed a highest compressive strength of 80 MPa with the influence of mineral admixtures.
59 The combined effect of pre-soaking, particle packing optimization and mineral admixtures shown
60 better influence on the concrete properties. It could be observed that the addition of RCA tend to
61 disturb the concrete properties, limiting its utilization. Several treatments were developed to RCA
62 with acids, polymers, slurries, carbonation, microbes etc. to enhance its quality.

63 Earlier studies with bacteria tend to improve the concrete properties through bio-deposition
64 of CaCO_3 (Bachmeier *et al.* 2002; Park *et al.* 2010; Majumdar *et al.* 2012). (Krishnapriya *et al.* 2016)
65 isolated *Bacillus megatherium*, *Bacillus licheniformis*, and *Bacillus flexus* and observed higher
66 strength with all three species than control concrete due to the CaCO_3 precipitation and among the
67 three, *Bacillus flexus* performed better due to its 100% higher similarity index with the parental
68 species. (Mondal *et al.* 2017) used *Bacillus Cereus* and *Bacillus Subtilis* at various concentrations
69 and observed that the concrete with 10^3 cells/ml of *Bacillus Cereus* and 10^5 cells/ml *Bacillus Subtilis*
70 of 10^5 cells/ml shows higher strength owing to the higher calcite precipitation by microbes that seals

up the micro-pores in the concrete. (Nain *et al.* 2019) used 0.8×10^9 cells/ml concentration of *B. Subtilis* and *B. Megaterium* and found that strength of the RAC was improved around 15% with both bacterial species. The microstructure revealed dense rhombohedra-shaped calcite crystals that deposit on the cracked surface and heal it. Thus it could be observed that microbes has the ability to improve concrete properties by sealing pores/voids in concrete through CaCO_3 deposition. The same technique was implemented to augment the quality of RCA to check its suitability in the concrete.

(Grabiec *et al.* 2012) used *Sporosarcina pasteurii* to enhance the RCA and found that the perviousness of RCA was decreased, and it is more efficient with smaller fractions of RCA. (Wu *et al.* 2020) inferred that the rate of absorption of water by RAC was reduced by 28.3%, and the strength of RAC was enhanced by 16.1% with the bio-deposition of RCA. The microbes precipitate the CaCO_3 crystals that seal the cracks on the cement mortar adhered to RCA. However, (Zhan *et al.* 2019) adopted microbial treatment to finer fractions of RCA and found a 32% improvement in the strength even with 100% of RCA. The solidification of the micro-structure through CaCO_3 precipitation augments the properties of the RAC. (Zhu *et al.* 2019) observed that microbe-induced CaCO_3 precipitation promotes the formation of CSH, CH, Aft, and AFm, which fill the cracks on the RCA and increase its strength. (Wu *et al.* 2018) developed an alternative technique with microbes through respiration and observed improved RCA properties, thereby enhancing RAC properties. However, fewer studies were performed to examine the influence of bio-treated RCA in the concrete mixture to study its mechanical and durability properties. This study used *Bacillus subtilis* as a microbe to enhance the quality of RCA and properties of RAC.

2. Experimental work

2.1. MATERIALS

This study utilizes 43 grades ordinary portland cement, river sand as natural fine aggregate (NFA) and river gravel as natural coarse aggregate. Construction wastes were procured from Structural Engineering Research Center (SERC), India, and only the concrete segments were sampled and used as RCA. The collected samples were crushed and sieved to fractions ranging from 4.75-mm

to 20 mm to eliminate finer dust particles. The properties of aggregates determined following IS 2386 (Part III)-1963 were given in Table 1. Table 1 shows the inferior properties of the RCA owing to its higher absorption, crushing index and less density than NCA as a result of smearance of mortar on RCA (Sivamani *et al.* 2020, Sivamani *et al.* 2021a, Abrahams *et al.* 2018). The photographic and microscopic observations of collected NCA and RCA samples are shown in Figure1 & Figure 2. It could be observed that the RCA particles are highly angular, with cementitious particles smeared on their surface. This is due to the repeated crushing of RCA to reduce the particles to size from 4.75- mm to 20 mm. The microscopic observation reveals the cement mortar remains on RCA and was visible through the loose irregular particles, whereas the images of NCA show fewer angular dense particles. The loose irregular RCA particles possess micro-cracks that increase the porosity of RCA. The XRD images of NCA and RCA are shown in Figure 3. The peak in NCA pattern specify the incidence of SiO_2 , $\text{NaAlSi}_3\text{O}_8$, and CaCO_3 , with the highest being SiO_2 . This could be favourable in the development of a C-S-H that promotes strength in the concrete. In RCA, the peak signify the calcite compound ensuing from the cement mortar and traces of SiO_2 .

Table 1. Aggregate Properties

Aggregate	Specific Gravity	Water absorption (%)	Relative density (kg/m^3)	Crushing index (%)
NFA	2.61	0.12	1651	19.43
NCA	2.74	0.87	1976	20.41
RCA	2.41	6.13	2431	25.23



Figure 1. Visual observation (a) NCA (b) RCA

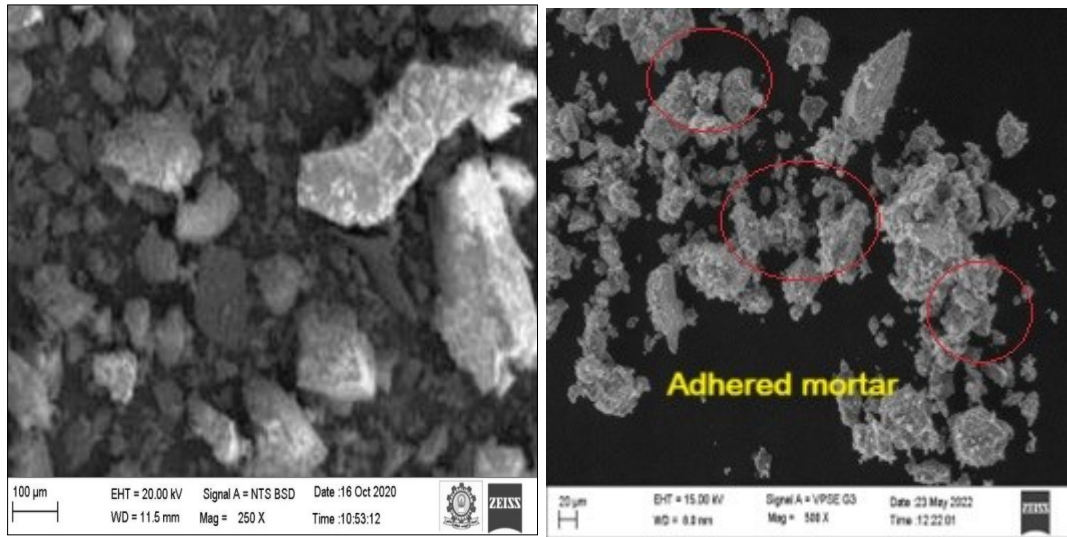


Figure 2. Microscopic observation (a) NCA (b) RCA

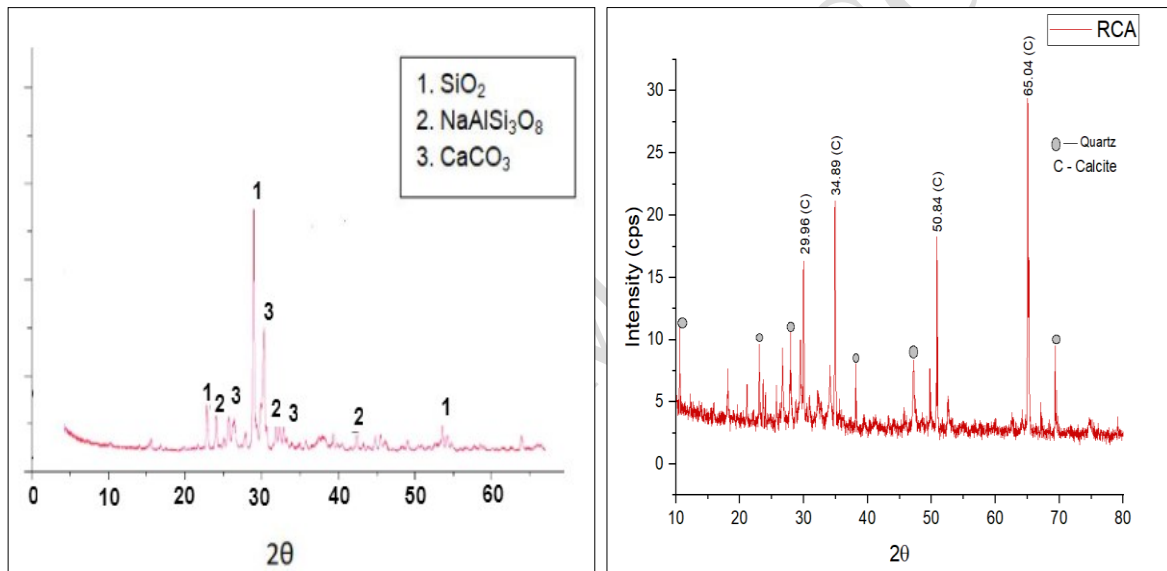
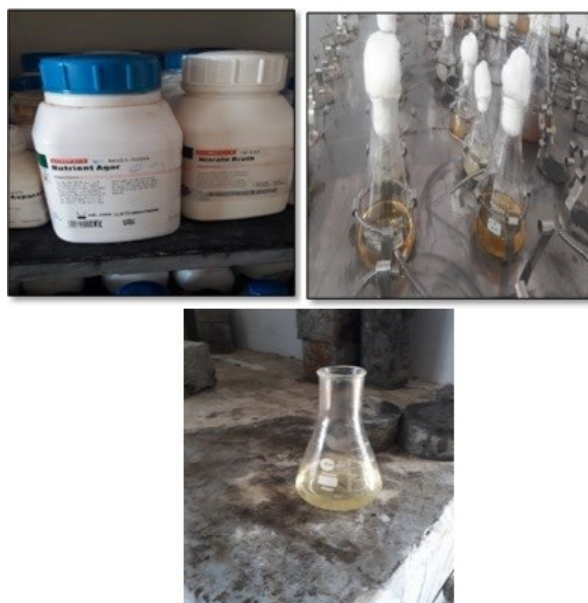


Figure 3. XRD patterns (a) NCA (b) RCA

2.2 BIO-DEPOSITION TREATMENT

The genetic variant of the *Bacillus subtilis* was obtained from a Gene bank in Mumbai, India (Gene ID: Chandigarh/ NCBI/EMBL/DDBJ/2341). The technique to culture *Bacillus subtilis* was performed with reference to the procedure (Sahoo *et al.* 2016; González *et al.* 2017). The strains of the *Bacillus subtilis* were preserved in a medium comprising 30 g/l urea and an extract of yeast (Y.E) each. The Y.E was diluted in 1000 ml of water, pasteurized at 110° C for 30 minutes, and cooled at room temperature. The urea was mixed in 0.1 liters of distilled water and diverse with pasteurized Y.E. The collected bacterial strains were put into the diluted solution and centrifuged at 200 rpm for

127 24 hours. It is then diluted to attain 10^1 , 10^3 , 10^5 , and 10^7 cells/ml concentration bacteria. The diluted
 128 concentrations of the bacterial strain were quantified by the optical density test method. Figure 4
 129 depicts the cultivation of microbes, and the cultivated microbial solution.



130
 131 **Figure 4.** Cultivation of microbes

132 The biotreatment to RCA was performed at room temperature with R.H >60%. The RCA was
 133 soaked in the bacterial culture of 10^1 , 10^3 , 10^5 cells/ml and 10^7 cells/ml concentration for 24 hours. The
 134 aggregates are then immersed in a bio-deposition medium containing 0.5 M of urea and calcium
 135 nitrate each for three days. After three days, the samples of the RCA were taken out, cleaned, and
 136 desiccated and used as bio-deposited RCA (BRCA) in the concrete.

137 *2.3 CONCRETE MIXTURES*

138 The concrete mix was prepared in 1:1.92:3.11 with 0.45w/c ratio as per IS 10262 (2009). The
 139 concrete mix was labelled as BR/R-x-N/S, where 'BR' represents the biotreated RCA, 'R' represents
 140 the RCA, 'x' represents the percentage of RCA and 'N/T' represents the normal mixing approach
 141 (NMA) / sand-enveloped mixing approach (SEMA). Table 2 depicts the quantity of concrete
 142 ingredients used in the study. The NCA and RCA were saturated in prior to achieve surface saturated
 143 dry (SSD) to avoid excess water absorption during the mixing of the concrete. The cementitious
 144 particles smeared on the RCA soak up additional water while mixing, affecting the workability of the

concrete. Henceforth, the concrete mixes were manufactured with SEMA technique that improves the workability of the RAC (Liang et al. 2015). Figure 5 depicts the illustration of NMA and SEMA.

Table 2. Concrete mix proportions

Mix ID	(Kg/m ³)					
	Cement	NFA	NCA	RCA	BRCA	Water
R-0-N	400	799	1029	0	0	180
R-0-S	400	799	1029	0	0	180
R-30-N	400	799	720	309	0	183
R-30-S	400	799	720	309	0	183
R-100-N	400	799	0	1029	0	190
R-100-S	400	799	0	1029	0	190
BR-30-S	400	799	720	0	309	180
BR-100-S	400	799	0	0	1029	180

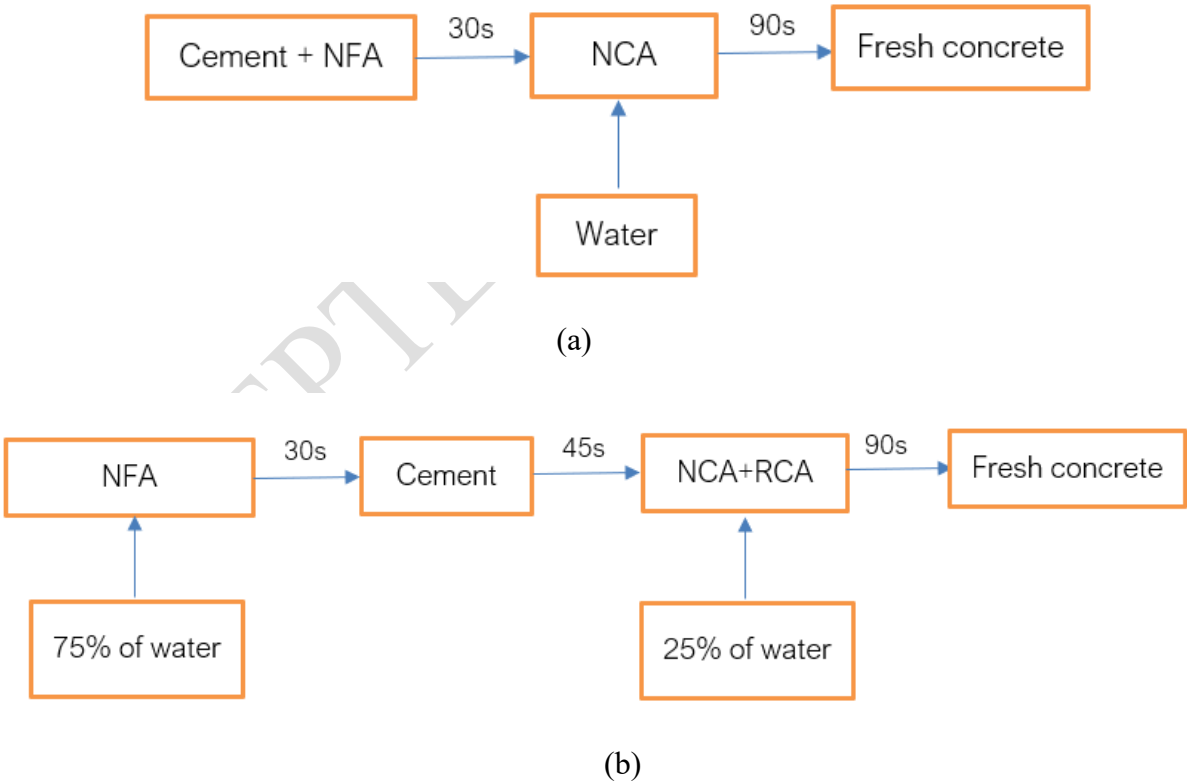


Figure 5. Schematic Illustration (a) NMA (b) SEMA

156 2.4 Testing methodology

157 The bio-treated aggregates used in the study were tested for its suitability by performing water
158 absorption, crushing index and density as per IS 2386 (Part III)-1963. A whole of 3 mix series were
159 prepared with NCA, RCA and BRCA. The concrete mixes are manufactured by NMA and SEMA
160 (Figure 3 and Figure 4) and casted into cubes (150 mm), cylinders (150 mm x 300 mm) and prisms
161 (500 mm x 100 mm x 100 mm). The fresh mixes were tested for its workability as per IS 1199 (1959)
162 and the hardened concrete were tested for strength and durability properties. The concrete cubes and
163 cylinders were tested for its compressive (CS) and tensile strength (TS) as specified in ASTM C39/
164 C39-M. The concrete prisms were tested for its flexural strength (FS) as specified in ASTM C469.
165 All the hardened property tests were performed in trios at 7 and 28 days.

166 The concrete cubes were tested for its water absorption (WA) at 28 days as specified in ASTM
167 C1585. Initially, dry samples were weighed and soaked in water for 28 days under laboratory
168 temperature. The samples were then taken out, allowed to dry and balanced and the variance in
169 balance before and after soaking depicts the water absorption. The concrete cubes were used to
170 measure the sorptivity as specified in BS 1881 (208). The carbonation resistance (CR) were
171 determined at 7 and 28 days (*Geng et al. 2013*). The cubes cured at room temperature were accelerated
172 with 25% CO₂ concentration. The carbonated samples were then cut and sprayed with
173 phenolphthalein indicator. The chloride penetration (CP) of the samples were tested at 28 and 90 days
174 as per ASTM C1202 (2019).

175 3. Results and Discussion

176 3.1. MATERIAL PROPERTIES

177 Table 3 depicts the properties of aggregates used in the concrete mixes. The significant change
178 in water absorption of BRCA could be observed, whereas the change in relative density and crushing
179 index was not significant. The relative density of BRCA was only 2.87% lower than the of RCA, and
180 the crushing index of BRCA was only 8.6% lower than RCA. The effect of CaCO₃ precipitation was
181 much reflected in water absorption compared to the relative density and crushing index (*Liu et al.*

2020). This is due to the insignificant change in the mass and volume of BRCA after CaCO_3 precipitation. The water absorption of BRCA is 33% lower than RCA. This is due to the CaCO_3 precipitation that fills the micro-pores and hence reduces the water absorption of RCA (Grabiec *et al.* 2012, Zhu *et al.* 2019, González *et al.* 2017, Qiu *et al.* 2014).

Table 3. Properties of aggregates

S. No	Aggregates	Water absorption (%)	Crushing index (%)	Relative density (kg/m^3)
1	RCA	6.3	26	2490
2	BRCA	4.2	23	2470
3	NCA	0.9	21	2200

3.2 CHARACTERIZATION STUDIES ON AGGREGATES

Figure 6 and Figure 7 show the SEM and XRD images of RCA and BRCA used in the study. From the SEM images, it is observed that the surface of BRCA was deposited with CaCO_3 precipitates, and the surface of the RCA was deposited with loose cement particles. Improved magnification of $5\mu\text{m}$ clearly shows the CaCO_3 precipitation on the RCA. The bio-deposits tend to seal the micro-cracks on the porous RCA, hence improving the quality of RCA. From XRD images, it is observed that the intensity of calcite precipitation for BRCA is 80000 cps and for URCA is 35000 cps. The maximum rate of CaCO_3 precipitation was evident in BRCA due to the microbial activity. The Ca^{2+} ions in the medium deliver appropriate positive ion source for urea hydrolysis, promoting CaCO_3 precipitation. Despite this, the calcite on the RCA was evident owing to the loose mortar on its surface.

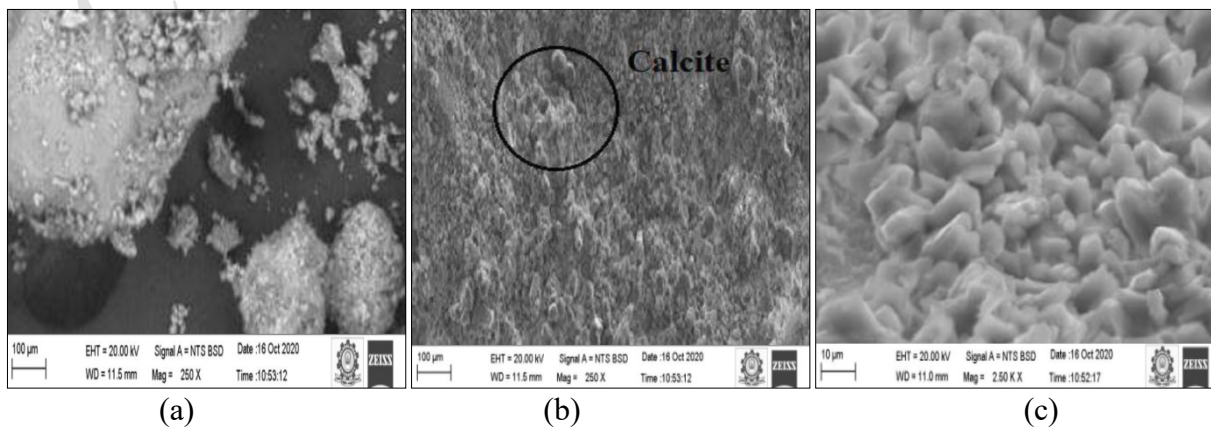


Figure 6. SEM images (a) RCA (100 μm) (b) BRCA (100 μm) (c) BRCA (10 μm)

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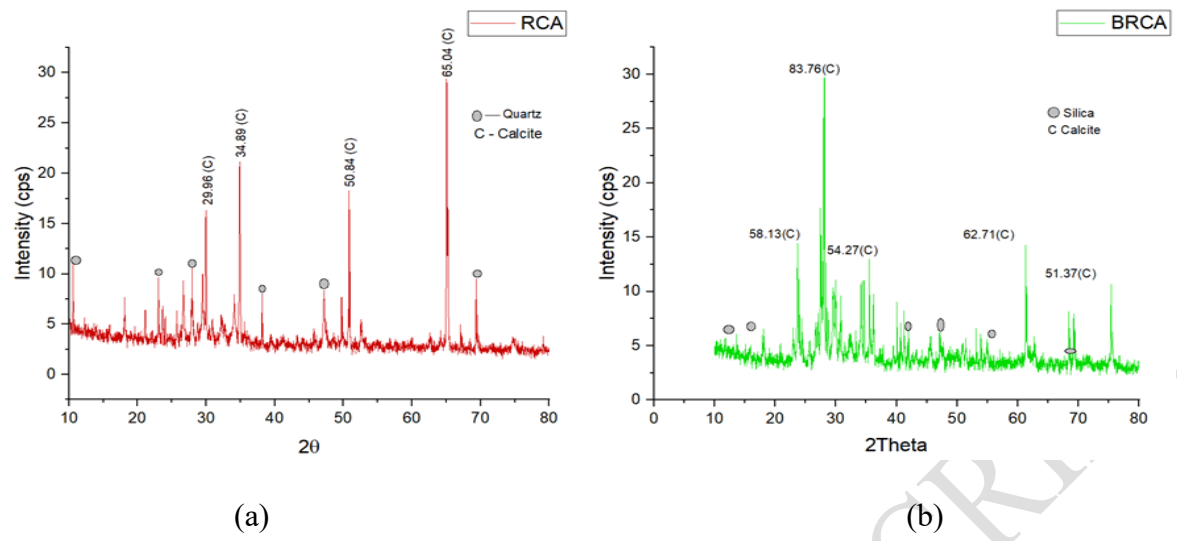


Figure 7. XRD patterns (a) RCA (b) BRCA

3.3 EFFECT OF BIO-DEPOSITION ON THE PROPERTIES OF RCA

The influence of bio-deposition on the properties of RCA was evaluated through weight increase, and water absorption is shown in Table 4. The increase in cell concentration increases the weight of the sample and decreases its water absorption. This is because as the bacterial concentration increases, the rate of CaCO_3 precipitation increases. The precipitated CaCO_3 crystals seal the pores on RCA, increasing its mass and decreasing the porosity. The optimal concentration of 10^5 cells/ml was observed as beyond which it increases the water absorption. At optimal concentration, CaCO_3 precipitate percolates inside the matrix and seals the surface whereas at 10^7 cells/ml CaCO_3 precipitate seals the surface rather than percolation into the matrix (*Jagan et al. 2022*).

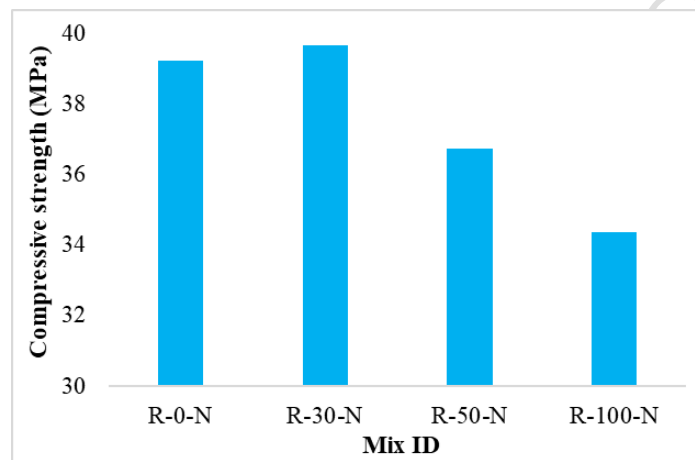
Table 4. Variation in mass and water absorption of BRCA

S. No	Concentration (cells/ml)	Mass increase (%)	Water absorption (%)
1	10^0 (RCA)	0.38	6.27
2	10^1	0.41	5.71
3	10^3	0.47	5.18
4	10^5	0.7	4.63
5	10^7	0.93	4.92

215

216 3.4 OPTIMIZATION OF RCA

217 Figure 8 depicts the compressive strength of RAC at 28 days. The ideal replacement of RCA
218 was observed to be 30%. The strength of R-10-N and R-30-N was 3.9% more than R-0-N at 28 days.
219 The trivial strength enhancement was due to the angularity of RCA ensuing from the recycling
220 process (Jagan *et al.* 2020, Sivamani *et al.* 2021a). The strength of R-50-N was reduced by 11.42%
221 and the strength of R-100-N was reduced by 20.06% at 28 days. The four phase system of RAC with
222 two ITZ, the weaker zone amongst old ITZ and new ITZ owing to higher porosity of RCA affects the
223 concrete strength (Thomas *et al.* 2018, Jagan *et al.* 2022).



224
225 **Figure 8.** Compressive strength of RAC at 28 days

226 3.5 MECHANICAL PROPERTIES WITH OPTIMIZED RCA

227 Figure 9(a) shows the variation in CS of the optimized mixes with respect to control mix. The
228 CS of R-0-S is 0.33% more than R-0-N at 28 days. The CS of R-30-N is 1.08% more than R-0-N,
229 while the CS of R-30-S is 4.75% more than R-0-N at 28 days. The minor improvement in the former
230 mixis due to the increase in angularity of the RCA owing to crushing (Sivamani *et al.* 2021b, Kirthika
231 *et al.* 2020), while in the latter mix, the SEMA coats the RCA with firm mortar that reduces its water
232 absorption during mixing (Liang *et al.* 2021, Sivamani *et al.* 2021b, Sivamani *et al.* 2022). However,
233 the CS of R-100-N is 12.41% more than R-0-N, but the CS of R-100-S is only 9.2% lesser than R-0-
234 N. Through bio-deposition, the CS of BR-30-S and BR-100-S was 8.17% and 12.63% more than R-
235 0-N. Also, the CS of BR-30-S was 19.6% and 16.6% more than R-100-N and R-100-S; CS of BR-
236 100-S was 23.5% and 20.65% more than R-100-N and R-100-S at 28 days. The CaCO₃ precipitation

237 by microbes lids the RCA surface, reducing its porosity and improves the concrete strength (*Sivamani*
 238 *et al. 2021a; Wang et al. 2017; Wu et al. 2018*)

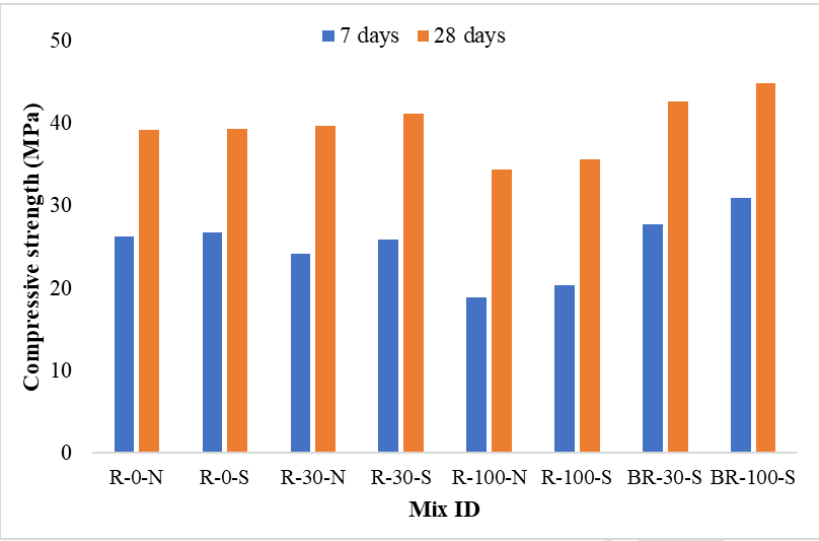


Figure 10(a). Variation in CS

241 Figure 9(b) shows the variation in TS of the optimized mixes with respect to control mix. The
 242 TS of R-0-S is 0.26% more than R-0-N at 28 days. The TS of R-30-N is 0.87% more than R-0-N,
 243 while the TS of R-30-S is 3.83% more than R-0-N at 28 days. However, the TS of R-100-N is 9.89%
 244 lesser than R-0-N, but the TS of R-100-S is only 7.31% lesser than R-0-N. Through bio-deposition,
 245 the TS of BR-30-S and BR-100-S was 6.62% and 9.93% more compared to R-0-N. Also, the TS of
 246 BR-30-S was 15.82% and 13.29% more than R-100-N and R-100-S; TS of BR-100-S was 19.14%
 247 and 16.71% more than R-100-N and R-100-S at 28 days.

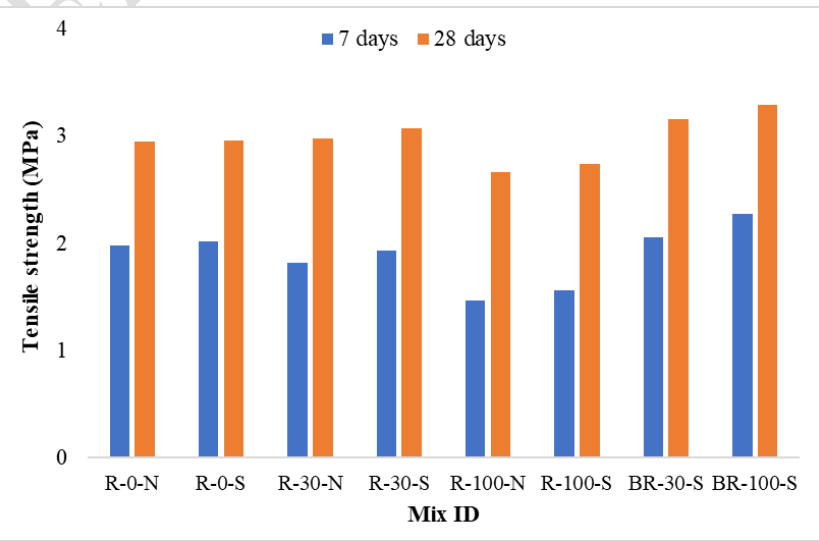
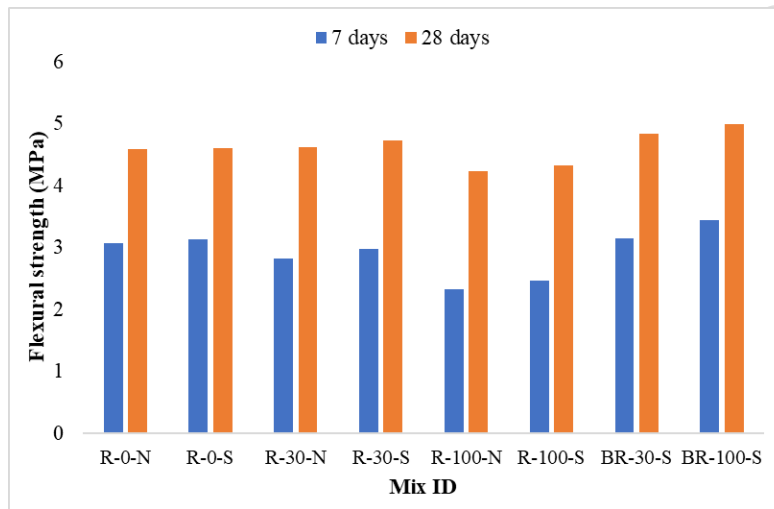


Figure 9(b). Variation in TS

250 Figure 9(c) shows the variation in FS of the optimized mixes with respect to control mix. The
 251 FS of R-0-S is 0.20% more than R-0-N at 28 days. The FS of R-30-N is 0.66% more than R-0-N,
 252 while the FS of R-30-S is 2.93% more than R-0-N at 28 days. However, the FS of R-100-N is 7.76%
 253 lesser than R-0-N, but the FS of R-100-S is only 5.7% lesser than R-0-N. Through bio-deposition, the
 254 FS of BR-30-S and BR-100-S was 5.06% and 7.91% more compared to R-0-N. Also, the FS of BR-
 255 30-S was 12.41% and 10.53% more than R-100-N and R-100-S; FS of BR-100-S was 15.03% and
 256 13.22% more than R-100-N and R-100-S at 28 days.



257
 258 **Figure 9(c). Variation in FS**

259 3.6 DURABILITY PROPERTIES WITH OPTIMIZED RCA

260 Figure 10(a) shows the WA of the optimized mixes at 28 days. The WA of R-0-S is 2.64%
 261 lesser than R-0-N at 28 days. The WA of R-30-N and R-30-S is 14.28% and 12.76% more than R-0-
 262 N. Similarly, the WA of R-100-N and R-100-S is 21.02% and 19.83% more than R-0-N. The increase
 263 in WA of RCA owing to the adhered mortar increases the WA of the RAC (*Thomas et al. 2018*,
 264 *Sivamani et al. 2021a*). The WA of the hardened concrete was influenced by the pore structure of the
 265 concrete. The bio deposition treatment blocks the micro-pores on the RCA surface through the
 266 precipitation of CaCO_3 crystals (*Grabiec et al. 2012*; *Qiu et al. 2014*). The WA of BR-30-S and BR-
 267 100-S is only 9.22% and 1.82% more compared to R-0-N. Also, the WA of BR-30-S and BR-100-S
 268 is 13% and 19.58% lesser than R-100-N and the WA of BR-30-S and BR-100-S is 11.87% and
 269 18.53% lesser than R-100-S at 28 days.

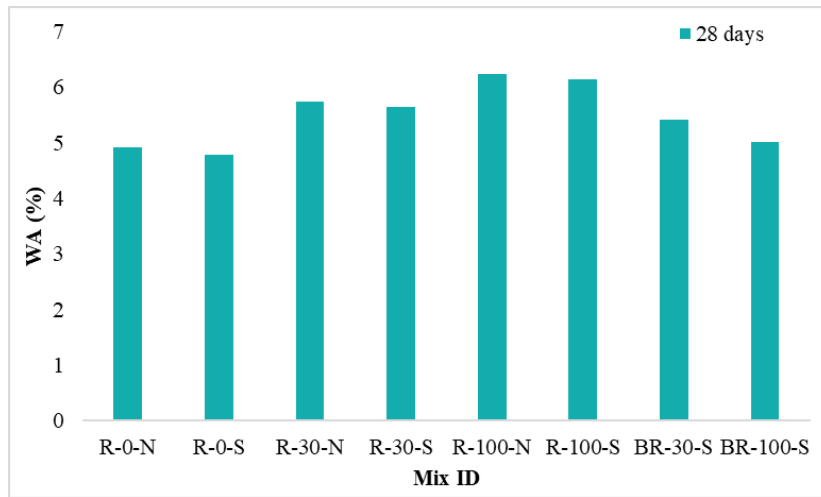


Figure 10(a). WA of the optimized mixes

Figure 10(b) shows the sorptivity of the optimized mixes at 28 days. The sorptivity of R-0-S is 1.71% lesser than R-0-N at 28 days. The sorptivity of R-30-N and R-30-S is 34.9% and 32.91% more than R-0-N. Similarly, the sorptivity of R-100-N and R-100-S is 38.9% and 37.73% more than R-0-N. The sorptivity is the capillary uptake of water which is influenced by the pores in the hardened concrete. The higher RCA content increases the pore volume in RAC and as a result sorptivity increases (*Kirthika et al. 2020*). The bio-treatment to RCA reduces the pore volume in RAC by reducing the water absorption of RCA and thus reducing the sorptivity. The sorptivity of BR-30-S and BR-100-S is only 6.64% and 1.26% more than R-0-N. Also, the sorptivity of BR-30-S and BR-100-S is 34.52 and 38.08% lesser than R-100-N and the sorptivity of BR-30-S and BR-100-S is 33.30% and 36.93% lesser than R-100-S at 28 days.

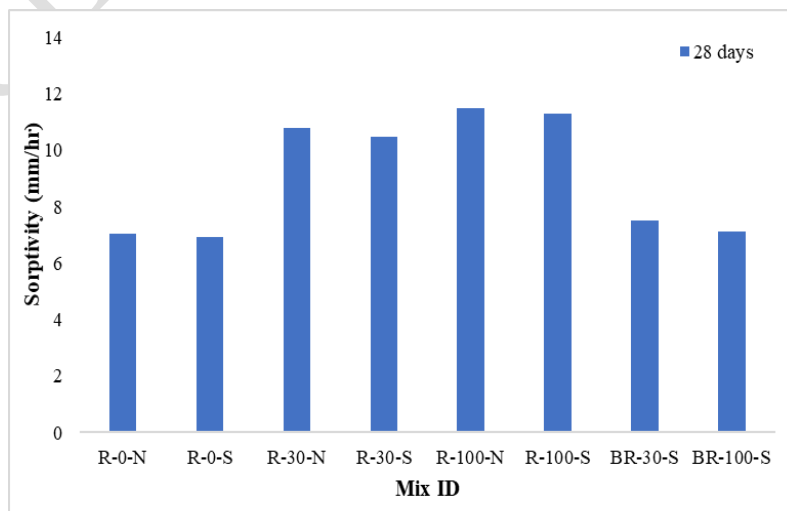
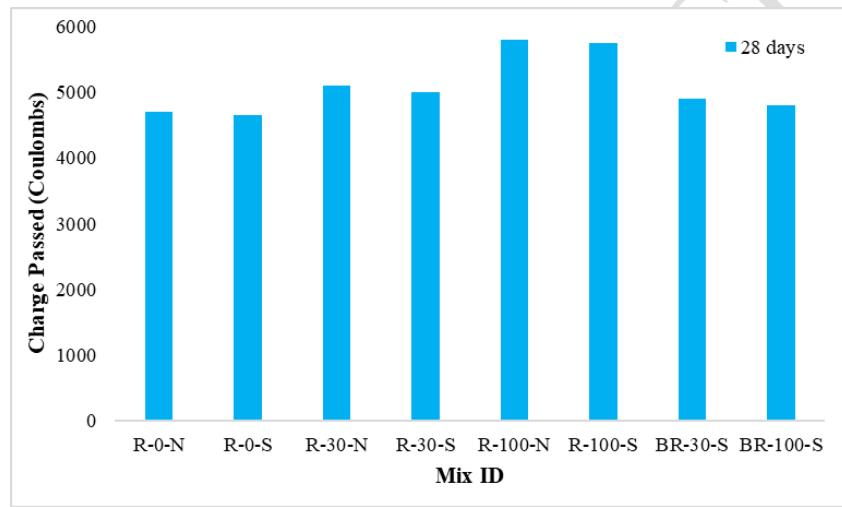


Figure 10(b). Sorptivity of the optimized mixes

284 Figure 10(c) shows the CP of the optimized mixes at 28 days. The CP of R-0-S is 1.06% lesser
 285 than R-0-N at 28 days. The CP of R-30-N and R-30-S is 7.84% and 6% more than R-0-N. Similarly,
 286 the CP of R-100-N and R-100-S is 19% and 18.26% more than R-0-N. The higher pore volume in
 287 RAC due to increase in the RCA increases the entry of Cl^- ions and affects the concrete. The CP of
 288 BR-30-S and BR-100-S is only 4.08% and 2.09% more than R-0-N. Also, the CP of BR-30-S and
 289 BR-100-S is 15.51% and 17.24% lesser than R-100-N and the CP of BR-30-S and BR-100-S is
 290 14.78% and 16.52% lesser than R-100-S at 28 days. The bio-treatment reduces the water absorption
 291 of RCA, reducing the pore volume in RAC and thus decreasing the Cl^- penetration.



292
 293 **Figure 10(c).** RCPT of the optimized mixes

294 Figure 10(d) shows the carbonation of the optimized mixes at 28 days. The carbonation of R-
 295 0-S is 3.84% lesser than R-0-N at 28 days. The carbonation of R-30-N and R-30-S is 13.3% and
 296 10.34% more than R-0-N. Similarly, the carbonation of R-100-N and R-100-S is 23.52% and 22.15%
 297 more than R-0-N. The higher pore volume in RAC due to increase in the RCA increases the entry of
 298 CO_2^- ions and increases the rate of carbonation in RAC (*Guo et al. 2018; Silva et al. 2015*). The
 299 carbonation of BR-30-S and BR-100-S is only 7.14% and 4.41% more than R-0-N. Also, the
 300 carbonation of BR-30-S and BR-100-S is 17.64% and 20% lesser than R-100-N and the carbonation
 301 of BR-30-S and BR-100-S is 16.16% and 18.56% lesser than R-100-S at 28 days. The bio-treatment
 302 reduces the water absorption of RCA, reducing the pore volume in RAC and thus decreasing the CO_2^-
 303 penetration.

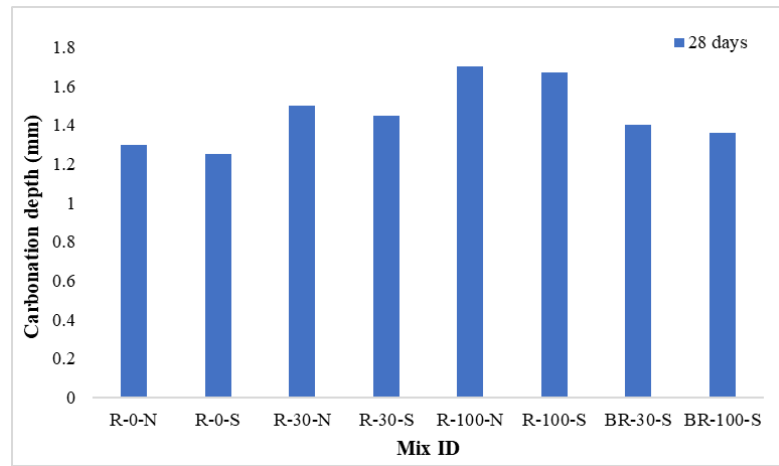


Figure 10(d). Carbonation of the optimized mixes

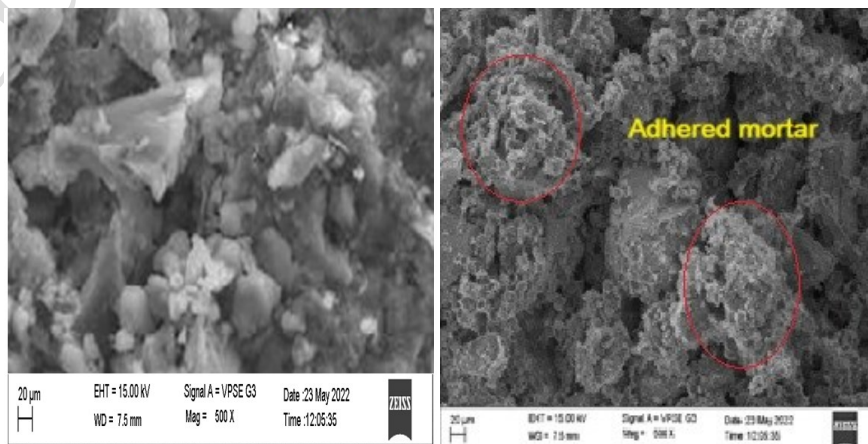
4. Discussions

The effect of bacterial treatment on the properties of URCA was assessed through weight increase, water absorption, and crushing index. It is inferred that bio-deposition increases the mass of the aggregates and reduces the water absorption and crushing index. The RCA used in the study was thoroughly washed and pre-saturated before bacterial treatment to remove the finer fractions adhered to the RCA surface (Wang *et al.* 2017). *Bacillus subtilis* was cultivated to investigate the bacterial species with optimal cell concentration for aggregate treatment. The increase in cell concentration increases bacterial precipitation in the order of 10^5 - 10^7 cells/ml. (Qui *et al.* 2014) inferred that incremental cell concentration improves the urea hydrolysis and the nucleation sites of CaCO_3 precipitation. This in turn increases the CaCO_3 precipitation and reduces the water absorption of BRCA. The influence of SEMA to enhance to quality of concrete mix tend to exhibit minor improvement in the concrete properties. In SEMA, $\frac{3}{4}$ th of the water added with NFA and cement produces stiff matrix that wraps the RCA added henceforth. The stiff wrap around RCA reduces the absorption of mixing water by RCA and thus improves the workability and strength of RAC (Liang *et al.* 2015, Sivamani *et al.* 2021a).

The improvement in the properties of BRCA eventually improves the properties of BRAC. The urea in the culture medium hydrolyse into NH_4 and CO_3^{2-} ions. The CO_3^{2-} ions reacts with $\text{Ca}(\text{OH})_2$

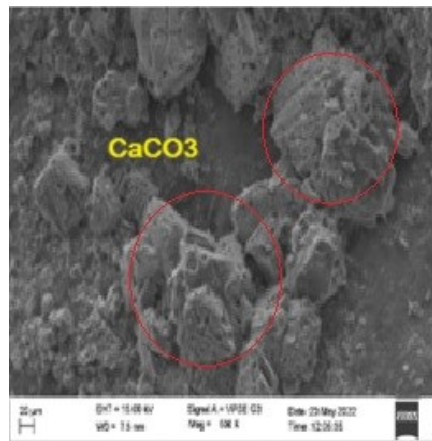
323 to precipitate CaCO_3 that deposits on the RCA. The higher CaCO_3 precipitation on the RCA firmly
 324 accumulates the micro-cracks on the RCA that resisted the shear exerted during the concrete mixing.
 325 In RAC, interfacial transition zone (ITZ) could be evident between RCA, fresh matrix and old matrix
 326 (Liu et al. 2020, Wu et al. 2020). The ITZ between fresh and old matrix becomes the weakest link
 327 due to the smeared mortar on the RCA. The CaCO_3 precipitated as a result of bio-deposition seals the
 328 micro-cracks on ITZ, endorsing hydration in ITZ and thus densified microstructure is formed
 329 enabling strength improvement in the RAC. Such microbial activity of *Bacillus Subtilis* improves the
 330 hardened properties of the RAC. The WA of BR-100-S was found to be lower than R-100-S and
 331 equivalent to R-0-N. When RCA was used, the pore volume of the concrete was increased that
 332 ultimately increases the WA of the RAC. Through bio-deposition, CaCO_3 lids the micro-cracks on
 333 RCA, eventually reducing the pore volume in RAC and hence WA of BR-100-S was equivalent to
 334 R-0-N (Liu et al. 2020, Wang et al. 2017). Other durability properties discussed in the study were
 335 direct reliant on the pore volume of the RCA, as which improvement was observed in bio-deposited
 336 mixes.

337 The effect of CaCO_3 precipitation on improving the properties of RAC was investigated
 338 through SEM. Figure 11 shows the SEM images of R-0-N, R-100-N and BR-100-S. It is observed
 339 that the mortar adhered to the RCA was highly porous, affecting the concrete's properties. Upon bio-
 340 deposition, dense CaCO_3 crystals were precipitated and deposited on the surface of the RCA (Grabiec
 341 et al. 2012, Wu et al. 2020, Liu et al. 2020, and Wang et al. 2017).



(a)

(b)



(c)

Figure 11. SEM images (a) R-0-N (b) R-100-N (c) BR-100-S

5. Conclusion

The experimental investigation on the properties of bio-deposited recycled aggregate concrete was performed. The study was performed to evaluate the properties of NCA, RCA and BRCA for its utilization in the concrete. The optimized concrete mixtures was evaluated for its hardened properties and durability properties at suitable ages. The microstructure investigations to evaluate the precipitation of CaCO_3 crystals by bacterial species were also conducted. The resulting inferences were made as follows:-

1. The CaCO_3 precipitation through urea hydrolysis by *Bacillus subtilis* reduces the RCA's water absorption.
2. Higher microbial concentration rises the CaCO_3 precipitation. This was evident through the increase in the mass and decrease in the absorption of BRCA.
3. The smearance of mortar on RCA decreases the replacement of RCA optimizing it to 30%, beyond which it results in strength reduction of RAC.
4. The strength of mixes with 30% of RCA, 30% of BRCA and 100% of BRCA was 4.75%, 8.17% and 12.63% more than control concrete while the strength of mixes with 100% of RCA was 9.17% lesser than R-0-N at 28 days.
5. The optimized concrete mixes with 100% of BRCA through shows improved durability due to the CaCO_3 precipitation and the densification of ITZ through SEMA.

365 The research provides an ideal solution to improve the RCA quality through bio-treatment
366 and found that the properties of BRAC were better compared to that of RAC and were comparable to
367 NAC. The technique of preparing the concrete mixture with BRCA through SEMA will extend the
368 utilization of RCA as substitute to NCA in construction. This, in turn will reduce the disposal
369 problems of construction wastes and promote sustainability in the construction.

370 **Conflicts of Interest**

371 The authors have declared no conflict of interest

372 **Data Availability Statement**

373 The data that support the findings of this study are available within the study and can be
374 collected from the corresponding author upon request.

375 **References**

- 376 Arunkumar G.E., Nirmalkumar K., Loganathan P. and Sampathkumar V. (2023). Concrete
377 constructed with recycled water to experimental analysis of the physical behavior of polypropylene
378 aggregate (PPA), *Global NEST Journal*, **25**, 126-135.
- 379 Andal J., Shehataa M.H. and Zacarias. P. (2016), Properties of concrete containing recycled concrete
380 aggregate of preserved quality, *Construction and Building materials*, **125**, 842-855.
- 381 Bachmeier K.L., Williams A.E., Warmington J.R. and Bang S.S. (2002), Urease activity in
382 microbiologically-induced calcite precipitation, *Journal of Biotechnology*, **93**, 171-181.
- 383 Garcia-González J., Rodríguez-Robles D., Wang J., De Belie N., Moran-del Pozo J.M., Guerra-
384 Romero M. I. and Juan-Valdés A. (2017), Quality improvement of mixed and ceramic recycled
385 aggregates by bio deposition of calcium carbonate, *Construction and Building Materials*, **154**,
386 1015-1023.
- 387 Geng J. and Sun J. (2013), Characteristics of the carbonation resistance of recycled fine aggregate
388 concrete, *Construction and Building Materials*, **49**, 814-820.

389 Grabiec A.M., Klama J., Zawal D. and Krupa D. (2012), Modification of recycled concrete
 390 aggregates by calcium carbonate bio deposition. *Construction and Building Materials*, **34**, 145-
 391 150.

392 Guo H., Shi C., Guan X., Zhu J., Ding Y., Ling T. C., Zhang H and Wang Y.(2018), Durability of
 393 recycled aggregate concrete – A review, *Cement and Concrete Composites*, **89**, 251-259.

394 Kirthika S. K. and Singh S. K. (2020), Durability studies on recycled fine aggregate concrete,
 395 *Construction and Building Materials*, **250**, Article no. 118850.

396 Krishnapriya S., Venkatesh Babu D.L., and Prince Arulraj G. (2015), Isolation and identification of
 397 bacteria to improve the strength of concrete, *Microbiological Research*, **174**, 48-55.

398 Lavado J., Bogas J., de Brito J and Hawreen A.(2020), Fresh properties of recycled aggregate
 399 concrete, *Construction and Building Materials*, **233**, Article no. 117322.

400 Liang Y. C., Ye Z. M., Vernerey F., and Xi Y. (2015), Development of processing methods to
 401 improve strength of concrete with 100% recycled coarse aggregate, *Journal of Materials in Civil*
 402 *Engineering*, **27**, 04014163.

403 Liu M., Xia J., Chin C. S and Liu Z. (2020), Improving the properties of recycled aggregate pervious
 404 pavement blocks through bio-mineralization, *Construction and Building Materials*, **262**, Article
 405 no. 120065.

406 Majumdar S., Sarkar M., Chowdhury T., Chattopadhyay B. and Mandal S. (2012), Use of bacterial
 407 protein powder in commercial fly ash pozzolana cements for high performance construction
 408 materials. *Open Journal of Civil Engineering*, **2**, 218-228. 10.4236/ojce.2012.24029.

409 Mavroulidou M. and Figueiredo J. (2010), Discarded tyre rubber as concrete: A possible outlet for
 410 used tyres. *Global NEST Journal*, **4**, 359-367.

411 Mi R., Pan G., Liew K. M. and Kuang T. (2020), Utilizing recycled aggregate concrete in sustainable
 412 construction for a required compressive strength ratio, *Journal of Cleaner Production*, **276**, Article
 413 no. 124249.

414 Mondala S., Das P and Chakraborty A. (2017), Application of Bacteria in Concrete. *Materials Today*
 415 *Proceedings*, **4**, 9833-9836.

416 Mwashia A and Ramnath R. (2018), Manufacturing Concrete with High Compressive Strength Using
 417 Recycled Aggregates, *Journal of Materials in Civil Engineering*, **30**, 04018182.

418 Nain N., Surabhi R., Yathish N.V., Krishnamurthy V., Deepa T and Tharannum, S. (2020).
 419 Enhancement in strength parameters of concrete by application of bacillus bacteria, *Construction*
 420 *and Building materials*, **202**, 904-908.

421 Naveen Arasu N., Natarajan M., Balasundaram N. and Parthasaarathi R. (2023), Utilizing recycled
 422 nanomaterials as a partial replacement for cement to create high-performance concrete, *Global*
 423 *NEST Journal*, **25**, 89-92.

424 Puthussery J.V., Kumar R and Garg A. (2017), Evaluation of recycled concrete aggregates for their
 425 suitability in construction activities: An experimental study. *Waste management*, **60**, 270-276.

426 Silva R. V., Neves R., de Brito J. and Dhir R. K (2015), Carbonation behaviour of recycled aggregate
 427 concrete, *Cement and Concrete Composites*, **62**, 22-32.

428 Qiu J., Sheng Tng D.Q. S and Yang, E. H. (2014), Surface treatment of recycled concrete aggregates
 429 through microbial carbonate precipitation, *Construction and Building Materials*, **57**, 144-150.

430 Jagan S., Neelakantan T. R. (2022), Performance Enhancement of Recycled Aggregate Concrete –
 431 An Experimental Study, *Applied Science and Engineering Progress*, **15**, 5212.

432 Sivamani J., Neelakantan T. R., Saravana Kumar P., Mugesh Kanna C., Vignesh Harish H. and Akash
 433 M. R. (2021a), Efficient Utilization of Recycled Concrete Aggregates for Structural
 434 Applications—An Experimental Study, *Lecture Notes in Civil Engineering*, **97**, 567-579.

435 Jagan S., Neelakantan T. R. and Lakshmikantha Reddy. (2020), Characterization study on recycled
 436 coarse aggregate for its utilization in concrete – A review. *Journal of Physics: Conference series*,
 437 **1706**, 012120.

438 Schandl H. and Krausmann F. (2017). The 20th century saw a 23-fold increase in natural resources
 439 used for building, *Environmental Newsletter (Down to earth)*, <https://www.downtoearth.org.in/>

440 Sivamani J. and Renganathan N.T. (2022). Effect of fine recycled aggregate on the strength and
 441 durability properties of concrete modified through two-stage mixing approach, *Environmental*
 442 *Science and Pollution Research*, **29**, 85869–85882

443 Sivamani J., Renganathan N.T. and Saravana Kumar P. (2021b), Enhancing the quality of recycled
 444 coarse aggregates by different treatment techniques—a review, *Environmental Science and*
 445 *Pollution Research*, **28**, 60346–60365.

446 Park S. J., Park Y. M., Chun W.Y., Kim W.J. and Ghim W.Y. (2010), Calcite-Forming Bacteria for
 447 Compressive Strength Improvement in Mortar, *Journal of Industrial Microbiology Biotechnology*,
 448 **20**, 782-788.

449 Thomas J., Thaickavil N. N. and Wilson P. M. (2018), Strength and durability of concrete containing
 450 recycled concrete aggregates, *Journal of Building Engineering*, **19**, 349-365.

451 TERI. (2022), “The Energy and Research Institute”, <https://terragreen.teriin.org/>

452 Wang J., Vandevyvere B., Vanhessche S., Schoon J., Boon N. and De Belie N. (2017), Microbial
 453 carbonate precipitation for the improvement of quality of recycled aggregates, *Journal of cleaner*
 454 *production*, **156**, 355-366.

455 Wu C. R., Zhu Y., Zhang X. T. and Kou S. C. (2018), Improving the properties of recycled concrete
 456 aggregate with bio-deposition approach, *Cement and Concrete Composites*, **194**, 248-254.

457 Wu C. R., Hong Z. Q., Zhang J. L. and Kou S. C. (2020), Pore size distribution and ITZ performance
 458 of mortars prepared with different bio-deposition approaches for the treatment of recycled concrete
 459 aggregate, *Cement and Concrete Composites*, **111**, 103631.

460 Zhan M., Pan G., Wang Y., Fu M. and Lu X (2019), Recycled aggregate mortar enhanced by
 461 microbial calcite precipitation, *Magazine of concrete research*, **72**, 622-633.

462 Zhu Y., Li Q., Xu P., Wang X. and Kou S. (2019), Properties of concrete prepared with recycled
 463 aggregates treated by bio-deposition adding oxygen release compound. *Materials*, **12**, 2147.1-13.
 464 10.3390/ma12132147