Bio-Based Cementitious Composites with Recycled Wastes

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy

By

Zuowei Liu

March 2022

PGR Declaration of Academic Honesty

NAME (Print)	Zuowei Liu
STUDENT NUMBER	1612461/201251948
SCHOOL/INSTITUTE	Department of Civil Engineering
TITLE OF WORK	Bio-Based Cementitious Composites with Recycled Wastes

This form should be completed by the student and appended to any piece of work that is submitted for examination. Submission by the student of the form by electronic means constitutes their confirmation of the terms of the declaration.

Students should familiarise themselves with Appendix 4 of the PGR Code of Practice: PGR Policy on Plagiarism and Dishonest Use of Data, which provides the definitions of academic malpractice and the policies and procedures that apply to the investigation of alleged incidents. Students found to have committed academic malpractice will receive penalties in accordance with the Policy, which in the most severe cases might include termination of studies.

STUDENT DECLARATION

I confirm that:

- I have read and understood the University's PGR Policy on Plagiarism and Dishonest Use of Data.
- I have acted honestly, ethically, and professionally in conduct leading to assessment for the programme of study.
- I have not copied material from another source nor committed plagiarism, nor fabricated, falsified, or embellished data when completing the attached material.
- I have not copied material from another source nor colluded with any other student in the preparation and production of this material.
- If an allegation of suspected academic malpractice is made, I give permission to the University to use source-matching software to ensure that the submitted material is all my own work.

SIGNATURE: Zuowei Liu

DATE: 2022/3/17

Abstract

The rapid economic and social development caused an increasing demand for industrial and civil construction. However, with the growing number of construction projects, the shortage of natural materials for mixing concrete has reached a crisis. There have been increasing studies focusing on introducing recycled wastes such as fly ash (FA) used for replacing cement and recycled coarse aggregates (RCA) used for replacing natural gravel with concrete. However, the inadequate mechanical performance and durability of FA concrete and RCA concrete (RCAC) have limited their widespread use. Biomineralisation has been evidenced as a helpful way to improve the mechanical performance and durability of concrete. Ureolytic bacteria are common strains used to induce biomineralisation. This research has preliminarily adopted three ureolytic bacteria strains for application in concrete. Adding bacterial solution into mixing water and pretreating RCA with bacteria solution and using these bio-treated RCA as the coarse aggregate concrete are the two approaches used in this project for applying the biomineralisation method to concrete. The results have shown that water absorption of RCA decreased by 17.3%, and the apparent density increased by 2.4% after the treatment. Meanwhile, applying the same type of bacteria with nutrition and extra calcium ions into fresh concrete, the compressive strength of FA concrete improved by 30%. The mechanical properties of concrete with 40% FA and 30% treated RCA enhanced by Sp bacterial solution can be close to normal concrete with the same mixing design. However, ammonia (NH_3) was generated unavoidably with an unpleasant smell. As part of the research focus, the use of denitrification bacteria to induce biomineralisation has also been investigated. Like the ureolytic bacterial approach, this approach also exhibited satisfactory enhancing effects and was

researched to enhance RCAC. Pre-treating the RCA by soaking in bacteria solution and mixing bacterial solution were proposed to apply to RCAC together, which was abbreviated as BTRCAC (bacterial treated RCAC). For BTRCAC, 30.3% and 19.2% improvements were recorded for compressive strength and tensile splitting strength, respectively. The calcium carbonate (CC) induced by the bacteria filled the open pores inside the concrete, which contributed to a 33.0% decrease in water absorption, impeding the absorption of outside water and reducing the swelling caused by water freezing. Bacteria bond the ITZs, resulting in higher cohesion between aggregates and matrix as well as enhancing the resistance to the pressure caused by freeze-thaw cycles. BTRCAC can withstand 75 more freeze-thaw cycles than RCAC. LCA analysis found that environmental impact (EI) contributions created by bacterial solution production were negligible. Though the EI caused by using nutrition and calcium sources is high, however using recycled wastes decreases EI and the biomineralisation method compensated for the inadequate performance caused by using the recycled wastes. Hence, compared with ordinary concrete, bio-based FA concrete with 100% RCA decreased 27.0% of Global Warming Potential (counted by kg CO2 eq). Moreover, the normalised EI value, which was obtained by weighted summing multi-EI categories including Global Warming Potential, Acidification Potential, Photochemical Ozone Formation Potential, Particulate matter, and Ionizing radiation according to EU Environmental Footprint 3.0, of bio-based recycled waste concrete was reduced by a maximum of 25.0%. Furthermore, if the nutrition and calcium ions can be replaced by the contaminant in wastewater, the EI of the bio-based recycled waste concrete can be further significantly decreased. Therefore, the bio-based recycled waste concrete w is a sustainable way to replace the traditional concrete.

Acknowledgements

I would like to express my sincere appreciation to my supervisors, Prof. Chee Seong Chin for his guidance and overall planning, Dr. Jun Xia for his support in my study and work, and Prof. Zhongwei Guan for his supervision.

Also, I would like to express my appreciation to all scholars who provided professional guidance in their area; especially Prof. Varenyam Achal, for the suggestion in microbiology, my senior colleagues, Dr. Xiang Wang, Dr. Yida Guo and Dr. Xinyi Wang, for imparting me the experience in concrete materials experiments and LCA analysis, and my friend Dr. Junnan Liu, for the help in the chemical area.

I am particularly grateful to all the staff involved in my PhD project from XJTLU, in particular, my colleagues in the Department of Civil Engineering; I am grateful for the time we did PhD together.

I would like to thank my roommates who have already worked in the construction industry. As a practitioner in the construction sector, your experience is precious for my research.

I would like to thank all my friends. I am deeply grateful for the friendship and mutual growing up that has been continuing for more than ten years.

Finally, my sincere appreciation goes to my parents, who have contributed so much during my academic career. Their inspiration and love are always the indispensable power guiding me forward.

Publications and Author's Contributions

1. Improving properties of recycled coarse aggregate (RCA) by biomineralization method. 2019, **ZW Liu**, CS Chin, J Xia, V Achal *Sustainable Buildings and Structures: Building a Sustainable Tomorrow*, 75-80 (Conference paper) **(Published)** Citations: 1

2. Utilisation of Biomineralisation Method in Recycled Coarse Aggregate Concrete with Fly Ash. 2021, **Z Liu**, CS Chin, J Xia *Building Materials for Sustainable and Ecological Environment*, 9-24 (Book chapter) **(Published)** Citations: 1

3. Improving recycled coarse aggregate (RCA) and recycled coarse aggregate concrete (RCAC) by biological denitrification phenomenon. 2021, **Z Liu**, CS Chin, J Xia ***Construction and Building Materials*** 301, 124338 (SCI Journal paper Q1 IF:6.141) **(Published)** Citations: 4

4. Novel Method for Enhancing Freeze-Thaw Resistance of Recycled Coarse Aggregate Concrete (RCAC) via Two-Stage Introduction of Denitrifying Bacteria, **Z Liu**, CS Chin, J Xia ***Journal of Cleaner Production*** Volume 346, 20 April 2022, 131159 **(Published)** (SCI Journal paper Q1 IF:9.297)

5. Improving the properties of recycled aggregate pervious pavement blocks through bio-mineralization M Liu, J Xia, CS Chin, **Z Liu** ***Construction and Building Materials*** 262, 120065 (Journal paper Q1 IF:6.141) **(Published)** Citations: 7

6. A method to enhance recycled aggregate by biomineralization induced by denitrification bacteria. **ZW Liu**, CS Chin, J Xia 2022, 202110112420.9 (patent) **(Pending authorization after revision)**

7. Life Cycle Assessment of Fly Ash Concrete and Recycled Coarse Aggregate Fly Ash Concrete Enhanced by Biomineralisation Method **Z Liu**, CS Chin, J Xia ***Journal of Cleaner Production*** (SCI Journal paper Q1 IF:9.297) **(Under review)**

Content

List of Abbreviations

AD:	Air-dried condition
AFm:	Monosulphate
AFt:	Ettringite
ATCC:	American Type Culture Collection optical density
Bm:	Bacillus megaterium de Bary
C&DW:	Construction and demolition wastes
CC:	Calcium carbonate
CH:	Calcium hydroxide
CLCD:	Chinese Life Cycle Database
CSH:	Calcium Silicate Hydrate
CUCG	China United Cement Group
EDS:	Energy dispersive spectrometry
EI:	Environmental impacts
EP:	Ecotoxicity
FA:	Fly ash
RI:	Particulate matter
GHG:	Greenhouse gas
GWP:	Global Warming Potential
ITZ:	Interfacial Transform Zone
IRP:	Ionizing radiation
LCA:	Life cycle assessment
LCIA:	Life cycle impact assessment
Lf:	Lysinibacillus fusiformis
NA:	Natural aggregates
OD:	Oven-dried

OPC:	Ordinary Portland cement
Pd:	Paracoccus denitrificans
PED:	Primary Energy Demand
Pf:	Pseudomonas fluorescens
POFP:	Photochemical Ozone Formation Potential
Psp:	Pseudomonas.Sp
RCA:	Recycled coarse aggregates
RCAC:	Recycled coarse aggregates concrete
RDME:	Relative dynamic modulus of elasticity
RFA:	Recycled fine aggregates
RI:	Respiratory Inorganics
SEM:	Scanning Electron Microscope
SP:	Sporosarcina pasteurii
SSD:	Saturated surface dry
TGA:	Thermogravimetric analysis
TR:	Treated RCA
TRCA(Sp):	RCA treated by Sp
TRCA(Psp):	RCA treated by Psp
UR:	Untreated RCA
XRD:	X-ray diffraction

List of Figures

List of Tables

Chapter 1. Introduction

Section 1.1 Background

Section 1.1.1 Concrete

Concrete, a composite mixture of cementitious materials, aggregates, and water in an appropriate proportion, is one of the most widely used materials in the construction area due to its high compressive strength and relatively low cost. However, cracks usually occur inside the concrete, which inevitably causes a reduction in mechanical performance and durability due to its weakness in tensile resistance and brittleness [1]. Furthermore, various adverse factors such as earthquakes, fire, frost, and penetration of chemical substances accelerate the damage to concrete, which has resulted in security threats and economic losses. Hence, it is the most fundamental requirement that concrete has adequate mechanical performance and durability in response to the load and the complicated environment. To evaluate whether the concrete can meet the requirements, various tests, including compressive strength, tensile splitting strength, elastic modulus, water permeability, chloride penetration, freeze-thaw resistance, and other properties, shall be conducted.

In addition, due to the rapid extension of urbanisation and infrastructure construction, rapidly increasing natural resources are being consumed by the construction industry. According to the statistics [2], 40% of global natural stone, gravel and sand, 25% of timber, 40% of total global energy consumption , and 16% of freshwater resources are consumed annually by the world's construction sector. The global production of concrete has exceeded 900 Gt since the industrial revolution began [3], and according to the statistics in 2021 [4], globally, annual concrete production is approximately 30 billion tonnes in recent years, creating an excessive consumption of resources and causing the deterioration of the environment [5]. Among the concrete compositions, cement production contributed to the environmental impacts (EI) most severely due to its high emission of CO_2, SO_2, NO_X and particulate [6–11]. The annual ordinary Portland cement (OPC) production arrived at 5 billion tonnes in 2021, accounting for about 5~8% of global CO_2 emissions [8,12]. Natural aggregates (NA), including natural sand and gravel, occupy 70% volume of the concrete [13]. In 2014, approximately 4 billion tonnes of NA were mined globally, and this valve is expected to arrive at 6 billion tonnes in 2025 [14]. Massively quarrying NA tends to deplete and causes environmental contamination [15]. Therefore, under the premise of meeting the mechanical performance and durability requirements, it is necessary to f replace the natural materials with recycled materials.

Section 1.1.2 Biomineralisation method for enhancing concrete

With the development of bio-science, many bio-technology is gradually applied in construction areas [16]. Take biomineralisation, a process by which hard biological materials consisting of inorganic mineral material are created under the interaction of organic protein molecules [17,18]. Some rigid materials, such as eggshells, mollusc shells, teeth and bones, are created by biomineralisation [19]. In 1973, Boquet et al. [20] found that bacteria are capable of forming calcium carbonate (CC) crystals, and they also demonstrated that over two hundred strains of bacteria that can form the crystals were isolated. These crystals are solid and have high compatibility with concrete [1]. Since then, many researchers have attempted to improve concrete by this method. In 1995, Gollapudi et al. [21] made the first attempt to control the leakage of the building matrix by using bacteria-induced CC deposition. In 2001, Santhosh et al. [22] innovatively put forward concrete remediation using microorganisms. Due to the rapid extension of the biological technique, bio-based cementitious composites show an increasingly significant potential for enhancing and healing concrete.

Section 1.1.3 Recycled wastes in concrete

The amount of solid wastes, such as fly ash (FA) and recycled aggregates (RA), including recycled coarse/fine aggregates (RCA/RFA), has been rapidly increasing [23–28] due to the growth of industry and rapid urbanisation [29]. Consequently, the management of solid wastes is increasingly challenging worldwide. Applying these recycled wastes into concrete to replace cement or aggregate is a way that saves natural resources and eliminates solid waste pollution.

FA is a kind of solid waste generated in coal power plants [30]. According to China Power Industry Annual Development Report 2021 [31], in China, coal fire power plants generated 4630 billion kWh coal power in 2020, resulting in approximately 700 million tonnes of FA production [32]. Each kWh of electricity produced consumes 313.6g of standard coal and creates 0.69 kg of CO_2 [33]. Not only does the emission of FA cause severe pollution, but the landfill itself also occupies limited and valuable land resources. Like the OPC, FA also has pozzolanic activity; hence, a large amount of research has attempted to use FA as cementitious materials in concrete to decrease waste disposal [34–36]. Furthermore, FA added to concrete makes cement particles diffuse and increases the workability of concrete, contributing to high compaction of fresh concrete; in the later period, FA has a secondary hydration reaction and produces Calcium Silicate Hydrate (CSH), which further improves the compaction and decrease the permeability of concrete; thus the long-term performance and durability can be enhanced by using FA in concrete [37–39]. In addition, FA can save 45% on cost and reduce 55% of CO_2 emissions compared to OPC [37].

RA, on the other hand, is gathered from crushed construction and demolition wastes (C&DW) [40]. At present, the annual production of C&DW in China is about 3.5

billion tonnes, with a storage capacity of about 20 billion tonnes and a land area of 500 million m^2, and it is rising at a rate of about 10% each year [41]. The large amount of C&DW is already a threat to the environment, and properly disposing of and using them has become an urgent issue. At the same time, the demand for coarse aggregate in the construction industry reached 10.3 billion tonnes in 2020 [42]. Using RCA in concrete not only effectively disposes of construction waste generated during urban construction but also reduces the dependence on natural resources, including natural sand and gravel. Previous research [43] reported that the greenhouse gas (GHG) emissions of producing each ton of NA were 46 kg CO_2 eq, whereas the GHG emissions for making each ton of RA were 2.4 kg CO_2. Replacing 50% NA with RA in construction projects could, therefore, potentially reduce GHG emissions by up to 23% [44]. In China, RA has already begun to be applied in concrete construction [25,44]; however, the recycling rate of C&DW has so far only reached 5%. Therefore, it is necessary to further research the properties of RA and concrete with RA (RAC) to promote the wider recycling of C&DW.

Although using recycled wastes, such as RCA and RA mentioned above, can decrease the cost of concrete, and avoid the wastes disposal issues, introducing recycled waste increases the complexity of concrete. For example, the pozzolanic activity of FA is lower than the OPC, which can delay the strength formation of concrete [45], and RCA can create a double layers of interfacial transition zone (ITZ) due to its loose old concrete layer, which is adverse to the strength and durability of concrete [34]. Hence it is necessary to research further the properties of recycled wastes, concrete, and corresponding improvement method.

Section 1.1.4 Sustainability analysis by Life cycle assessment (LCA)

Besides the performance, sustainability is an indispensable property of concrete. There is now a global consensus for controlling the concentration of carbon dioxide in the atmosphere within a specific time frame and at an appropriate level. The construction sector, one of the major engines of global economic development, consumes 35% of the world's energy and accounts for 40% of total CO_2 emissions [7]. The construction sector is a crucial area for promoting sustainable development and achieving carbon peaking and carbon neutrality. Ordinary Portland cement (OPC) production occupied approximately 90% of carbon emissions in concrete production [46].

Life cycle assessment (LCA) was first introduced in the early 1960s to rationalise the energy consumption of buildings and has since developed into a broader concept that integrates all environmental impacts and is one of the main tools used for pollution prevention [47].. Standards ISO 14040:2006 [48] and ISO 14044:2006 [49] are the rules to guide the LCA analysis. Generally, a cradle to grave analysis involves various stages of a product system, from raw material extraction to final disposal. Environmental impacts of each unit process can be quantified based on the material input, energy flows and pollutant emissions to air and water. LCA methodology is usually used to compare different production processes [50]. Due to the increasing demand for sustainable development, sustainability is gradually becoming a crucial indicator for evaluating concrete. Hence, the LCA method is also adopted to obtain and compare the sustainability of concrete with various substitutions. LCA analysis is usually calculated and evaluated using software programs such as GaBi®, SimaPro® and openLCA. Various EI assessment methods characterize different environmental concerns [51]. According to ISO 14044:2006 [49], there are four parts: “the goal and scope definition, cataloguing analysis, environmental impact assessment, and result interpretation in processing the LCA analysis". After the LCA analysis, the sustainability of each kind of concrete can be characterized, thus making it feasible to come to a judgment and draw a comparison. Therefore, to explore the sustainability of bio-based cementitious composites with recycled wastes, it is imperative to conduct an LCA analysis.

Section1.1 Aims and objectives

This project aims to create sustainable concrete by applying biomineralisation technology to recycled waste concrete. The primary objectives of this research are:

- To introduce recycled wastes, including FA and RCA, into concrete and obtain their influence on the mechanical performance and durability of concrete.
- To explore the suitable composition of bio-agent for enhancing the inadequate performance of recycled waste concrete, including the strain and dosage of bacteria, nutrition, and calcium ion supplier.
- To measure the influence of the bio-agent on mechanical performance, durability, and micro-structure of concrete with FA and RCA for providing data for practical application.
- To summarise the effects and drawbacks of the ureolytic bacterial biomineralisation method and select novel strains to induce biomineralisation.
- To apply denitrification bacteria into RCAC and obtain its mechanical performance, durability, and micro-structure.
- To quantify the sustainability of recycled waste concrete with the biomineralisation method by LCA analysis. To illustrate the environmental interest of this project by quantified EI values obtained by weighted summing multi-EI categories according to EU Environmental Footprint 3.0.

Chapter 2. Literature Review

Section 2.1 Introduction

As two common recycled wastes, FA and RCA are usually used as substitutes for cementitious materials and natural gravel, respectively. However, introducing these recycled wastes may affect the performance of concrete. Qualified concrete should meet the loading and durability requirements. Only increasing the usage amount of recycled wastes is hard to obtain sustainable concrete with competent performance. The Biomineralisation method can improve the mechanical performance and durability of concrete. Hence, combining these two research areas may create a new kind of high sustainability and qualified performance.

This project, bio-based cementitious composites with recycled wastes, needs to interdisciplinary research, including exploring the method of biomineralisation and properties of concrete with recycled wastes. Although many researchers have focused on these two topics, they have only focused on them independently, and thus research on combining these two topics is limited. This chapter aims to analyse the feasibility of biomineralisation to improve recycled waste concrete by reviewing and evaluating the previous investigations on bio-based cementitious material and recycled waste concrete. This chapter reviewed previous research in biomineralisation, recycled waste concrete, mechanical and durability performance, and LCA of concrete. It provided the latest achievements, orientation, and guidelines from various areas for the whole project.

Section 2.2 Biomineralisation method in concrete

Section 2.2.1 Mechanism of concrete enhanced by biomineralisation

Biomineralisation in concrete aims to fill the voids and connect the cracks inside concrete by CC induced by some microbes [16,36,52]. Bacteria with approximately 0.5–5.0 μm in length have movement ability, except for a few particular strains [53].

Hence, bacteria cells could arrive at the voids and pores inside the concrete, and the biomineralisation procedure could occur. The complete process of biomineralization is presented in Figure 1. To create biomineralisation in concrete, besides the adequate calcium (Ca^{2+}), dissolved inorganic carbon (CO_3^{2-}) should be supplied [54,55]. In the microbial world, photosynthesis, sulphate reduction, urea hydrolysis and denitrification are four common metabolic pathways that can drive the formatting process of carbonate ions [52]. The chemical equations of carbonate ions creation derived from the three metabolic pathways are shown in Eq. 1 ~ Eq. 2, Eq. 3 ~ Eq. 4, Eq. 5 ~ Eq. 9 and Eq. 10 ~ Eq. 14, respectively [56–61]. At the same time, the cell walls of the bacteria usually have negative charges. Thus, calcium ions are attracted from the environment and deposited on the cell wall [62]. As a result, calcium ions and carbonate ions combine into CC precipitation, as shown in Eq. 13 [62,63]. With the deposition of CC, the bacteria finally become a crystal filling the void inside the concrete. In summary, the mechanism of concrete enhanced by biomineralisation is that organic carbon and calcium ions are combined into CC, which then fills the voids inside concrete induced by the metabolism of microbes. The CC induced by bacteria can enhance the cement matrix and ITZ between the cement matrix and aggregate [64–66].

Photosynthesis:

$$CO_2 + H_2O \rightarrow CH_2O + O_2 \quad (1)$$

$$2HCO_3^- \leftrightarrow CO_2 + CO_3^- + H_2O \quad (2)$$

Sulphate reduction:

$$2(CH_2O) + SO_4^{2-} \rightarrow HS^- + HCO_3^- + CO_2 + H_2O \quad (3)$$

$$HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O \quad (4)$$

Urea hydrolysis:

$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3$ (5)

$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$ (6)

$H_2CO_3 \leftrightarrow HCO_3^- + H^+$ (7)

$2NH_3 + 2H_2O \leftrightarrow 2NH_4+ + 2OH^-$ (8)

$HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O$ (9)

Denitrification:

$2HCOO^- + 2NO_3^- + 2H^+ \rightarrow 2CO_2 + 2H_2O + 2NO_2^-$ (10)

$HCOO^- + 2NO_2^- + 3H^+ \rightarrow CO_2 + 2H_2O + 2NO$ (11)

$HCOO^- + 2NO + H^+ \rightarrow CO_2 + H_2O + N_2O$ (12)

$HCOO^- + N_2O + H^+ \rightarrow CO_2 + H_2O + N_2$ (13)

$CO_2 + 2OH^- \rightarrow CO_3^{2-} + H_2O$ (14)

Calcium carbonate deposition:

$CO_3^{2-} + Ca^{2+}\text{-Cell} \rightarrow CaCO_3 + \text{Cell}$ (15)

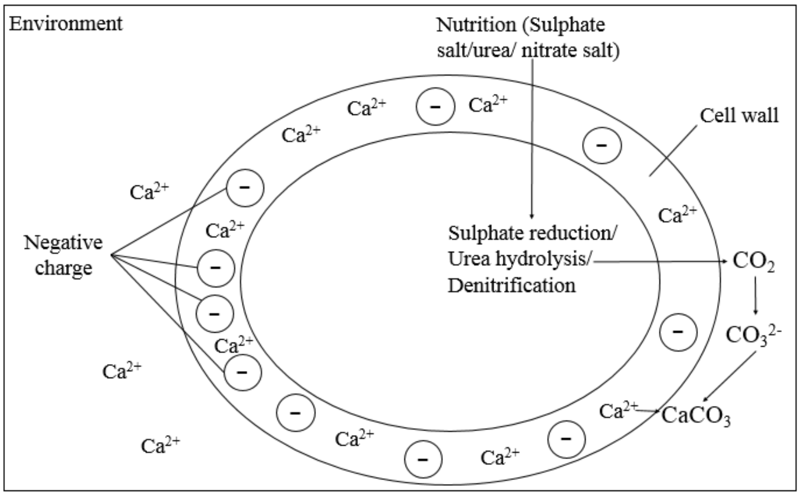


Figure 1. The schematic presentation suggested bacterial calcium metabolism and subsequent $CaCO_3$ precipitation under high-pH and high-Ca^{2+} extracellular conditions.

Section 2.2.2 Chemical composition and microstructure of bacterial concrete

Previous research [67–71] explored the chemical composition and microstructure by energy-dispersive spectrometry (EDS), X-ray diffraction (XRD), scanning electron microscope (SEM) and Thermogravimetric analysis (TGA). SEM, XRD and EDS belong to qualitative methods. By EDS analysis, previous research [1,72,73] presented that after introducing bacteria into concrete, some products consisting of Ca, C, and O have appeared. Researchers [74–76] suggested that the products were likely to be $CaCO_3$ by the further analysis of XRD. Due to various environmental factors, such as PH, temperature and other ions in solution, CC crystal exists in different morphologies [77–79]. In general, CC exists in three types of crystals: calcite, aragonite and vaterite [79–81]. The SEM and XRD patterns of each kind of crystal are shown in Figure 2 [81]. By SEM and XRD analysis, Muynck et al. [82] observed calcite and small quantities of vaterite were produced after applying *Bacillus sphaericus* to concrete.

In summary, the bio-induced CC in concrete mainly existed as calcite and depended on different calcium resources and bacteria strains. According to previous research, aragonite and vaterite also existed [69,82–85]. In addition, TGA can be used for dectecting the amount of each chemcial composation. The amount of CC in concrete can be calculated by the mass loss caused by CC decomposes into CaO and CO_2 in the temperature range from 650 to 800 °C [86,87]. Wang et al. [86] detected a 26 % weight loss of concrete with bacteria during 650 to 800 °C, while the negligible mass loss was detected in control groups. Chaurasia et al. [66] reported that a 34% higher amount of CC in the bacterial group was observed compared to the control group.

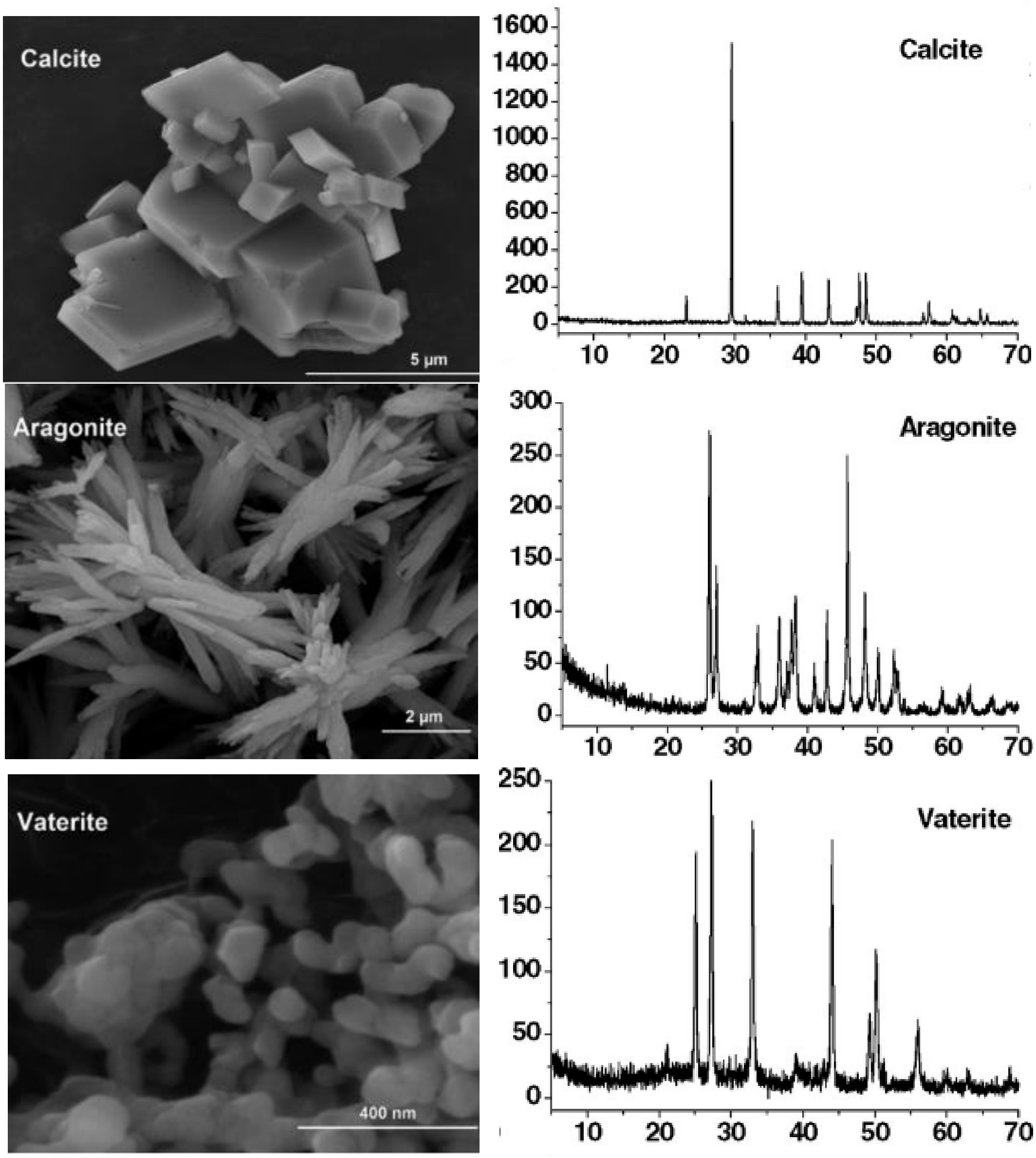


Figure 2. Representative SEM and XRD patterns of the phase-pure CC polymorph [81].

Section 2.2.3 Applying the biomineralisation method in concrete

The environmental situation inside concrete should be considered to create biomineralisation inside the concrete. Photosynthesis needs sufficient light; however, it is hard to shoot enough light inside concrete specimens, especially during the curing time. Sulphate ions are required to be introduced if "sulphate reduction" is adopted to drive the biomineralisation; however, mixing sulphate ions into concrete can significantly degrade its quality, because the sulphate ions can corrode the concrete [88–91]. Hence, few researchers utilize these two mechanisms in concrete production.

On the contrary, according to Eq. 5 ~ Eq. 9, urea hydrolysis did not have an adverse effect on the properties of concrete. Bacteria that can hydrolyse urea to supply energy for their life activities are named ureolytic bacteria [92]. Ureolytic bacteria are widely spread in soil and wastewater and survive in a harsh environment [55]. Hence, ureolytic bacteria are usually used to induce biomineralisation in concrete research. Table 1 summarises several studies of applying ureolytic bacteria to enhance the performance of concrete [65,84,93–103]. It was found that various ureolytic bacteria exhibited enhancement on concrete, and Charpe et al. [102] found that natural bacteria in soil screened by urea also improved the strength and reduced water absorption of concrete. The bacteria were usually added to mixing water of concrete, and from 10^5 to 10^7 CFU/ml, mixing water was a common concentration. Directly adding bacterial solution when mixing fresh concrete was the most common way to introduce bacteria [65,104]. Introducing ureolytic bacteria into concrete could improve the compressive and tensile strength, which many researchers have widely evidenced; water absorption and chloride permeability were also reduced significantly by using bacteria [65,94,97–103]. There is a lack of freeze-thaw resistance research on bacterial concrete, which may be because the freeze-thaw resistance needs long-term and continually observing and recording. However, in many regions, temperature varies above and below 0 °C throughout the year. In these regions, concrete is susceptible to the damage caused by freeze-thaw cycles [105,106], adversely affecting the safety, serviceability, and durability of concrete structures [107]. Traditional methods for improving freeze-thaw resistance, such as reducing the water-cement ratio and increasing the usage of chemical admixtures (air-entraining and plasticising agents), are merely palliative methods. These methods may increase the utilisation of cementitious materials with chemical substances; consequently, the requirement of sustainable development cannot be satisfied. Moreover, the introduction of organic substances (air-entraining agent) may also reduce concrete strength [108]. Therefore, research on improving the freeze-thaw resistance of concrete using the biomineralisation method is also indispensable.

Table 1 Studies of applying ureolytic bacteria to enhance the performance of concrete.

Bacteria strain	The optimum concentration (CFU/ml)	Compressive strength improvement	Tensile strength improvement	Water absorption reduction	Chloride permeability reduction	Year	Reference
Sporosarcina pasteurii	10^5	16.7%	N/A	17.4%	50.3%	2013	[101]
Bacillus subtilis	10^7 for water absorption, 10^5 for strength	27.0%	N/A	27.0%	N/A	2016	[98]
AKKR5	10^5	10.5%	N/A	23.1%	15.5%	2016	[100]
Bacillus Subtilis	10^5	27.4%	13.3%	23.5%	N/A	2017	[99]
Bacillus megaterium	10^7	30.2%	N/A	22.0%	N/A	2018	[65]
Bacillus megaterium	3×10^6	24.0%	N/A	N/A	N/A	2018	[95]
Soil leaching solution	OD600 = 0·898	25.0%	11.0%	14.0%	N/A	2019	[102]
Sporosarcina pasteurii	10^7	N/A	N/A	N/A	37.0%	2019	[103]
Bacillus sphaericus	10^5	9.5%	9.6%	N/A	N/A	2019	[93]
Sporosarcina pasteurii	10^5	26.6%	34.4%	12.7%	N/A	2019	[97]
Bacillus megaterium	10^7	22.6%	18.3%	N/A	N/A	2019	[96]
Bacillus subtilis	10^5	32.0%	14.0%	N/A	N/A	2020	[84]
Bacillus subtilis	10^7	27.0%	N/A	N/A	39.9%	2020	[94]

With further research on ureolytic bacterial concrete, several researchers [58,59,109] have pointed out that ammonia (NH_3) were generated inevitably during the urea hydrolysis, which may pollute the environment. Urea is not an environmentally friendly material [60,110], and the UK limited the production and usage of urea

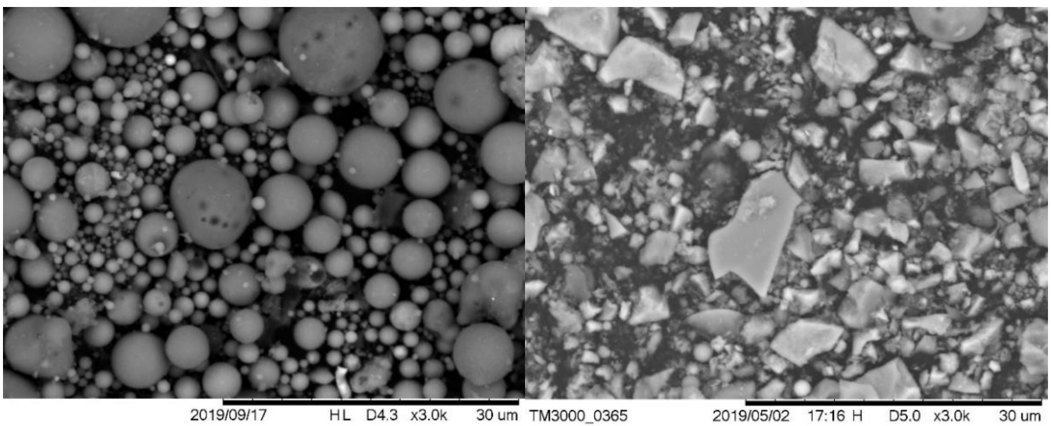
[111,112]. Therefore, it is necessary to find an alternative metabolic pathway to advance the biomineralization method for enhancing concrete. According to Eq. 10 ~ Eq. 14, the denitrification process does not create environmentally harmful by-products. The nutrition and calcium sources of denitrification included calcium formate ($Ca(HCOO)_2$) and calcium nitrate ($Ca(NO_3)_2$), which are common commercial concrete admixtures [36,113–115].

Furthermore, nitrite ions were created during the denitrification process, and this method also inhibited steel corrosion [60]. In addition, denitrification bacteria have higher viability in an oxygen-limited environment than ureolytic bacteria [52,98], which implies that these bacteria can maintain activity deep inside concrete when oxygen is depleted. More importantly, relative high PH is not a challenge for denitrification processes [116]; microbiological research [116,117] pointed out that denitrification bacteria can use up most nitrate ions when PH is approximately 10, which gives the stronger viability in an alkaline environment. Therefore, denitrification should be the more suitable metabolic pathway that can be utilized to induce biomineralisation in concrete. Despite these potential benefits of denitrification bacteria, previous research nevertheless mainly focused on using ureolytic bacteria, with few researchers studying denitrification bacteria in concrete. Except for Ersan et al. [60,109,118],few researchers studied denitrification bacteria in concrete. They applied [119] *Diaphorobacter nitroreducens* in concrete and obtained a maximum 21.1% increase in compressive strength; the biomineralisation was driven by nitrate ions reduction instead of urea decomposition. Other properties, such as tensile strength, elastic modulus, water absorption, durability, and sustainability, of concrete amended by denitrification bacteria, however, have not been explored.

Section 2.3 Fly Ash concrete

FA is the ash waste generated in coal power plants and is mainly composited with aluminosilicate compounds [120,121]. Replacing cement with FA increases the workability, long-term strength, and durability of concrete [37,38]. These improvements are caused by the reason that introducing FA can increase the volume of fresh cement slurry and the slump of fresh concrete by reducing the viscosity of the cement pastes [122] due to the smooth shape and low fineness of FA particles compared with OPC [14], as Figure 3 shows, and the high workability made the concrete easier to the full filled the moulds contributing higher compaction [123]. FA can react with $Ca(OH)_2$ created by hydration of cement and gradually promote the formation of secondary hydration substances in the long term [124]. In addition, replacing OPC with FA decreases cement usage and avoids the end-of-life disposal cost of FA, which significantly benefits the environment and economy [39,46]. Seto et al. [46] compared the sustainability of FA concrete with ordinary concrete by applying LCA analysis; they simulated various scenarios and presented that the FA can decrease up to 43% of environmental impact (based on the Global Warming Potential Index).

On the other hand, the pozzolanic reaction of FA is slow, which accounts for the low early age strength of FA concrete [38,125–127]. Kocak and Nas reported that, compared with their control mortar, the compressive strength of specimens with 25% of FA by mass decreased 26%, 21%, 18% and 18% at 2, 7, 28, 56 and 90 days, respectively, and showed that the strength gap between FA concrete and OPC mortar was decreased with the increase of age. Nevertheless, FA concrete might not reach the same strength level as OPC concrete without any remedy methods, even after one year [128]. Hence, some chemical agents such as sodium/potassium silicate (Na_2/K_2SiO_3), sodium/potassium hydroxide (Na/KOH) and calcium/sodium sulphate (Ca/Na_2SO_4) have been used to activate the FA [129–131]. In addition, Hosan and Shaikh [45] found that introducing 1% of nano-$CaCO_3$ increased the compressive strength of concretes with FA by 28%, which led to the compressive strength of concrete with FA reaching the same level as the OPC concrete. Applying FA in concrete can decrease the heat of hydration [132,133] and alleviate the alkalinity of the cement matrix [134], which implies that the introduction of FA may alleviate the injury of bacteria caused by the hydration heating and alkaline environment. Chahal et al. [101] found that 10^5 cells/ml of *Sporoscarcina pasteurii* in concrete with 10% of FA led to a 20% increase in compressive strength, while for concrete without FA, the improvement was 16.67%. By applying the biomineralisation method, the mechanical performance of FA concrete can achieve the same level as normal concrete. Jagannathan et al. [36] reported that concrete with 10% FA and *Bacillus Sphaericus* is superior to concrete without FA or bacteria regarding compressive, tensile splitting and flexure strength at 28 days. Kadapure et al. [97] presented that concrete with 30% of FA can also achieve equal strength with normal concrete after enhancing by *Sporoscarcina pasteurii* bacteria.



(a) (b)

Figure 3. SEM photos of FA (a) and OPC (b).

Section 2.4 Recycled coarse aggregate concrete (RCAC)

Section 2.4.1 Properties of RCA

RCA usually contains concrete products, unbound aggregate, clay masonry units, bituminous materials and other materials [135]. Old concrete particles generally occupy the highest ratio of the total amount of RCA [136,137]. As shown in Figure 4, RCA particles consist of original aggregate and old cement matrix and an old ITZ between them [138]. The old cement matrix with cracks and loose structure results in the rough texture and irregular shape of RCA [138]. The amount of the old cement matrix depends on the performance of the raw concrete, the crushing process, and the particle size of the RCA. There is always a reciprocal relationship between this adhering old cement matrix and the properties of the RCA [138]. Compared with the NCA, RCA shows lower bulk density, higher water absorption, higher porosity and higher crushing value [139,140]. These properties of RCA showed considerable influence on the mechanical performance and durability of RCAC [139].

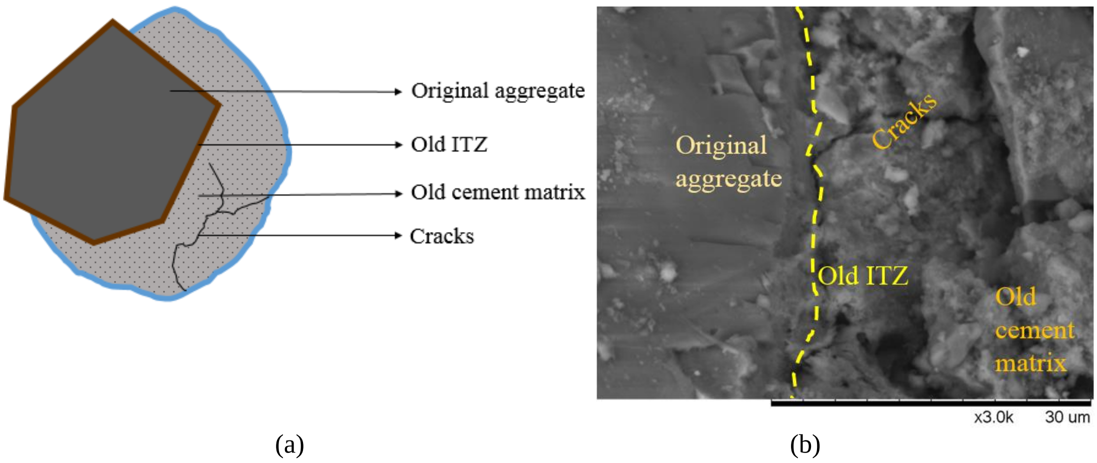


Figure 4. Sketch (a) and SEM photo (b) of RCA.

Section 2.4.2 Water absorbed by RCA in mixing RCAC

Due to the complexity of RCA, the producing process shows more influence on the performance of RCAC compared with NC. The water absorption of RCA ranges from 5% to 15% [14,141,142]. Hence, it is necessary to consider the absorbed water, especially before designing RCAC. Firstly, when designing the concrete composition, the amount of aggregate is considered in saturated surface dry (SSD) mass, air-dried condition (AD) mass, or oven-dried (OD) mass resulting in diverse results. However, if the RCA were not in SSD condition, mixing water would be absorbed during concrete mixing, thus resulting in variation of "effective water-cement ratio." When the water-cement ratio was high, reducing the ratio increased the strength; however, when the water-cement ratio was low, the water absorbed by RCA resulted in decreasing in fresh RCAC fluidity, which was adverse to the bond between the recycled aggregate and the cement paste; thus its compressive strength was decreased [143]. Hence, when designing mix composition, using SSD mass shows its superiority in avoiding the variation of water-cement ratio caused by water absorption of RCA.

In addition, the initial moisture of RCA also considerably influenced the properties of RCAC. In other words, the method of introducing extra water that was used to saturate the aggregate during fresh concrete mixing also showed significant effects on the test results [144–146]. Brand et al. [145] compared to concrete with OD aggregate and SSD aggregate and presented that for RCAC, the compressive strength and slump of the OD aggregate group decreased by 14.1% and increased by 130.7% compared to the SSD aggregate group. At the same time, for NC, no noticeable variation was detected. This result was because the incomplete absorption of the extra water increased the total water-cement ratio [146]. Poon et al. [144] also reported that the initial variation of moisture influenced the performance through the same mixing design. Therefore, when conducting the fresh RCAC mixing, it is necessary to control the variation of initial moisture of RCA.

These properties of aggregates, such as grading, moisture, water absorption, shape, and texture, influenced the workability of concrete. As discussed above, due to different considerations of measuring mass (OD mass, AD mass and SSD mass) in mixing design and initial moisture of RCA, there is a disagreement on the influence of RCA on workability. When considering SSD mass in mixing design, both Poon et al. [144] and Brand et al. [145] argued that the workability showed a negative relationship with the initial moisture; however, Brand et al. [145] suggested that the workability of RCAC was always higher than that of NC, in contrast, Poon et al. [144] proposed that if the initial moisture of aggregate reached SSD state, the workability of RCAC was lower than that of NC. Kisku et al. [147] reported that the OD RCA led to higher initial workability but loss more quickly than AD and SSD RCA due to the water being absorbed continually by RCA.

Section 2.4.3 Mechanical properties of RCAC

The mechanical properties of RCAC have long been the subject of criticism by researchers due to its double ITZ layers [107]. As Figure 5 depicts, the new hydration products are poorly connected to the surface of the old cement matrix resulting in a new ITZ in RCAC, which is regarded as the weakest region [148,149]. The porosity increases considerably because of the loose structure and cracks of adhered mortar on RCA [150]. The high porosity of RCAC and its double ITZs contributed to the significant difference from NC in terms of mechanical performance and durability in previous studies [136,148,151].

The compressive strength is the most important property of concrete. Due to the variation in the quality of the RCA and water-cement/binder ratio, the compressive strength of RCAC varied considerably in different research. Several researchers claimed that replacing NCA with RCA showed limited influence on compressive strength, even slightly increasing the compressive strength of concrete [152–154].

However, most researchers [144,155–159] presented that RCAC was considerably less than the compressive strength of NC. The anomalous results may be because the water absorbed by RCA was ignored [152], or the grading of RCA was much better than NCA [153]. As shown in Figure 6, there is a general descending tendency with the increased replacement ratio and compressive strength [144,153,155–159]. It was found that when the replacement rate of RCA is below 30%, the effect on the compressive strength of concrete is slight; however, when the ratio reaches 100%, the compressive strength is reduced significantly. Wang et al. [160] summarized that if 100% of NCA was replaced with RCA, the decline in the compressive strength, elastic modulus, flexural strength, and the splitting tensile strength might reach approximately 40%, 40%, 20%, and 40% respectively.

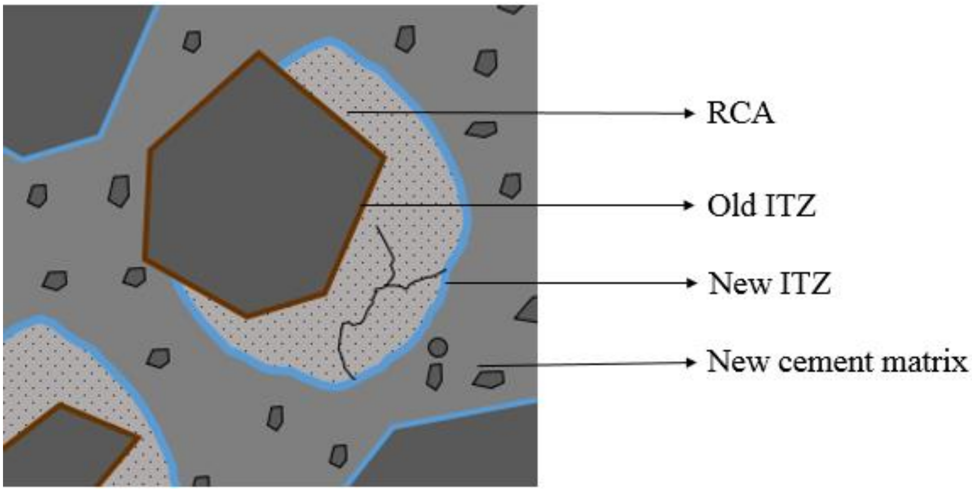


Figure 5. Sketch of a section of RCAC.

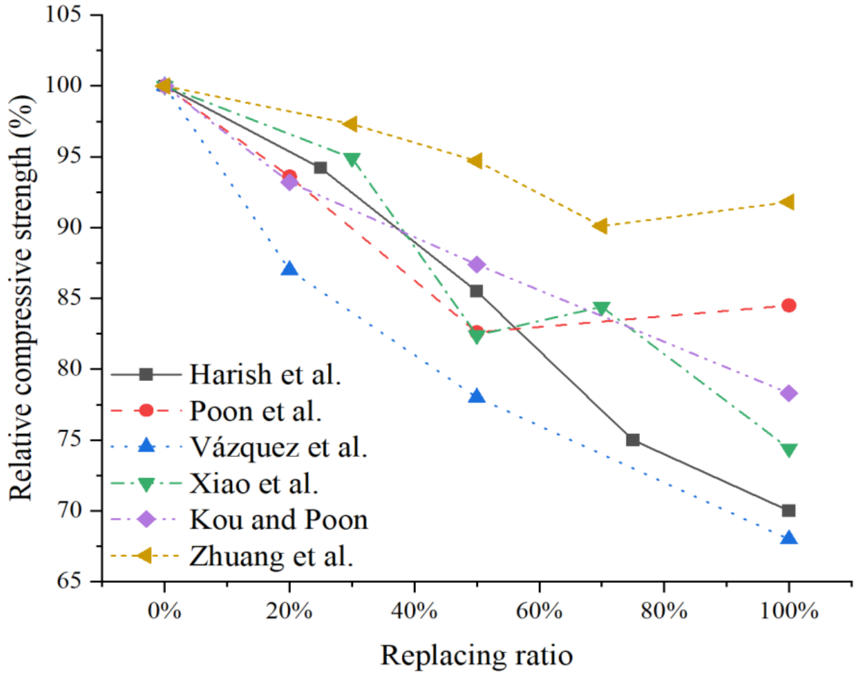


Figure 6. Relative compressive strength of RCA with different replacing ratios in various research.

Section 2.4.4 Durability of RCAC

Due to the loose structure of RCAC, foreign substances, such as oxygen, water and chloride ion, pass through pores more easily and remain inside RCAC compared to NC [147]. The permeability of these foreign substances usually proportionally increases with the increase of the replacing ratio of RCA, water/cement ratio and age [161–163]. With the accumulation of these substances, RCAC is, therefore, more susceptible to the reduction of durability such as water penetration [164], chloride ion penetration [140,156] and freeze-thaw cycles [154,165–173].

Thomas et al. [162] reported that after replacing 100% NCA with RCA, the water permeability at 28 days was almost doubled. The gap declined with the increase of age and decrease in the water-cement ratio. Applying RCA into concrete also negatively influences chloride ion penetration resistance. However, the deterioration of this resistance was not as much as that of water permeability [140,147,163]. The significant increase in the water permeability can cause much more amount of water to enter RCAC compared to ordinary concrete. Hence, in frost areas, the freeze-thaw resistance of RCAC is suspected to be de deteriorated. Suffering freeze-thaw cycles contribute to damage to the durability and strength of concrete. The damage degree is detected by measuring the mass change and dynamic modulus of elasticity change. Introducing RCA aggravated this damage due to the high water absorption and low strength [154,165–173]. Luan et al. [168] found that, compared with NC, the loss rate of relative dynamic modulus of elasticity (RDME) and compressive strength of RCAC increased by 25% and 20%, respectively, after only six freeze-thaw cycles. Kazmi et al. [167] presented that the weight loss rate increased from 3% to 5%, and the RDME loss rate increased from 50% to 65% after 66 freeze-thaw cycles if the NCA was 100% replaced by RCA. Gokce et al. [170] reported that the RDME of concrete with 12.5%, 25%, 50% and 100% weight of RCA reduced to less than 45% of the initial value after being subjected to 210, 180, 180 and 90 freeze-thaw cycles. In comparison, the RDME of the NCA concrete still maintained over 90% of the initial value even after 500 freeze-thaw cycles.

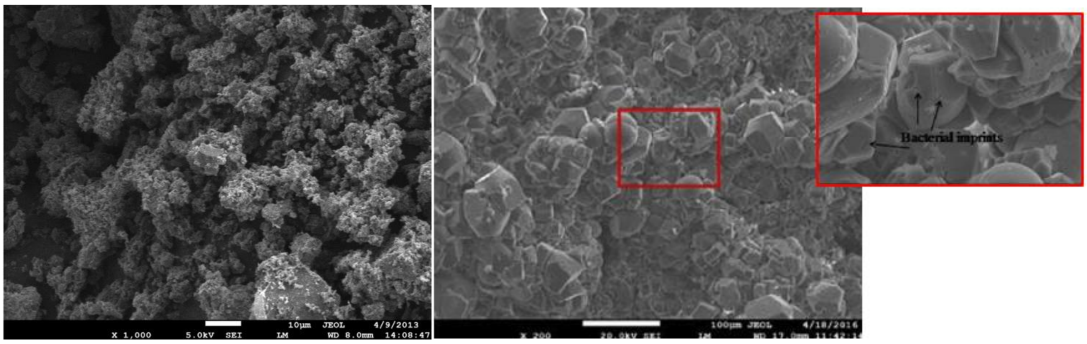
Section 2.4.5 Enhancing the properties of RCA and RCAC

As reviewed above, RCAC shows weakness in mechanical performance and durability. The critical factor that caused the poor property of RA is that the old mortar adhered to the original aggregate, and a weak ITZ is usually formed between them [174–178]. Peeling the adhered mortar was one method to solve this defect. Li et al. [179] applied the calcination-ball milling method to remove the adhered mortar and reduce water absorption. Wang et al. [73] applied mechanical rubbing on RCA that had been soaked in acetic acid solution to obtain RCA with a less adhered mortar and lower water absorption. In addition, heating [180], ultrasonic [174] and microwave [177] have been separately applied to remove the adhered mortar, and the proposed methods were shown to be effective in decreasing the water absorption and crushing value. However, all these peeling methods required a complicated process, expensive equipment, and high energy consumption, contrary to the primary objective—sustainability.

Furthermore, the peeling process may commonly cause internal damage to RCA [181]. Coating the RA was another method to improve its properties. Zhang et al. [176] utilized sulphoaluminate cement slurry to coat the surface of RA, which contributed to a denser microstructure of ITZ. Kong et al. [178] improved RA by coating FA and fine-grounded slag on its surface and found that RAC with treated RA showed a considerable improvement in compressive strength and durability. In addition, using microbial carbonate precipitation is also an effective way to coat and improve RCA. Since the discovery of the biomineralisation phenomenon [20], applying bio-method in the construction area has attracted growing interest.

The biomineralisation method was also applied to enhance RA. Bacteria can enhance RCAC in two ways. One is pre-treating the RCA particles with the bacterial solution to improve its properties; the other is mixing bacterial solution into fresh concrete to strengthen the RCAC. The pre-treating RCA method is usually achieved by soaking RCA in a bacterial solution or spraying the bacterial solution on the RCA particles and curing them at suitable temperatures and humidity. Researchers attempted this method by using ureolytic bacteria, as Table 2 summarises. According to the previous research [141,148,182–185], the bacterial solution included bacteria strains, nutrients, such as urea, yeast and whey, and calcium ions; and the optimum PH of the bacterial solution was found to be 9.5 [141]. The treatment time ranged from one day to twenty-one days. During the treatment period, the bacterial solution is absorbed by RCA. As shown in Figure 7, CC crystals are induced by bacteria filling and filling the loose space of the old cement matrix, resulting in an increasingly compact RCA surface. These crystals exhibit high cohesion and a strong bond with the RCA particles, which significantly improves the resistance to fragmentation [183]. After the treatment, the properties of RCA and RCAC with these treated RCA are improved. In addition, some bacteria still survived in the RCA; biomineralisation further occurred in concrete and further enhanced concrete [183]. In the new ITZ in particular, as Wu et al. detected [184] in Figure 8, CC offered a nucleation site for hydration products and remedied the weakest place, thus contributing to a dense connection between aggregate and matrix. Skevi et al. [186] also presented that the bacteria accelerated the formation of hydration products connecting the voids and cracks and improving the cohesion of the matrix.

The theory of mixing bacterial solution into fresh RCAC is the same as in Section 2.2. Sahoo et al. [187] directly mixed 10^6 cell/ml *Bacillus subtilis* into fresh RCAC and observed a 20% improvement in compressive strength.



(a) (b)

Figure 7. SEM photos of the surface of untreated RCA (a) [141] and treated RCA (b) [183].

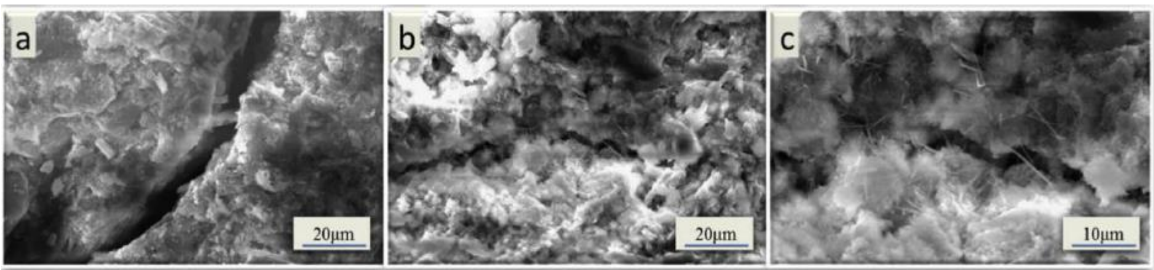


Figure 8. SEM images of the ITZ in (a) commonly recycled mortar; and (b,c) recycled mortar prepared with B-RCA [184].

Table 2 Water absorption improvement effort of RA experimented by different researchers.

Bacteria strains	The optimum concentration (CFU/ml)	Treating time (days)	Properties of aggregate			Concrete performance		Reference
			Density improvement	Water absorption decrease	Crushing value improvement	Compressive strength improvement	Water absorption decrease	
Bacillus pasteurii	$9{,}0 \times 10^6$	5	N/A	14.5%	N/A	Equal to corresponding NC	N/A	Grabiec et al. [182]
Sporosarcina pasteurii	10^8	3	1.0%	15.0%	N/A	N/A	N/A	Qiu et al. [141]
Bacillus sphaericus	3×10^8	1	2.0%	10.0%	N/A	40.0%	27.0%	Wang et al. [183]
Bacillus sphaericus	10^8	4	16.0%	18.0%	N/A	N/A	N/A	Julia et al. [188]
Bacillus pseudofirmus	10^7	20	5.0%	23.0%	12.0%	35.5%	34.9%	Wu et al. [184]
H4 bacillus	10^7	20	N/A	28.3%	10.8%	20.2%	N/A	Wu et al. [148]
Bacillus megaterium	10^5	21	0.0%	10.4%	9.5%	28.5%	N/A	Liu et al. [185]

Section 2.5 Life cycle assessment (LCA) on concrete

Section 2.5.1 Definition of goal and scope

According to the reviews above, applying the biomineralisation method can improve the performance of concrete, which implies that a smaller amount of concrete is needed to reach the same construction requirement as before, thus resulting in less pollution. However, the production of bacteria and nutrients caused by this method may create an extra environmental problem. There is little research focus on bio-based concrete, which may be because this method used in concrete is relatively new. Hence it is necessary to fill this gap by analysing the EI of bio-based concrete. Likewise, whether recycled waste can increase the sustainability of concrete also needs to be quantitatively demonstrated. Using FA in concrete can increase the sustainability of concrete even considering the negative influence on its strength [37,189,190]. While for RCA, it is controversial. Schepper et al. [191] presented that RCA significantly reduces the GWP of 1 m^3/MPa concrete. However, Yazdanbakhsh et al.[192] presented that RCA decreases the sustainability of concrete due to the low performance.

In fact, whether the RCA can increase the sustainability of unit MPa concrete mainly depends on the performance of RCAC [193]. Opportunely, LCA is a tool for qualifying the EI of the whole products system [190,194]. Therefore, the goal of conducting LCA on concrete is to quantify the overall EI [50,190]. Though "cradle to grave" includes the entire life cycle, most of the literature [9,51,195] considered "cradle to gate" as the system boundary due to the following reasons: a) the maintaining stage of concrete is significantly uncertain [195,196], b) the EI created in maintenance, and end-of-life stages are negligible or insignificant [197,198] and c). the difference in EI among various concretes are similar [199]. Hence, when applying the LCA analysis on concrete, the scope should include the production of all raw materials used in the concrete mixing and the EI created by transporting all these materials from the raw materials production place to the concrete mixing place.

A simple way for the functional unit is to use the 1 t or 1 m^3 of concrete [10,136,200]. However, this consideration ignores the effect of the performance of concrete on the EI. It is obvious that applying recycled wastes can decrease the EI of a unit volume of concrete, but the performance may decrease; the biomineralisation method can improve the performance of concrete but producing bacteria and corresponding chemical agents may create extra EI. Hence comparing the EI of unit volume or mass of concrete with different performance is not persuasive. Thus, many researchers are using a specific structural element, such as a column, beam and slab, which can withstand a given loading in a given condition as the functional unit [190]. This consideration visibly reflects the different LCA outputs of concrete with various characters [190,201,202].

Section 2.5.2 Life cycle inventory (LCI)

A different group of EI categories may be chosen in various sectors. Global Warming Potential (GWP), Ionizing Radiation Potential (IRP), Particulate matters, as well as, Respiratory Inorganics (RI), Primary Energy Demand (PED) and Photochemical Ozone Formation Potential (POFP) are commonly chosen EI categories for the construction sector [51,203–206]. Initial data for LCA analysis can be collected from questionnaires, environmental reports, environmental product declarations and LCA databases [190]. After obtaining the data, attention should be paid to allocating them to different raw materials [10]. Allocation by mass, economic value, and no allocation (considered as recycled wastes) show significantly different results, and they have their advantages and disadvantages [10,202]. Allocated by mass is a sample, and the results are stable, but it neglects the economic factors; however, allocated by the economic method may create significant fluctuations in the outcome [10,50,202]. No allocation is only suitable for recycled waste; however, whether a material 100% belongs to recycled waste rather than a by-product usually lacks consensus [50,190]. Generally, in a world context influenced by trade offers and flows of goods, the economic allocation method is more appropriate than the other two methods because it considers the economic values, which is the most important engine force of the development, added to the main products and by-products [202]. Hence, this project prefers the economic allocation method.

Section 2.5.3 Life cycle impact assessment (LCIA)

According to ISO 14040:2006 [48] and ISO 14044:2006 [49]: LCIA assesses the results of the potential EI of each base flow identified in the LCI and characterizes the indicator of each EI category. Sometimes, this phase also needs to normalise the results in different EI categories to make it possible to compare severity among different EI categories.

Section 2.5.4 Interpretation

Interpretation includes a) identifying significant issues based on the results of the LCI and LCIA phases; b) assessing the uncertainties, sensitivities and consistency checks; and c) making conclusions and giving suggestions and guidelines to construction projects [190].

Section 2.6 Summary

Many researchers have introduced ureolytic bacteria in concrete. The biomineralisation method has obtained a considerable improvement in mechanical performance and the durability of concrete, water absorption/permeability and chloride penetration resistance. However, most researchers have either not resolved or simply neglected the problems caused by the usage of urea and the creation of ammonia; in addition, the influence of freeze-thaw resistance has hardly been explored. Denitrification bacteria can induce biomineralisation and only release nitrogen (N_2). However, the research on biomineralisation induced by denitrification bacteria is limited in the biological research area. There seems to be a lack of systematic research on the influence of denitrification bacteria on the performance of concrete.

In addition, to increase the sustainability of concrete, some recycled wastes are applied to the concrete. However, these recycled wastes may be adverse to the performance of concrete, and the methods of remedying this are mainly limited to adding chemical agents or physical milling. Although some researchers have tried to adopt the biomineralisation method to improve concrete with recycled waste, these studies, nevertheless, are still preliminary, and only a limited number of specific properties have been researched. Therefore, systematic research, including mechanical performance, durability, and sustainability, on applying the biomineralisation method to improve the performance of concrete with some recycled waste is necessary.

Chapter 3. Methodology

Section 3.1 Materials preparation

Section 3.1.1 Bacteria strains selection, preservation, and cultivation

Three strains of ureolytic bacteria, including *Lysinibacillus fusiformis* (Lf) (MN097919), *Bacillus megaterium de Bary* (Bm) (ATCC 25300) and *Sporosarcina pasteurii* (SP) (ATCC 11859), and three strains of denitrification bacteria, including *Paracoccus denitrificans* (Pd) (ATCC19367), *Pseudomonas.Sp* (Psp) (ATCC13867) and *Pseudomonas fluorescens* (Pf) (ATCC35858) were used in this project. *Lf* was isolated from sludge by East China Normal University. Bm, Sp, Pd, Psp and Pf were imported from the American Type Culture Collection (ATCC) and subcultured by bio-company. All bacteria ranked biosafety level 1, which means that the bacteria used for the experimental studies are a wide range of microbial substances with all known properties and proven to be non-disease causing.

The procedure of bacterial preparation is shown in Figure 9. The bacteria purchased from bio-company was in freeze-dried powders status and needed to recover in a liquid medium, consisting of "Lab-Lemco" powder (1 g/L), yeast extract (2 g/L), peptone (5 g/L), NaCl (10 g/L) and distilled water. After the liquid medium became turbid, the bacteria were inoculated and cultivated in a solid medium which was convenient to subculture and could keep the activity of bacteria for approximately six months. Bacteria in solid mediums were kept in a room at 4 °C. When bacteria were needed in the experiment, bacteria were inoculated in the liquate medium and conical flasks and incubated in a shaker incubator at 30 °C until they arrived at the required concentration. Two kinds of liquid culture medium were used: one without urea and the other with urea. The non-urea medium was the same as the liquid medium used for bacteria recovery; all strains were cultured in this medium. For the urea medium, peptone (5 g/L) was substituted by filter-sterilised urea (20 g/L). The concentration of the bacterial solution was detected by observing the optical density (OD) value at 600 nm. Once the OD value reached 1, the bacteria cells were directly isolated from the medium by 15-minute centrifugation (6000 rpm, 20 °C). For ureolytic bacteria, to screen the bacteria with high urea utilizing ability, a part of the bacterial solution was removed to an empty liquid medium consisting of urea (OD_{600}=0). It was cultured until the OD_{600} arrived at 1 again. The same operation was taken to get the bacterial cells. These bacteria were labelled. Each gram of these centrifugated solid contained approximately 10^{12} bacterial cells. The isolated bacteria were stored in the plastic centrifuge tube and prepared for concrete mixing.

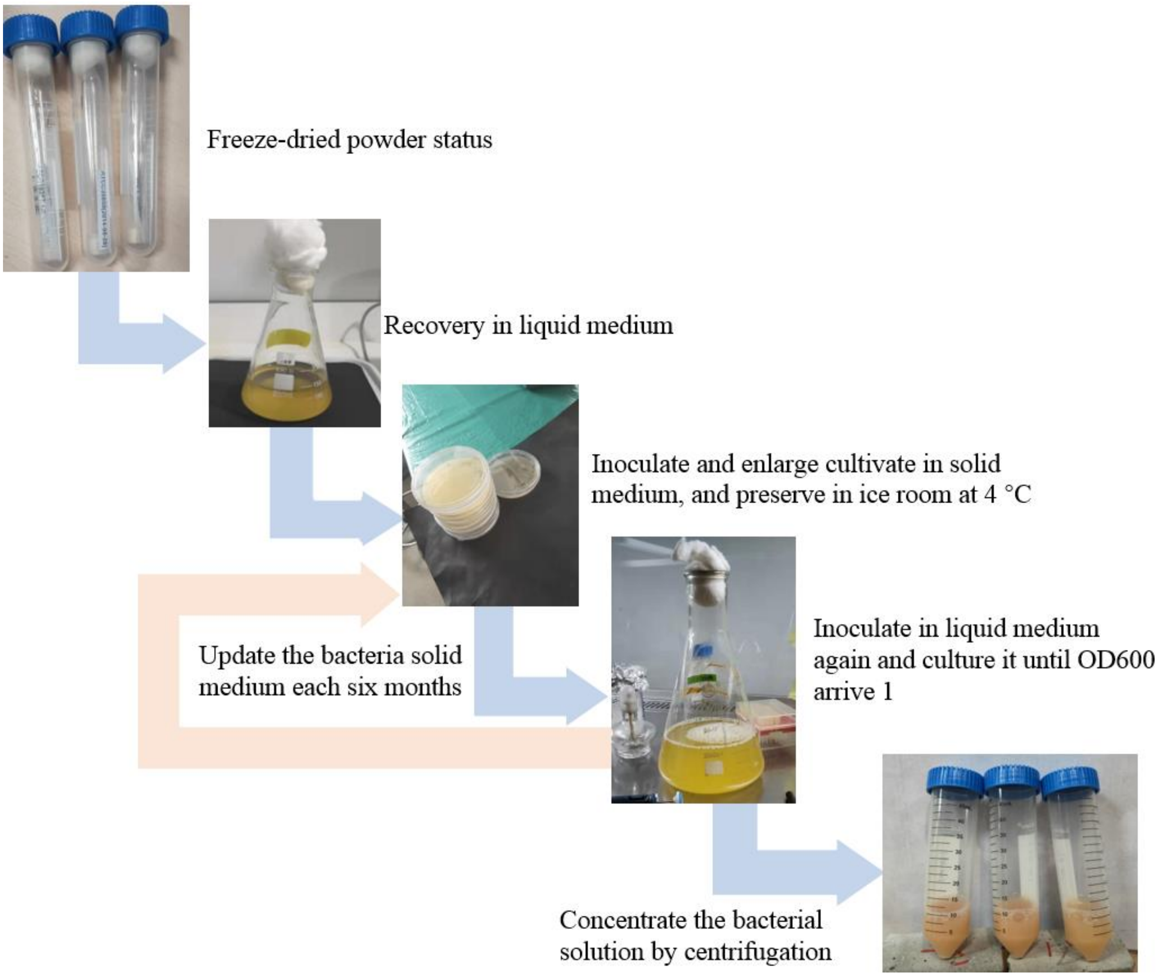


Figure 9. The procedure of bacterial preparation.

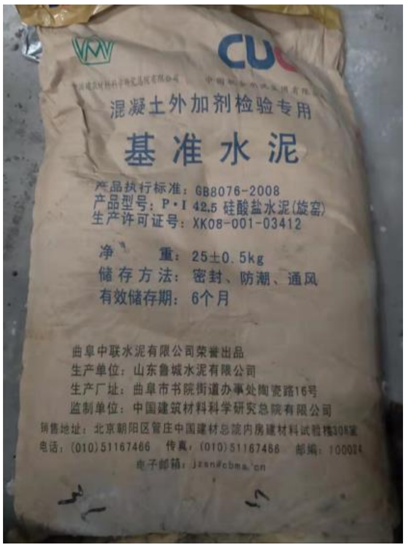
Section 3.1.2 Cementitious materials

Portland cement (P. I 42.5R) purchased from China United Cement Group (CUCG) was used in this research. FA was purchased from Wuhan Huashan Intelligent Technology. The chemical composition and photos of the cement and FA are shown in Table 3 and Figure 10, respectively.

Table 3 Chemical composition of cement and FA (%).

Composition	CaO	SiO_2	Al_2O_3	Fe_2O_3	MgO	SO_3	K_2O	Na_2O	LOI*
Cement (%)	55.32	25.44	7.06	2.89	2.25	2.77	0.67	0.49	2.23
FA (%)	0.98	62.40	23.70	3.19	0.55	0.24	1.38	0.63	4.00

*Loss on ignition



(a)

(b)

Figure 10 Photos of OPC (a) and FA (b) used in this project.

Section 3.1.3 Aggregates

Section 2.7.3.1 Natural coarse aggregate (NCA) and Natural fine aggregate (NFA)

Crushed gravel purchased from CUCG. Two kinds of particle sizes of NCA: 0 ~ 5 mm (NCA5) and 5 ~ 10 mm (NCA10), were received, respectively. River sands purchased from the local market in Suzhou, China, were chosen for NFA in this project. Their photo shows in Figure 11 particle size distribution is shown in Figure 13.

(a) (b) (c)

Figure 11 Photo of NFA (a), NCA5 (b) and NCA10 (c) used in this project

Section 2.7.3.2 Recycled coarse aggregate (RCA)

C&D wastes were collected from Suzhou, China. The constituents were measured according to BS EN933-11:2009 [135]. The photo of the RCA is shown in Figure 14. 20 kg of the RCA was sampled uniformly from the C&D wastes pile, and particles passing the 4 mm sieve were recorded and removed. The reminding sample was immersed in the water, and floating particles were recorded and removed. 5 kg of non-floating particles (approximately 1000 particles) were further analysed. The sampled particles were dried and separated into concrete, unbound aggregate, clay masonry units and bituminous materials, and the mass of each constituent was weighed. The constituent of RCA was obtained, as shown in Figure 12. Before experiments, the soil, plastic, mud, metal, and wooden particles were removed through soaking and washing processes. The washed RCA was dried in an oven. Because the mass of all aggregate in the mixing design was considered in SSD condition, extra water calculated by corresponding water absorption was added into the RCA before the concrete mixing. The particle size distribution of aggregate is shown in Figure 13. The gradation of concrete showed a non-negligible influence on the strength of concrete. Hence, 40% of NCA5 and 60% of NCA10 were mixed by mass. By this process, the particle distribution of the two aggregates was almost equivalent.

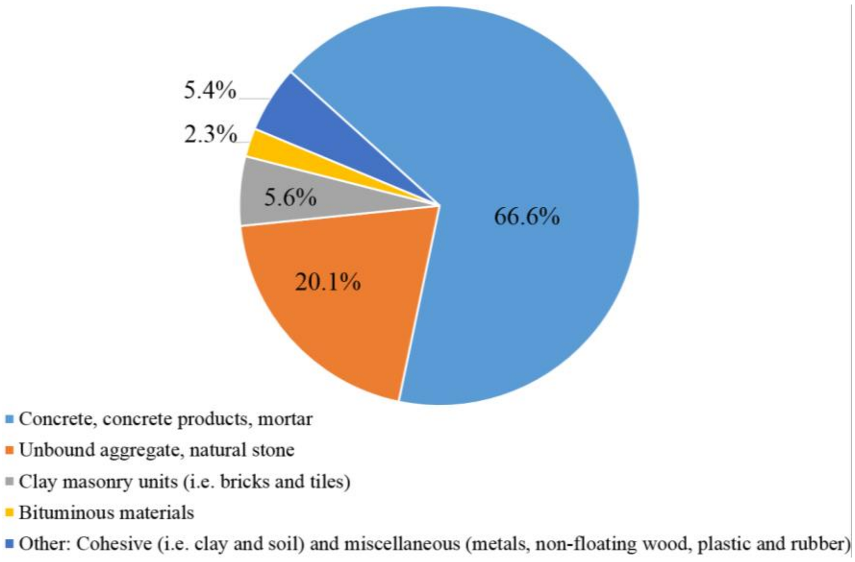


Figure 12. Constituent of RCA.

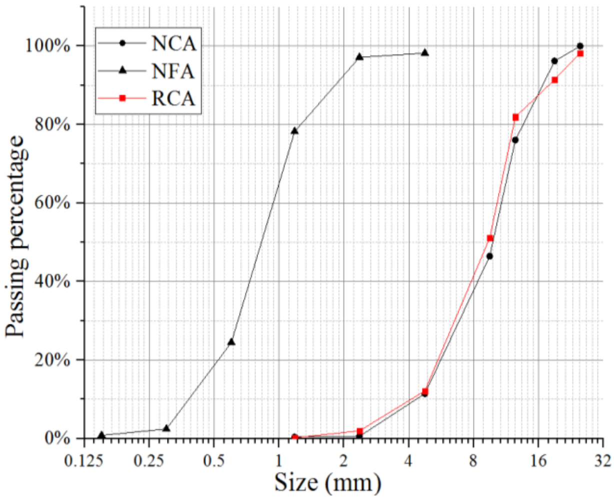


Figure 13. The particle size distribution of aggregate.

Figure 14 Photo RCA used in this project

Section 3.1.4 Mixing water and admixture agent

In this project, bacteria and their corresponding calcium source and nutrition were directly added to the mixing water and introduced into concrete with the mixing water. Tap water in the lab was used as the mixing water for concrete casting. The concentrated bacterial solution was dissolved into the mixing water until the concentration of bacteria arrived at the required value. Calcium nitrate ($Ca(NO_3)_2$) were selected as a calcium source for bacterial concrete after considering that organic calcium salts, such as calcium lactate and calcium gluconate, are much more expensive than inorganic calcium salts, and calcium chloride ($CaCl_2$) may introduce chloride ion in concrete. Urea and broth were used as the nutrition for ureolytic bacteria. According to Eq. 10 ~ Eq. 14, calcium formate ($Ca(HCOO)_2$) and $Ca(NO_3)_2$ provided both calcium ions and nutrition for denitrification bacteria. In addition, glucose was added, which could provide energy for bacteria and increase the activity of bacteria in a harsh environment.

Section 3.1.5 Mixing design

The mixing design of the control group mortar adopted the recommended composition in BS EN 196-1:2016 [207]: the mass ratio of cement, water, and sand are 2:1:3, and the target strength of this mortar is 35 MPa. The mixing design of the control group concrete was calculated according to the Design of normal concrete mixes [208] and shown in Table 4. The target strength of this concrete is 46 MPa. Moreover, some substitutions were made to the control mixing design in order to obtain the influence of FA, RCA, and bacteria on the properties of concrete, respectively. And those specific mixing designs are listed and elaborated in chapters 4, 5 and 6, respectively.

Table 4 Mixing designs of reference concrete

Material	Cement kg/m^3	Water kg/m^3	Fine aggregate kg/m^3	Coarse aggregate kg/m^3
RC	433.6	195.1	620.6	1152.5

Section 3.1.6 Mortar and concrete casting and curing

All the mortar specimens were cast according to BS EN 196-1:2016 [207]. Mortar specimens were cast using uniform moulds of 40 mm × 40 mm × 160 mm, as shown in Figure 15 (a). A mortar mixer (as shown in Figure 16 (a)) with a stainless steel bowl with a capacity of about 5 L and a stainless steel blade meeting the requirement of BS EN 196-1:2016 [207] was used for mortar mixing. Sands and cement were pre-mixed for the 60s, and the water with bacteria solution (if needed) was gradually added into the mixer for a 90 s mixing. Fresh mortar was removed from the moulds, and a 30 s variation was applied. The demoulding time was chosen 48 h due to the crispiness of the mortar specimen.

All the concrete specimens were cast according to BSI 1881-125:2013 [209]. A concrete mixer with a volume of 200 L meeting the requirements of BSI 1881-125:2013 [209] was used for concrete mixing. Before concrete mixing, the water content of each kind of aggregate was measured. According to the water absorption of each type of aggregate, extra water was added to the aggregates used for concrete mixing until the aggregates arrived in SSD condition. Concrete specimens were cast in various moulds with fresh concrete made in the lab. Fine and coarse aggregates were mixed up for 60 s at first, and cement and other cementitious materials (where needed) were pre-mixed in the concrete mixer for 60 s. After that, water with bacterial solution (if required) was added gradually and conducted another 90s mixing. Having mixed fresh concrete was removed from the mixer to their corresponding moulds, and a 120 s vibration was applied for compaction, as shown in Figure 15 (b). Cube specimens were used for compressive strength and tensile splitting strength, cylinder specimens were used for water permeability test, and prism specimens were used for freeze-thaw resistance test. And the testing detail is presented in the following section. After 24 h, specimens were demoulded. As shown in Figure 17, all specimens were cured in a chamber at 30 °C, and relative humidity was 95% until the testing began.

(a)

(b)

Figure 15. Mortar (a) and concrete (b) specimens in moulds.

(a) (b)

Figure 16. Mortar (a) and concrete (b) mixer.

Figure 17. Curing chamber.

Section 3.2 Testing method

Section 3.2.1 Properties of Aggregate test method

The water absorption and density test were carried out on aggregates according to BS EN 1097-6:2013 [210]. Aggregates soaked for 48 h and wiped in SSD station were weighted and filled in pyknometer, then poured water until the pyknometer was full and weighted. Then, the aggregates were taken out and oven-dried at 105±5°C for 24h.

At the same time, the mass pyknometer full filled with water was measured. The water absorption (%) and apparent density (kg/m³) were calculated according to Eq. 16 and Eq. 17. And according to the ISO/DIS 20290-3 [32], the crushing value of aggregate was tested. The aggregate with particle sizes from 10 mm to 14 mm, was uniformly filled in the steel mould three times, as shown in Figure 18. Then, the mould was inserted into the compression machine and loaded at a uniform increasing pressure with 40 kN/min for 10 mins and a stable 400kN pressure for 5 s. The crushed samples were taken out of the mould and screened by a 2.36mm sieve. Mass of sample passed the sieve were weighted, and the crushing value of RCA was acquired according to Eq. 18.

$$WA = \frac{100\times(M_4-M_1)}{M_4} \tag{16}$$

$$\rho_a = \rho_w \frac{M_4}{M_4-(M_2-M_3)} \tag{17}$$

Where WA is the water absorption (%), M_1 is the mass of the saturated and surface-dried aggregate in the air (g), M_2 is the mass of the pyknometer containing the sample of saturated aggregate and water (g), M_3 is the mass of the pyknometer filled with water only (g); M_4 is the mass of the oven-dried test portion in the air (g), ρ_a is apparent particle density (kg/m³), ρ_w is the density of water, at 20°C, taken as 998 kg/m³.

$$ACV = \frac{M_2}{M_1} \tag{18}$$

Where ACV is the total crushing value (%), M_1 is the mass of the test specimen; M_2 is the mass of the material passing the 2,36 mm test sieve.

(a) (b)

Figure 18. Crushing value test apparatus (a) and installation method (b).

Section 3.2.2 Slump test

Slump tests were carried out on fresh concrete, according to BS EN 12350-2:2009 [211]. The slump barrel was filled with fresh concrete three times after filling with a pounding hammer from the outside to the inside of the barrel wall evenly from 25 blows. Then the barrel was pulled up, and the height of the barrel (300mm) minus the height of the highest point of the concrete after the collapse was the value of slump.

Section 3.2.3 Compressive and flexure strength test of mortar specimens

The mortar tests were followed by BS EN 196-1:2016 [207]. The tools aided these two tests as shown in Figure 19, specimens were applied to the flexure test first, and specimens were fluxed into two parts which supplied samples for the compressive test. The flexure and compressive test loading speeds were 1mm/min and 0.5 MPa/s, respectively.

(a) (b)

Figure 19. Tools for testing the compressive strength (a) and flexure strength (b) of mortar.

Section 3.2.4 Compressive strength and tensile splitting strength tests

Three 150mm cubes were prepared for each type of test. Each mixing design contained three specimens. The compressive and tensile splitting strength testing follow BS EN 12390-3:2009 [212] and BS EN 12390-6:2009 [213], respectively. The set-up of tensile splitting is shown in Figure 20. The loading speeds of the compressive and tensile splitting strength test were 0.5 MPa/s and 0.05 MPa/s, respectively.

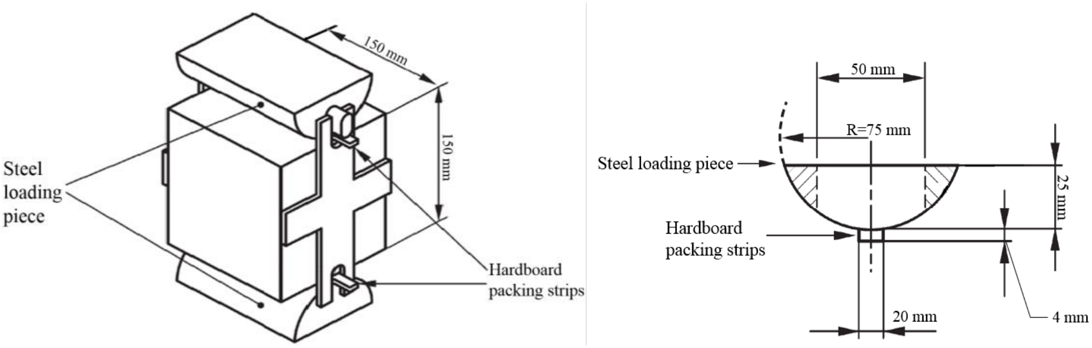


Figure 20. The set-up of tensile-splitting apparatus in this research [213].

Section 3.2.5 Elastic modulus of concrete test

Six prisms with a size of 100 mm × 100 mm × 300 mm were prepared for the elastic modulus test. The tests followed BS EN 12390-13:2013 [214]. Three prisms were used to test the prism compressive strength. The others, having been installed inducer, as shown in Figure 21, were placed in the testing machine, and a change loading shown in Figure 22 was applied. At the same time, the formation of the prism was recorded by the inducer. The elastic modulus ($E_{C,0}$) was calculated by Eq. 19.

Figure 21. Specimen with holder.

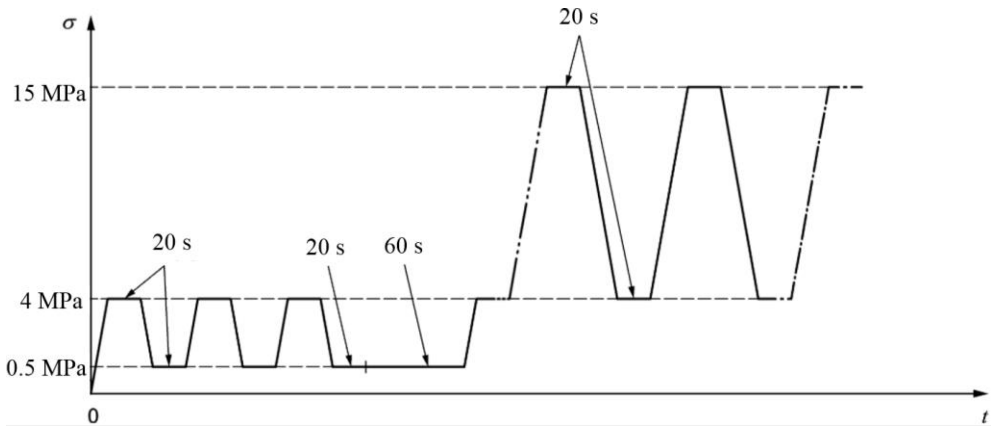


Figure 22. Loading process curve.

$$E_{C,0} = \frac{\Delta\sigma}{\Delta\varepsilon_0} \tag{19}$$

Where $\Delta\varepsilon_0$ is the strain difference during the loading cycle and $\Delta\sigma$ is the difference between measured stresses.

Section 3.2.6 Ultrasonic pulse velocity test

Ultrasonic pulse velocity test as a non-destructive detecting method has been verified to help evaluate the quality and performance of concrete [215,216]. The velocity showed a positive relationship with the compactness, compressive strength and elastic modulus [216,217]. Three 100mm×100mm×300mm prisms were prepared for each group to conduct UPV tests at 28 days. The test refers to BS EN 12504-4:2004 [218]. The transducer arrangement shows in Figure 23, and the frequency of the pulse generated by the transducer was 50 kHz in this research.

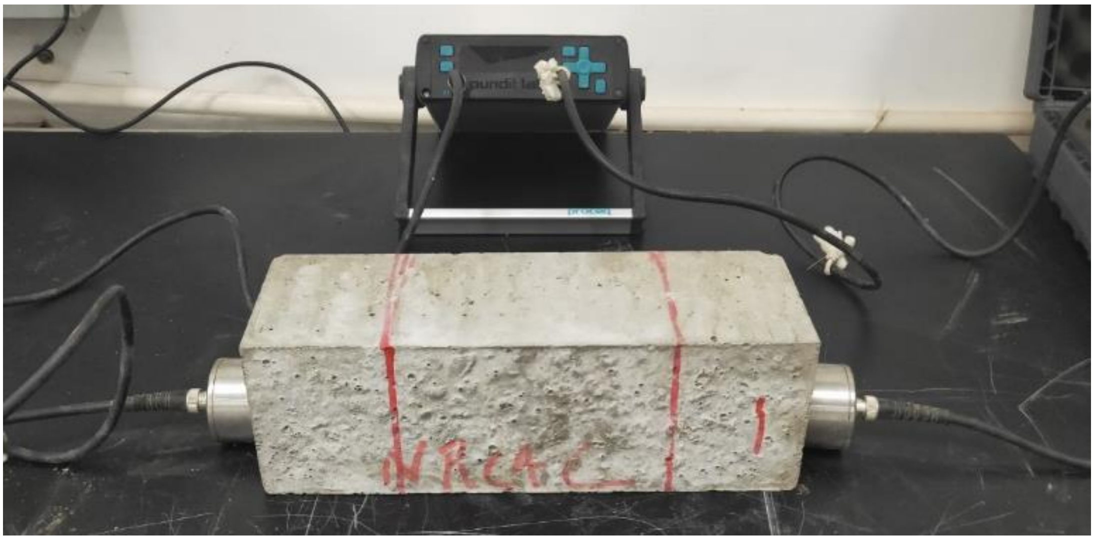


Figure 23. Apparatus and specimen setting of ultrasonic pulse velocity test.

Section 3.2.7 Depth of penetration of water under pressure

The specimens were tested according to BS EN 12390-8:2009 [219] and the test machine, as shown in Figure 24 (a). Each mixing design contained three specimens. Specimens were fixed on the steel moulds, and a 500 ± 50 kPa water pressure was applied to the specimens from the bottom for 72 h. After 72 h, the specimens were removed from the machine and split into two parts; the maximum depth of the penetration was marked and measured.

(a) (b)

Figure 24. Depth of penetration of water under pressure machine (a) and installation tools (b)

Section 3.2.8 Freeze-thaw resistance test

The modified machine is shown in Figure 25, which can meet the requirement of a fast freeze-thaw resistance test (GB/T 50082-2009 [220]). Three beam specimens of each mixing design with a dimension of 400 mm × 100 mm × 100 mm were prepared for a fast freeze-thaw resistance test. The freeze-thaw resistance was evaluated by the RDME (P) and mass loss (ΔW_{n}) according to Eq. 20 and Eq. 22, respectively. After each twenty-five freeze-thaw cycle, the value RDM and mass lost ratio were measured, respectively.

$$P = \frac{1}{3}\sum_{i=1}^{3} P_i \quad (20)$$

$$P_i = \frac{f_{ni}^2}{f_{\mathrm{oi}}^2} \times 100 \quad (21)$$

Where, P_i is the RDME after n cycles of freezing and thawing (%); f_{oi} is the fundamental transverse frequency before the freeze-thaw resistance test (Hz); f_{ni} is the fundamental transverse frequency after n freeze-thaw cycles (Hz).

$$\Delta W_{\mathrm{n}} = \frac{\sum_{i=1}^{3} \Delta W_{\mathrm{ni}}}{3} \times 100 \quad (22)$$

$$\Delta W_{\mathrm{ni}} = \frac{W_{oi} - W_{\mathrm{ni}}}{W_{0i}} \times 100 \quad (23)$$

Where, ΔW_{ni} is the mass loss (%) of the i-th concrete specimen after n freeze-thaw cycles, to 0.01; W_{0i} is the mass of the i-th concrete specimen before the freeze-thaw resistance test, in g; W_{ni} is the mass of the i-th concrete specimen after n freeze-thaw cycles, in g.

(a) (b)

Figure 25. Freeze-thaw resistance test machine (a) and ice chamber (b).

Section 3.2.9 Scanning Electron Microscope (SEM) Observation

TM3000 desktop electron microscope, as shown in Figure 26, was used to examine the microstructure of the samples. The dried coarse aggregate particles were inserted into the vacuum chamber of SEM. Due to size limitations, square samples 50 mm long and 5 mm thick were prepared for the observation. The SEM photos were taken with an acceleration voltage of 15 kV, and the magnification scales were 5000 times.

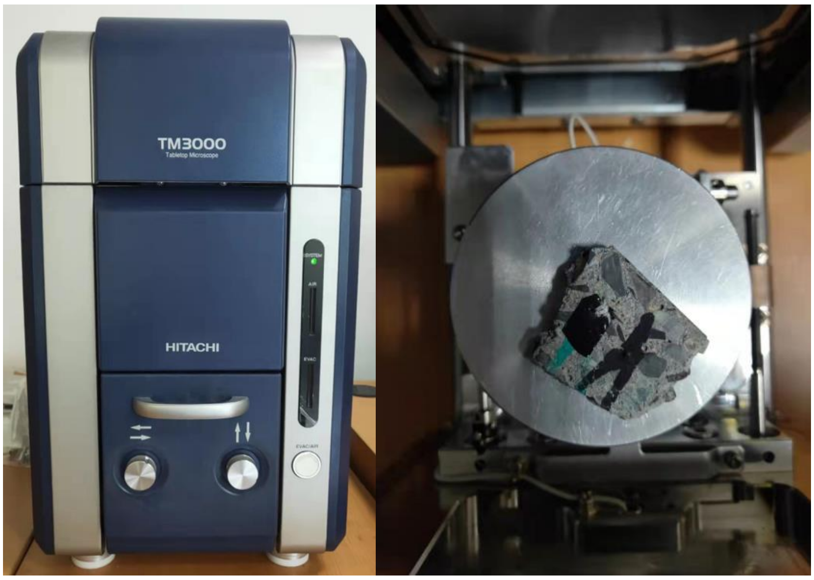


Figure 26. TM3000 desktop electron microscope.

Section 3.2.10 X-ray diffraction (XRD) analysis

Broken concrete fragments after finishing the 28-day compressive test were collected. A cement matrix without aggregate was peeled from the fragments and milled into powders. The powders passing the 0.1mm sieve were collected to perform XRD analysis. As shown in Figure 27, The X-ray Diffraction machine, D8 ADVANCE Plus, produced by Bruker Corp, was used in this research.

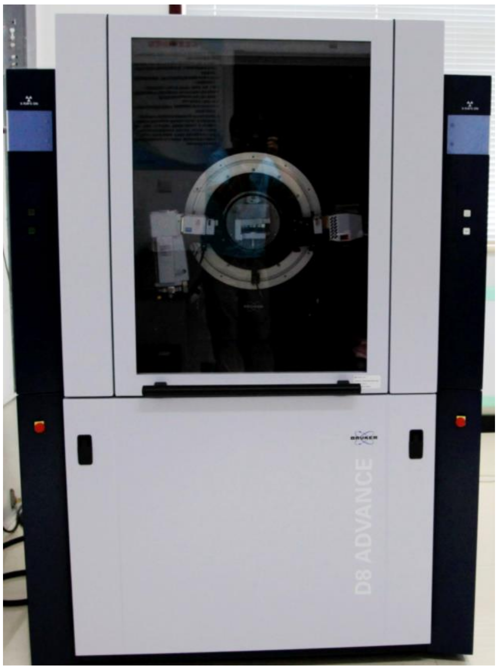


Figure 27. X-ray Diffraction machine, D8 ADVANCE Plus

Section 3.2.11 Thermogravimetric analysis (TGA)

A Netzsch STA 449 F3, a thermal Analysis Instrument for TGA, was used to detect the composition of hardened concrete such as calcium hydroxide (CH) and $CaCO_3$. The cement matrix of RCAC, BRCAC and BTRCAC were sampled and milled into powders. The sampling positions were the matrix next to the newly formed ITZ in the corresponding concrete, as shown in Figure 28. The samples were exposed to a continuous heating environment inside the instrument ranging from 20 °C to 900 °C with a 5 °C per minute heating rate in the N_2 atmosphere.

Figure 28. A Netzsch STA 449 F3, a thermal Analysis Instrument.

Chapter 4. Result and discussion of ureolytic bacterial recycled waste concrete

Section 4.1 Introduction

Ureolytic bacteria have been evidenced to be helpful for improving the performance of concrete. FA and RCA are also commonly used in concrete to enhance sustainability. This chapter elaborates on the application of ureolytic bacteria in concrete with FA and RCA. The influence of three strains of bacteria, including Lf, Bm and Sp, on the workability, water absorption and permeability, compressive strength, tensile splitting strength and microstructure of concrete were explored.

Section 4.2 FA concrete with ureolytic bacteria

Section 4.2.1 Mixing design

Reference concrete (RC) without any additives was used as the control group. NFC represented concrete, with 40% of cement replaced by FA. And NFC-NCa means nutrition and claim source were added in NFC. The mix design of bio-based FA concrete was designed by replacing 40% of cement with FA by mass. Three strains of bacteria at two concentration levels were introduced into concrete. The mix proportions for each group of specimens are shown in Table 5, where N means nutrition which includes nutrition broth and urea, and Ca means additional calcium source (calcium nitrate). Based on the experiment results above, three groups of bacteria cultured in a medium with urea were applied and named ULF7-NCa, UBM5-NCa and USP7-NCa and listed in Table 5. According to the test results of the first twelve groups, the more suitable concentration was chosen and used in another three groups, which were designed to explore the influence of nutrition and calcium nitrate, including LF7-W, BM5-W and SP-W, and listed in Table 5. According to compressive testing results of groups LF5-NCa, LF7-NCa, BM5-NCa, BM7-NCa, SP5-NCa and SP7-NCa, the concentration of the Lf and Sp bacterial solution was 10^7 CFU/mL, while for Bm bacteria, the concentration was 10^5 CFU/ml.

Table 5 Mixing designs of each group.

Label	Strain	Concentration	Broth	Urea	$Ca(NO_3)_2$	OPC	FA	NFA	NCA	Water
		CFU/ m^3	g/m^3	kg/m^3	kg/m^3	kg/m^3	kg/m^3	kg/m^3	kg/m^3	kg/m^3
RC	None	0	0.0	0.0	0.0	415.0	0.0	620.6	1152.5	195.1
NFC	None	0	0.0	0.0	0.0	249.0	166.0	620.6	1152.5	195.1
NFC-NCa	None	0	13.0	3.9	2.3	249.0	166.0	620.6	1152.5	195.1
LF5-NCa	*Lf*	2.0×10^{10}	13.0	3.9	2.3	249.0	166.0	620.6	1152.5	195.1
LF7-NCa	*Lf*	2.0×10^{12}	13.0	3.9	2.3	249.0	166.0	620.6	1152.5	195.1
BM5-NCa	*Bm*	2.0×10^{10}	13.0	3.9	2.3	249.0	166.0	620.6	1152.5	195.1
BM7-NCa	*Bm*	2.0×10^{12}	13.0	3.9	2.3	249.0	166.0	620.6	1152.5	195.1
SP5-NCa	*Bm*	2.0×10^{10}	13.0	3.9	2.3	249.0	166.0	620.6	1152.5	195.1
SP7-NCa	*Sp*	2.0×10^{12}	13.0	3.9	2.3	249.0	166.0	620.6	1152.5	195.1
ULF7-NCa	*ULf*	2.0×10^{12}	13.0	3.9	2.3	249.0	166.0	620.6	1152.5	195.1
UBM5-NCa	*UBm*	2.0×10^{10}	13.0	3.9	2.3	249.0	166.0	620.6	1152.5	195.1
USP7-NCa	*USp*	2.0×10^{12}	13.0	3.9	2.3	249.0	166.0	620.6	1152.5	195.1
ULF7-W	*Lf*	2.0×10^{12}	0.0	0.0	2.3	249.0	166.0	620.6	1152.5	195.1
UBM5-W	*Bm*	2.0×10^{10}	0.0	0.0	2.3	249.0	166.0	620.6	1152.5	195.1
USP7-W	*Sp*	2.0×10^{12}	0.0	0.0	2.3	249.0	166.0	620.6	1152.5	195.1

Section 4.2.2 The slump of fresh concrete

As previous research has concluded [121,221], FA was able to increase the workability of fresh concrete. This superiority also worked in bacterial FA concrete. Figure 29 compares the slump of reference concrete, FA concrete, FA concrete with 10^5 CFU/ml bacterial solution and FA concrete with 10^7 CFU/ml bacterial solution. All the groups with FA showed higher workability than the RC groups. In addition, groups with bacteria showed higher workability than NFC groups, and a higher concentration of bacteria showed a higher slump. These phenomena might cause by the surrounding capsular smoothing the bacterial cells and lubricating each fresh concrete composition [222]. Hence, the bacterial solution played the role of lubricant and increasing the concentration of bacteria caused an increasing improvement in the workability of fresh concrete. Therefore, with the common effecting of bacteria and FA, bacterial FA concrete, especially with the concentration of 10^7 CFU/ml, showed a considerable improvement in the workability of fresh concrete.

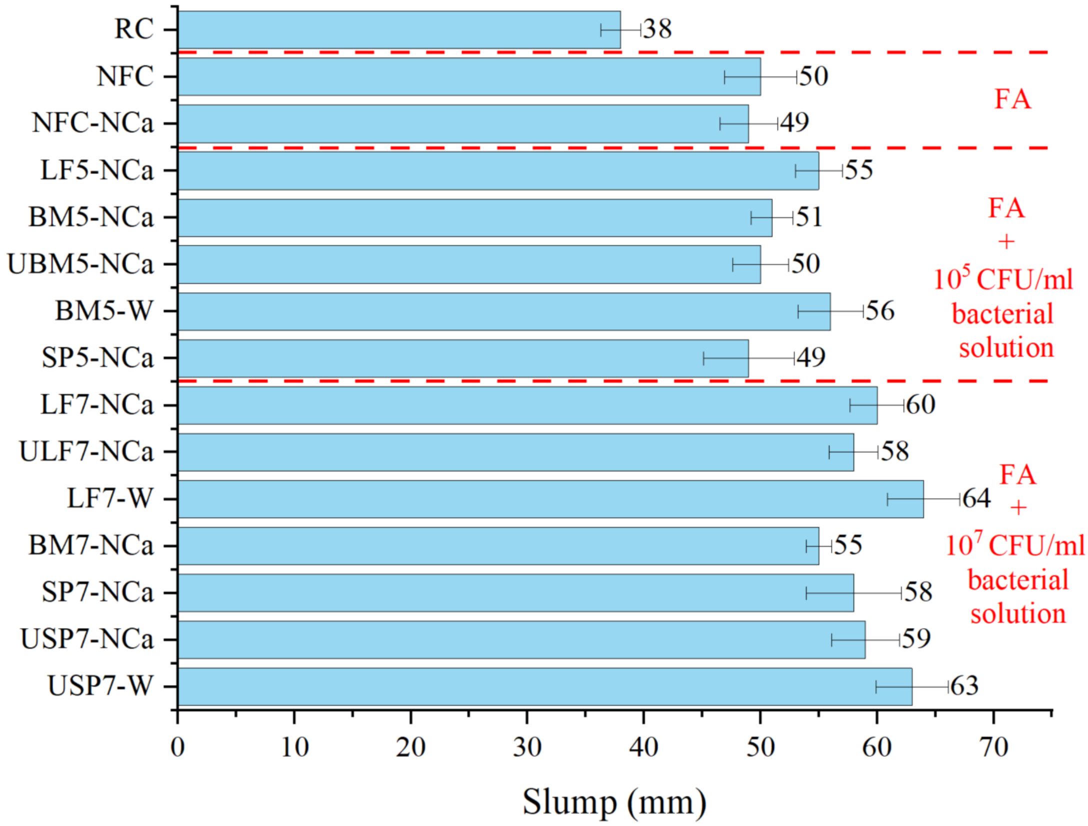


Figure 29. Slump of fresh concrete in each group.

Section 4.2.3 Compressive strength testing result

The compressive strength testing results are presented in Table 6. Generally, replacing cement with FA delayed strength development due to the low pozzolanic activity of FA [127,223]. Replacing 40% cement with FA decreased 32.7% and 18.9% of the 7 days’ and 28 days’ compressive strength respectively. By comparing FA concrete groups with and without nutrition (NFC and NFC-NCa groups), it can be found that introducing nutrition into concrete decreased the compressive strength by 9.2%, 6.5% and 4.8% after 7-day, 28-day and 56-day curing, respectively. In three tested ages, every bacterial group showed higher compressive strength than the NFC group. For ULF7-NCa, UBM5-NCa and USP7-NCa groups, compressive strength at 28 days and 56 days exceeded the values obtained from specimens in the RC group. The applied biomineralisation method increased up to 24.7% of compressive strength and fully compensated for the strength reduction of FA as cement supplementary material. It shall be noted that various effects may influence the effectiveness of biomineralisation, such as strain and concentration of bacteria and whether the culture medium consisted of urea.

The impact of concentration on the strength enhancement was different when using different bacteria strains shown in Figure 30. 10^7 CFU/ml concentration for Lf showed a slightly higher strength at 28 days (10.5%). Moreover, at 28 days and 56 days, the difference (1.8% and 3.1%) between these two concentrations was neglectable. The 10^5 CFU/ml concentration for Bm showed higher compressive strength at three tested ages. For Sp, 10^7 CFU/ml concentration showed higher compressive strength in all three ages. Ideally, all bacterial cells are supposed to become a calcium carbonate ball filling the pores and voids of concrete. However, a large number of bacteria were inactivated due to the harsh environment. The inactivated bacterial cells as an organic substance are adverse to the strength formation of concrete. Hence, bacteria with different characteristics and activities have different suitable concentrations.

Culturing medium showed considerable influence on strength enhancement. Figure 31 compares the differences in compressive strength between groups with urea medium and ordinary medium. Although The difference at 7 days compressive strength was neglectable, the compressive strength at 28 days and 56 days was quite different. Because, bacteria cultured in a medium consisting of urea showed a more apparent effect than that grown in an ordinary medium. Compared with the NFC group, the compressive strength of the ULF7-NCa, UBM5-Nca and USP7-Nca groups increased by 10.6%, 16.7%, 11.6%, 21.5%, 24.4%, and 24.7%, and 13.4% 22.4% and 23.0%, in 7 days, 28 days and 56 days, respectively. The compressive strength of the specimens was equivalent to the RC group after introducing bacteria culture in a urea medium. It is speculated that urea would become the leading energy supplier for the metabolism of bacteria after using up the added concrete. When cultured in a urea medium, bacteria with powerful ureolytic capacity were more natural to survive and reproduce. Therefore, bacteria cultured in urea were more adaptive to extensive biomineralisation in concrete.

In addition, the nutrition (urea) and additional calcium source (calcium nitrate) also played an essential role in improving concrete. Figure 32 compares the 28 days' compressive strength, with and without nutrition and additional calcium source. It was found that the improvement of compressive strength was limited without providing the nutrition and additional calcium source for the three strains. These three strains could not work efficiently unless both calcium ions and nutrition were supplied. However, if urea was applied, pungent gas was smelled, especially when the specimen collapsed, due to the metabolism of ureolytic bacteria.

Table 6 Compressive strength test results on 7 days, 28 days, and 56 days.

Label	Compressive strength (MPa)								
	7 days	STD	Change compare to NFC	28 days	STD	Change compare to NFC	56 days	STD	Change compare to NFC
RC	32.1	1.7	48.6%	38.5	3.2	23.4%	41.6	3.3	16.5%
NFC	21.6	1.3	0.0%	31.2	2.5	0.0%	35.7	2.3	0.0%
NFC-Nca	19.6	1.8	-9.3%	29.8	1.9	-4.5%	33.8	2.3	-5.3%
LF5-Nca	20.9	1.3	-3.2%	32.9	2.1	5.4%	34.6	1.9	-3.1%
LF7-Nca	23.1	1.0	6.9%	32.5	2.1	4.2%	35.7	2.4	0.0%
BM5-Nca	25.5	0.9	18.1%	34.3	2.5	9.9%	36.7	2.4	2.8%
BM7-Nca	23.3	1.7	7.9%	28.8	1.6	-7.7%	33.8	2.8	-5.3%
SP5-Nca	21.9	1.8	1.4%	29.8	1.3	-4.5%	37.6	1.1	5.3%
SP7-Nca	26.1	1.6	20.8%	35.4	2.9	13.5%	39.7	1.8	11.2%
ULF7-Nca	23.9	1.2	10.6%	37.9	2.2	21.5%	40.5	2.6	13.4%
UBM5-Nca	25.2	1.4	16.7%	38.8	2.1	24.4%	43.7	2.6	22.4%
USP7-Nca	24.1	0.7	11.6%	38.9	2.8	24.7%	43.9	1.8	23.0%
ULF7-W	19.9	1.1	-7.9%	32.3	2.1	3.5%	N/A	N/A	N/A
UBM5-W	21.9	1.2	1.4%	30.1	2.0	-3.5%	N/A	N/A	N/A
USP7-W	20.3	0.9	-6.0%	31.3	1.9	0.3%	N/A	N/A	N/A

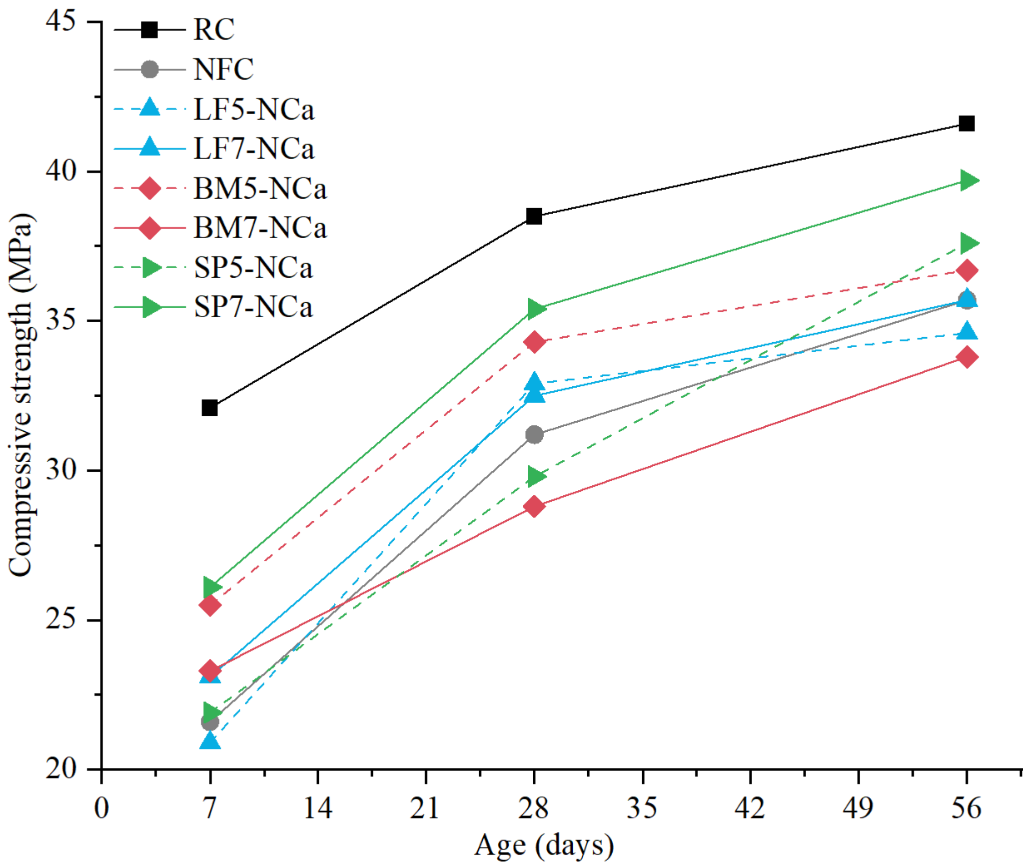


Figure 30. Compressive strength of bacterial concrete groups with different concentrations at 7 days 28days and 56 days.

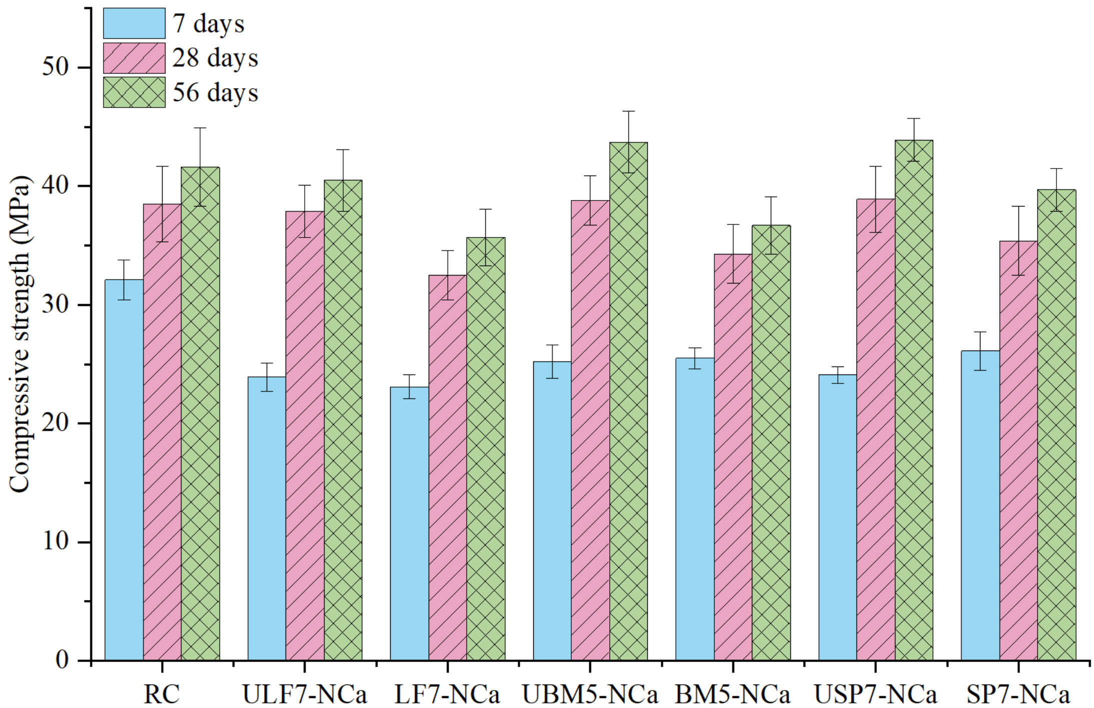


Figure 31. Compressive strength of concrete with bacteria cultured in urea/non-urea medium at 7 days 28days and 56 days.

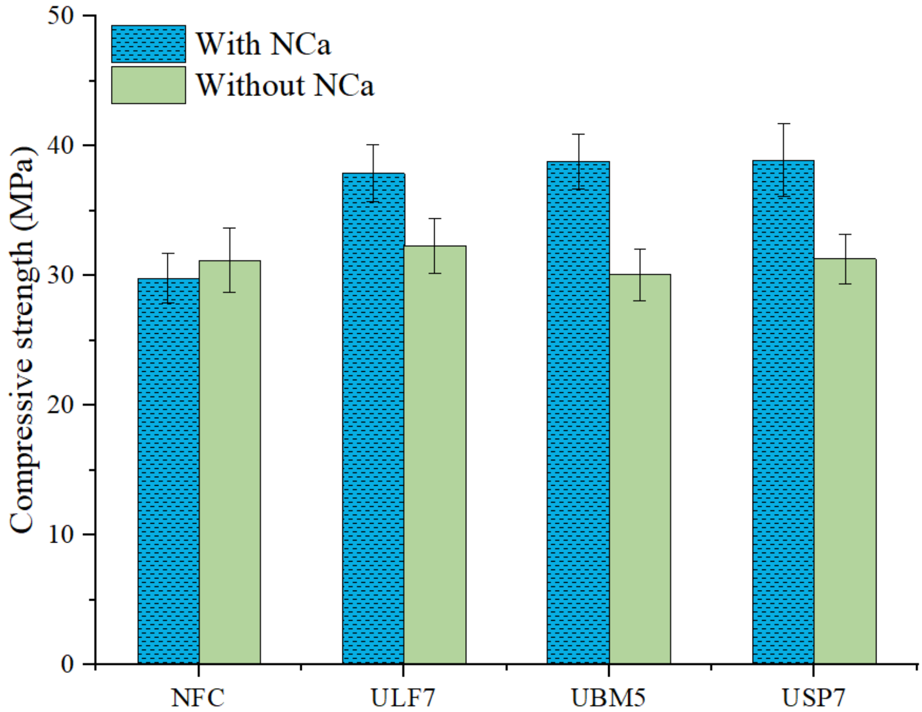


Figure 32. Compressive strength of concrete with/without Nca at 28days.

Section 4.2.4 Depth of penetration of water under pressure and water absorption

The depth of penetration of water under pressure is summarised in Table 7. Different from the compressive strength, the effect of introducing FA was limited in water permeability. Compared with the NFC group, the water permeabilities of bacterial groups reduced 20.3%, 16.9%, and 25.4% at 28 days for Lf, Bm, and Sp. The water absorption of each group is presented in Figure 33. Compared with the non-bacteria groups (RC, NFC and NFC-Nca), a considerable decrease in water absorption was found in all bacterial groups. Compared with non-bacterial groups, USP7-Nca group achieved the minimum water absorption in 7 days which was 5.3%. At 28 days and 56 days, the minimum water absorption was 4.0% and 3.0%, recorded from the ULF7-Nca and USP7-Nca group, respectively. Compared with the NFC group, water absorption at 7 days, 28 days and 56 days of ULF7-Nca USBM5-Nca and USP7-Nca groups were reduced by 13.9%, 29.7% and 26.8%, 16.3%, 26.6% and 25.6%, and 18.0%, 26.3% and 26.0%, respectively. Compared with the RC group, the reduction rates at the three ages of three groups were 12.4%, 30.1% and 30.6%, 13.5%, 27.0% and 27.1%, and 13.8%, 26.6% and 29.0%, respectively. This improvement was caused by the deposition of bio-induced CC, which embedded in the void and plugged the capillary pores inside the concrete. This microstructure change was confirmed by SEM observation and is discussed in Section 4.2.5. Given these results, all bacterial strains significantly reduced water permeability and absorption.

Table 7 Depth of water penetration at 28 days.

	Depth of water penetration (mm)	STD
RC	60	9.7
NFC	59	8.3
ULF7-Nca	47	10.2
UBM5-Nca	49	7.4
USP7-Nca	44	8.7

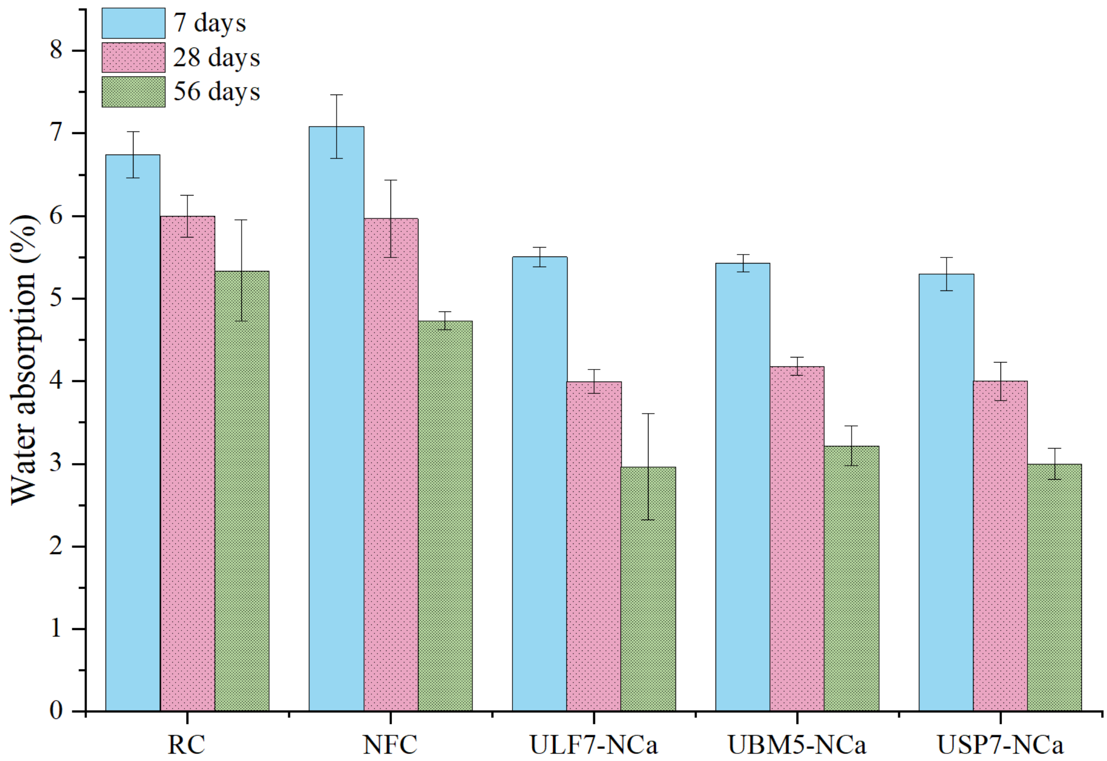


Figure 33. Water absorption of each group at 7, 28 and 56 days.

Section 4.2.5 SEM Observation

SEM images for RC, NFC, ULF7-Nca, UBM5-Nca and USP7-Nca are shown in Figure 34. The RC and NFC groups detected calcium hydroxide (CH) and calcium silicate hydrate (CSH). For ULF7-Nca, UBM5-Nca and USP7-Nca groups, besides CSH and CH, CC crystals (CC) were detected, while CH was not found. Hence, SEM observation demonstrated that CC was generated in bacterial groups. Compared with the NFC group, there are fewer voids in bacterial groups. Furthermore, the SEM photos show in Figure. 34 (c), (d) and (e), denser crystals and less void were detected than in other bacterial groups. Hence, voids in the matrix filled by bio-induced CC efficiently densified concrete microstructure and contributed to higher strength and lower water absorption. However, a limited improvement in accelerating the hydration of FA particles. Many unhydrated particles were detected in NFC, ULF7-Nca, UBM5-Nca and USP7-Nca groups, respectively, as shown in Figure 35. When comparing the slump shown in Figure 29 and compressive strength listed in Table 6, it was found that introducing bacteria improved the workability and compressive strength at the same time, while FA improved the workability but decreased the compressive strength. This distinction can be explained by the SEM observation results. The bacterial cells lubricated the fresh concrete in the cell state, and during the curing phase, bacteria gradually became CC blocks combined with the matrix well [224]; however, some FA particles were difficult to hydrate, reducing the viscosity of the cement pastes was affected continuously. Therefore, the intruding bacteria solution improved the workability of fresh concrete and the strength of hardened concrete.

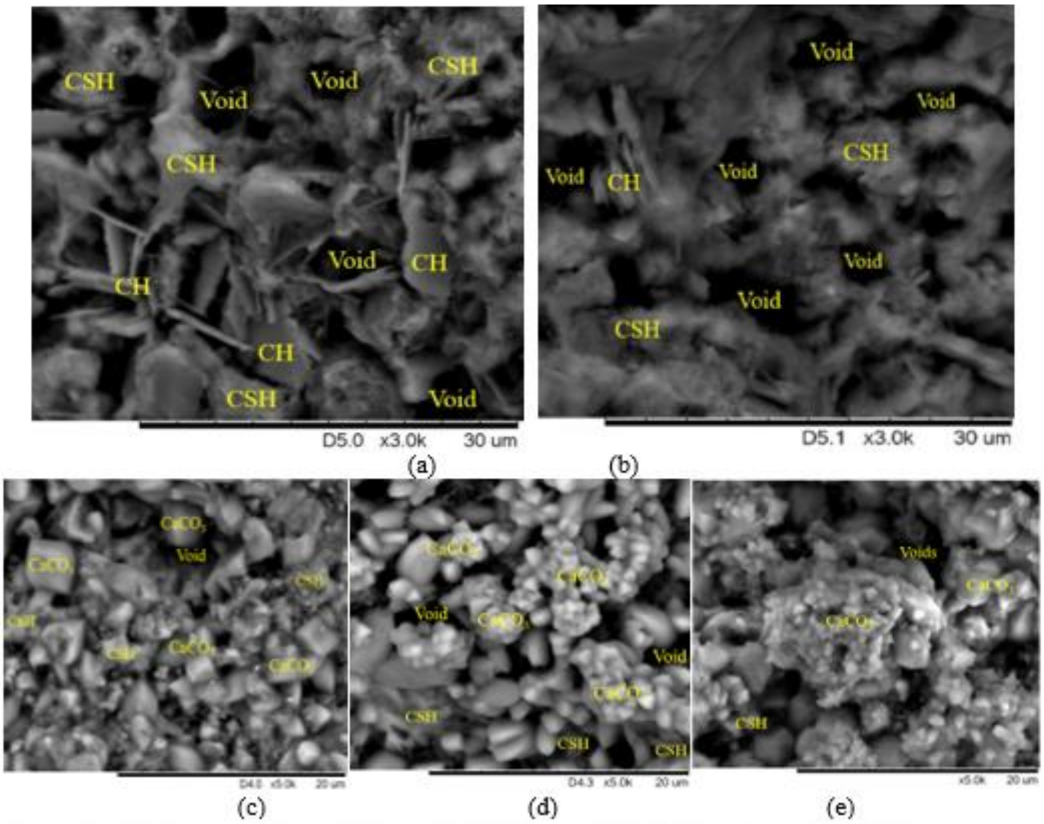


Figure 34. SEM photos RC (a), NFC (b), ULF7-Nca (c), UBM5-Nca (d) and USP7-Nca (e) groups.

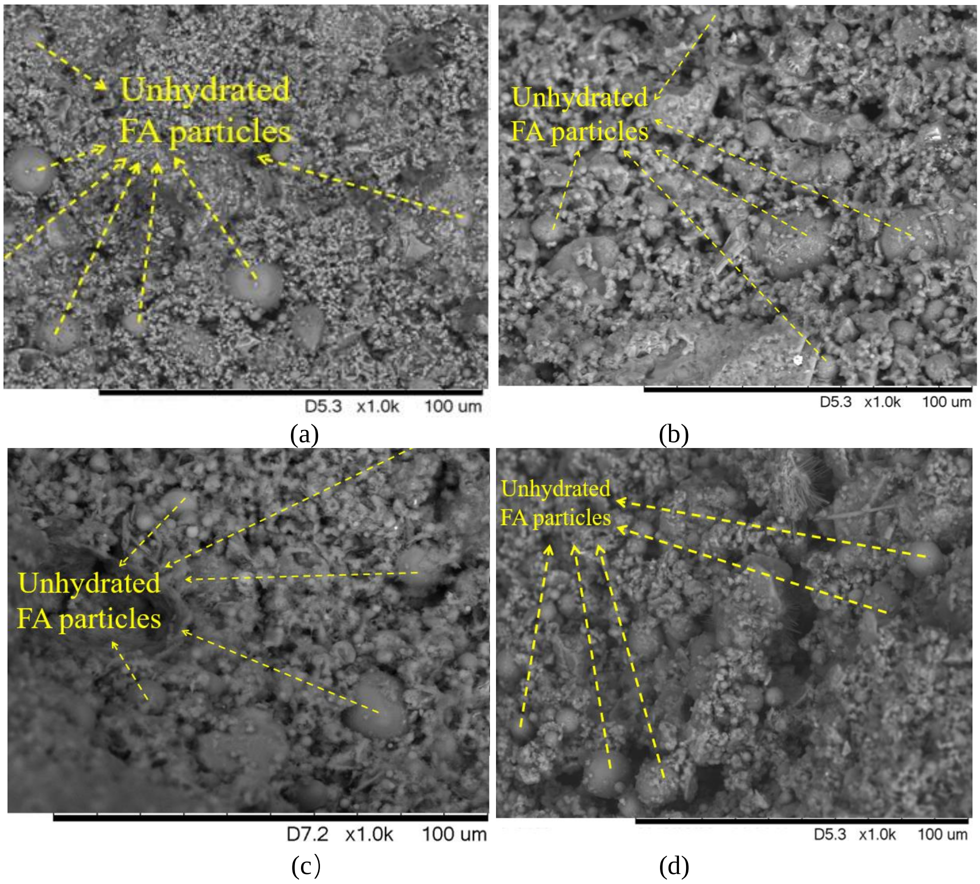


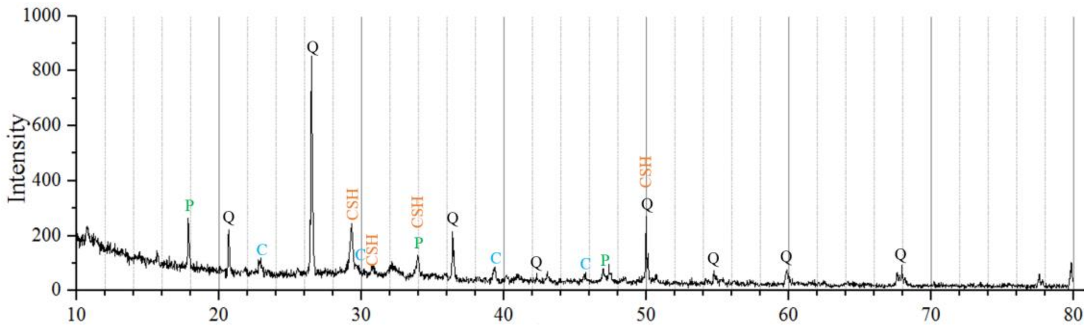
Figure 35. SEM photos of Unhydrated FA particles were observed in NFC (a), ULF7-Nca (b), UBM5-Nca (c) and USP7-Nca (d) groups, respectively.

Section 4.2.6 XRD analysis results

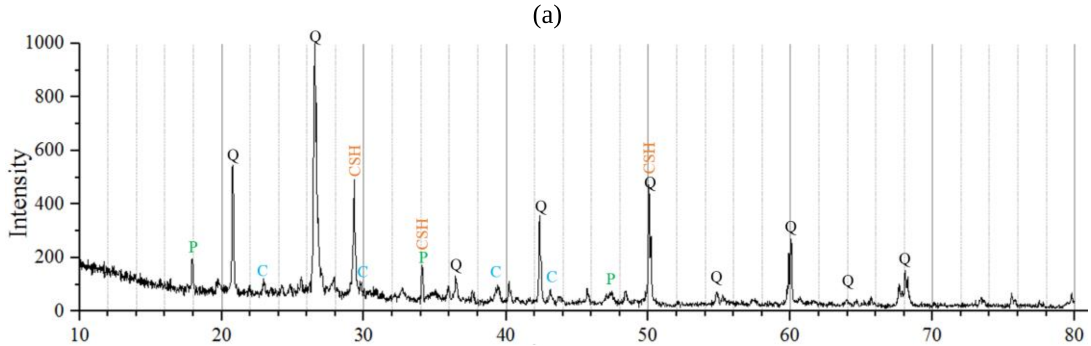
The XRD spectra of the five groups are shown in Figure 36. After comparing the peaks intensity (2θ) of each crystal that might be created in concrete with previous research [87,225–227], it was found that all three groups of concrete exhibited silica dioxide crystal (quartz 2θ ≈ 27°, 21°, 36.5°), calcium hydroxide crystal (CH 2θ ≈ 18°, 34°, 47°) and calcium silicate hydrate (CSH 2θ ≈ 29°, 34°, 50°). In all three groups, the peak values of quartz had the highest intensity. The quartz mainly came from FA and fine aggregate, including a high proportion of silica dioxide. The peak of CH was not observed in the bacterial groups (ULF7-Nca, UBM5-Nca and USP7-Nca). It means that the bacteria consumed CH by creating carbon dioxide.

Due to various environmental factors, such as PH, temperature and other ions in water, CC crystal exists in different morphologies [77,78]. Based on previous research

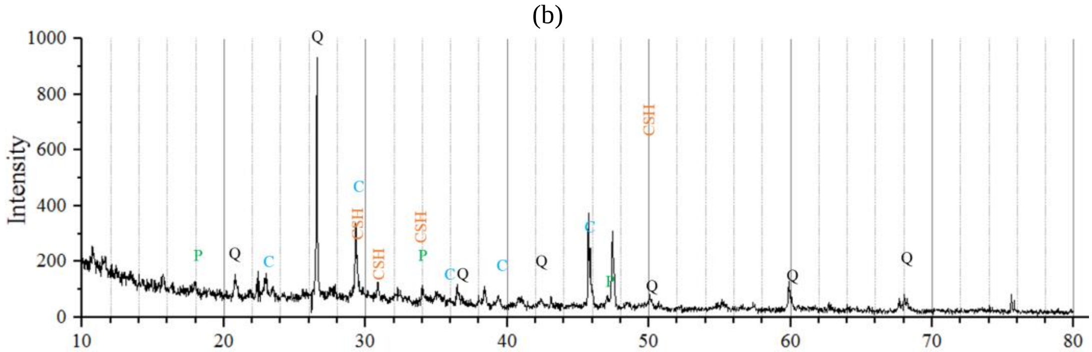
[77,78,228], bacteria created CC through Eq. 5 ~ Eq. 9. After comparing the XRD spectra of the bacterial concrete with the XRD of the three pure morphologies, it was found that the peaks of calcite were apparent. In contrast, peaks of aragonite and vaterite were not apparent. Moreover, Lf, Bm and Sp are aerobic bacteria that absorb water and oxygen in the air and release carbon dioxide, which reacts with calcium hydroxide producing CC; hence, the peak value of calcium hydroxide in bacteria groups was inconspicuous.



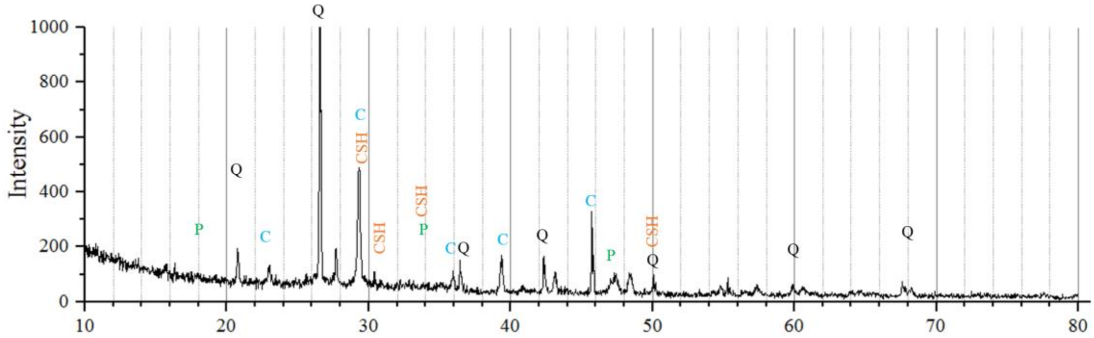
(a)



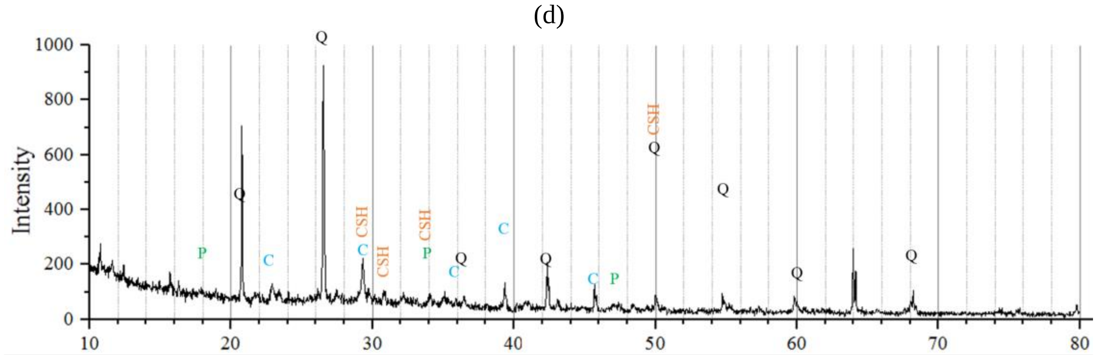
(b)



(c)



(d)



(e)

Figure 36. XRD spectra of RC (a), NFC (b), ULF-Nca (c), UBM-Nca (d) and (e) USP-Nca.

Section 4.3 RCA with ureolytic bacteria

Section 4.3.1 Bacterial treatment of RCA

Before the RCA was soaked, aggregates were washed by spraying water until the dust and mud on their surface were cleaned up. After that, RCA were put into four tanks, and three kinds of bacterial solution and water were poured into these tanks, respectively until the liquid submerged all the RCA particles. The soaking continued at 30 °C for 72 h. The compositions of the bacterial treatment solution are shown in Table 8, and the treatment device is shown in Figure 37.

Table 8 Composition of four kinds of soaking solution.

Name	Nutrition broth (g/L)	Urea (g/L)	$CaCl_2$ (g/L)	Bacteria strain	Concentration (CFU/mL solution)
NC	0	0	0	No bacteria	0
LF	0.26	20	11.1	Lf	10^7
BM	0.26	20	11.1	Bm	10^7
SP	0.26	20	11.1	Sp	10^7

Figure 37. Photo of the treatment device.

Section 4.3.2 The influence on RCA properties

The water absorption and apparent density of four aggregates groups are shown in Figure 38 (a) and (b). It can be found that soaking recycled coarse aggregate in bacterial solution could increase the density and decrease the water absorption of RCA. Reductions of 15.8%,18.4% and 15.7% in water absorption were observed in LF, BM, and SP groups, respectively. For apparent density, there were 1.72%, 2.02% and 2.35% increases in Lf, Bm and Sp groups, respectively. Ureolytic bacteria can hydrolyse urea into ammonium and carbonate ions. At the same time, the cytomembrane of bacteria with negative charges can attract the calcium ion, which can make a bacteria cell a micro-CC filler to fill the void and hole in cementitious materials. Hence, the biomineralization ability of bacteria precipitated CC in a mortar with RCA to improve the engineering properties of the resulting materials. However, little influence was found in the crushing value, as shown in Figure 38 (c). This result may be caused by insufficient soaking time. CC forming on the surface of RCA mainly influences the absorption, while CC forming inside of RCA mainly influences the crushing value [184]. In this sense, enhancing crushing value needs a longer soaking term. In addition, a pungent smell is also created while soaking the RCA due to the metabolism of ureolytic bacteria.

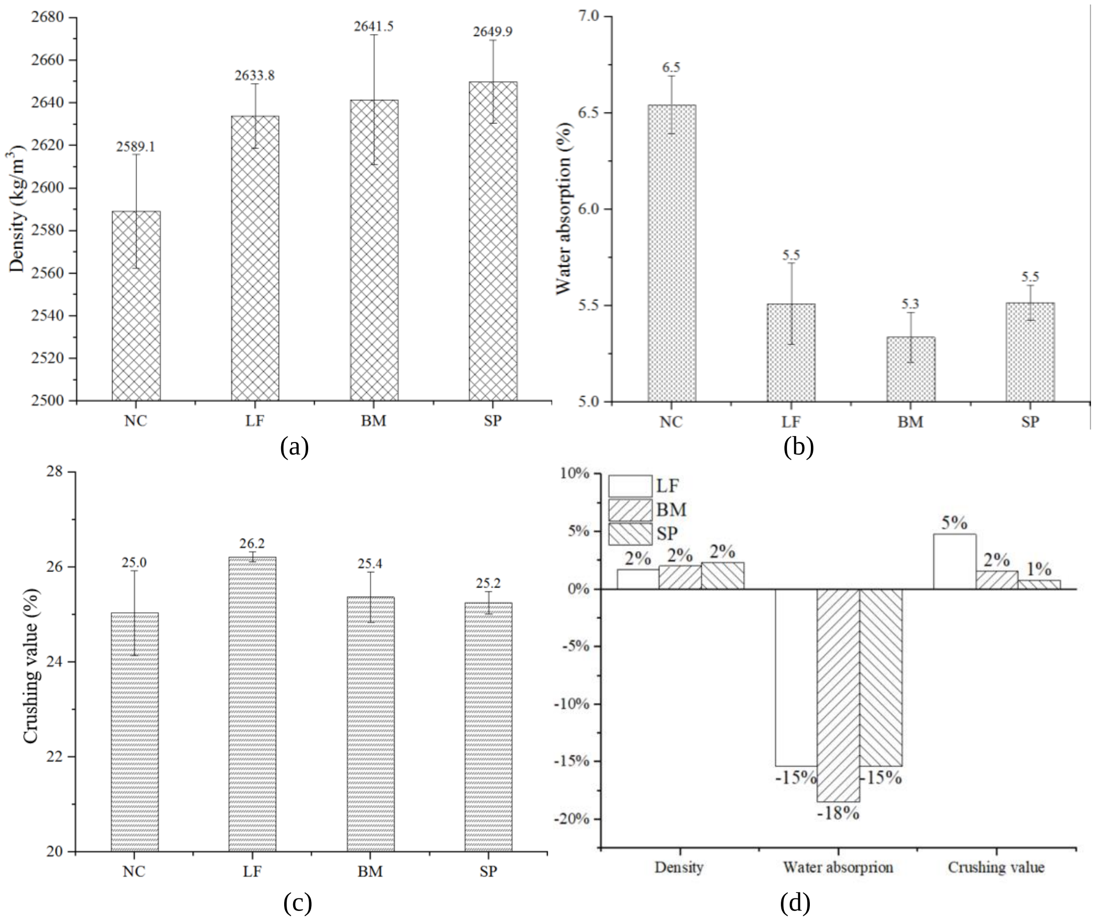


Figure 38. Water absorption (a), apparent density (b), crushing value (c) and percentage changes against NC of each group after 72 h.

Section 4.3.3 SEM observation on RCA particles

Micro-structures of RCA in NC, LF, BM and SP groups are compared. The surface of URCA was loose, and many voids were observed; on the contrary, deposition of calcium carbonate crystals was observed on the surface of bacterial-treated RCA. The CC crystals embedded in the voids and thus blocked the spaces that would absorb and collect water. Moreover, due to the creation of calcium carbonate crystals, voids and pores were filled, leading to increased apparent density.

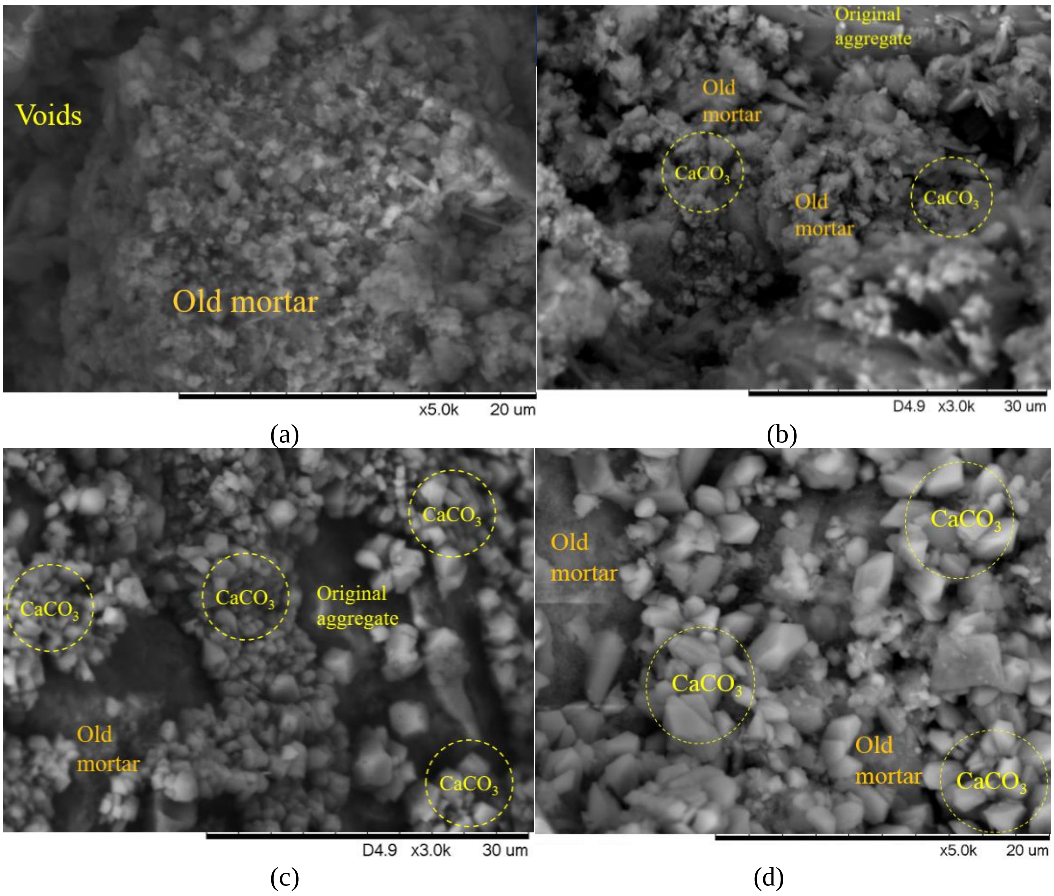


Figure 39. Micro-structures of RCA particles in NC (a), LF (b), BM(c) and SP (d) groups

Section 4.4 FA and RCA concrete improved by optimized bacterial solution

Section 4.4.1 Concrete preparation and mixing design

After achieving the results above, Sp cultured in a urea medium (USp) with the concentration of 10^7 CFU/ml was selected to be applied into concrete with both FA and RCA. Several specimen groups were designed to explore the influence of bacteria on RCA concrete with FA, as shown in Table 9. Where NRC-NA group was the baseline control group in accordance with other experiments; "B" represents bacteria introduced in concrete by directly adding into mixing water with nutrition and calcium source, and the concentration was 10^7 CFU/ml, and "N" represents no bacteria; "F" represents 40% of OPC was replaced with FA; and NA, UA and TA represent concrete with 100% NCA, 30% untreated RCA, and 30% treated RCA respectively. The treated method followed the method given in 4.4.1. The replacing ratio of FA was according to mass, while RCA was by volume.

Table 9 Mixing designs of each group.

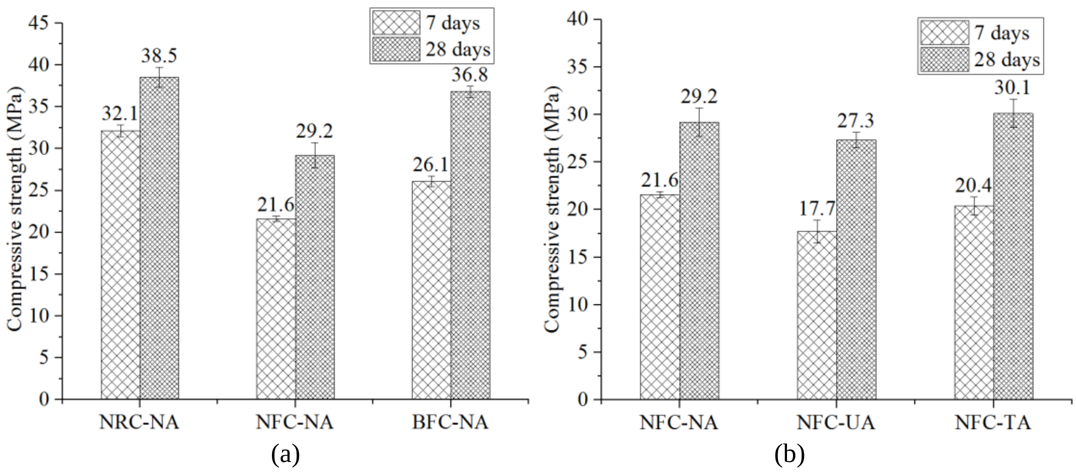
Label	Bacterial solution	Water	OPC	FA	NFA	NCA	TRCA	URCA
		kg/m3	kg/m3	kg/m3	kg/m3	kg/m3	kg/m3	kg/m3
NRC-NA	None	195.1	415.0	0.0	620.6	1152.5	0.0	0.0
NFC-UA	None	195.1	249.0	166.0	620.6	806.8	0.0	316
NFC-TA	None	195.1	249.0	166.0	620.6	806.8	323.6	0.0
BFC-NA	USp bacterial solution	195.1	249.0	166.0	620.6	1152.5	0.0	0.0
BFC-TA	USp bacterial solution	195.1	249.0	166.0	620.6	806.8	323.6	0.0

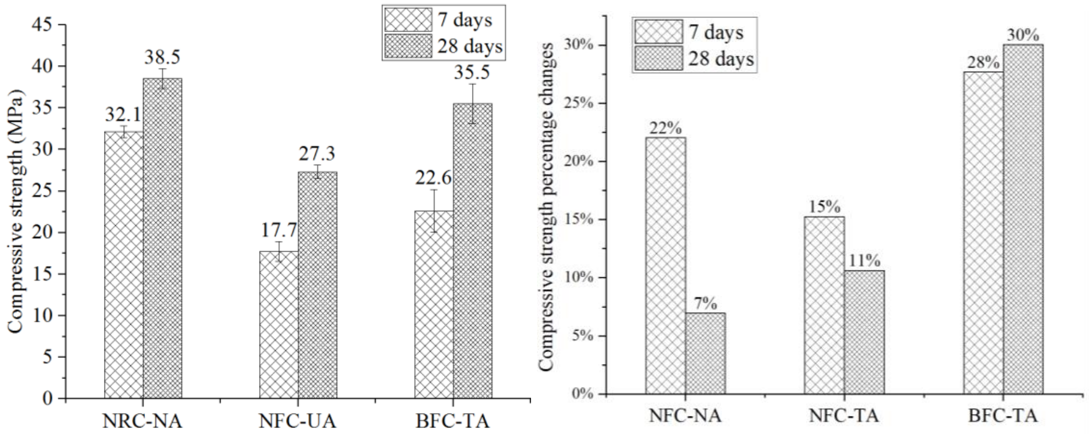
Section 4.4.2 Mechanical Properties of Concrete

Figure 40 (a) shows the influence of bacteria on FA concrete. It was found that replacing 40% OPC with FA reduced the strength of concrete. The compressive strength of the NFC-NA group at 7 and 28 days decreased 32.7% and 24.2%, respectively, compared with the NRC-NA group. However, if the bacteria solution was added to the FA concrete, only 18.7% and 4.4% decreased at 7 and 28 days. In other words, by introducing bacteria into the FA concrete, the compressive strength increased 20.8% and 26.0% at 7 days and 28 days, respectively. A similar level of improvement was present in previous research [70]. Figure 40 (b) compares the compressive strength of FA concrete with no RCA, 30% URCA, and 30% TRCA. According to the results, replacing 30% of NA with URCA led to an 18.1% and 6.5% decrease in concrete strength at 7 days and 28 days, respectively; however, if the RCA was treated by bacteria solution, the negative impact of replacement regarding compressive strength can be mitigated. Sahoo al. [187] and Wang al. [183] also confirmed that introducing bacteria into fresh concrete and treating RCA with bacterial solution increases the compressive strength. Figure 40 (c) shows the experiment results with both methods applied simultaneously. It was found that replacing both OPC and NA reduced compressive strength. For the NFC-UA group, the compressive was only

17.7 MPa and 27.3 MPa at 7 days and 28 days, respectively, 44.9% and 29.1% lower than the NRC-NA group. With the combined utilization of FA and bacteria, the 7-day and 28-day compressive strength of the BFC-TA group increased by 27.7% and 30.0%. The improvement ratio of the summation methods was higher than that of either single method.

The tensile splitting strength of each group is shown in Figure 41. Unlike what was observed in compressive strength, FA did not lead to an apparent decrease in tensile splitting strength. Applying bacteria into fresh concrete (BFC-NA) increased the tensile splitting strength by 7.9% compared with the NFC-NA group. Moreover, the tensile splitting strength of TRCA concrete (NFC-TA) was 8.3% higher than that of URCA concrete (NFC-NA). By applying both methods, the tensile splitting strength of BFC-TA increased by 16.7% compared with the NFC-UA group. Therefore, both methods could improve the tensile splitting strength of concrete. Figure 42 shows the micro-structure comparison between concrete matrices with and without bacteria. CC crystals were observed in the concrete matrix with bacteria. These crystals bonded with CSH and filled in the void inside the concrete, contributing to a compact structure inside the concrete.





(c) (d)

Figure 40. Compressive strength (a), (b), (c) and compressive strength percentage changes against NFC-UA (d) of each group.

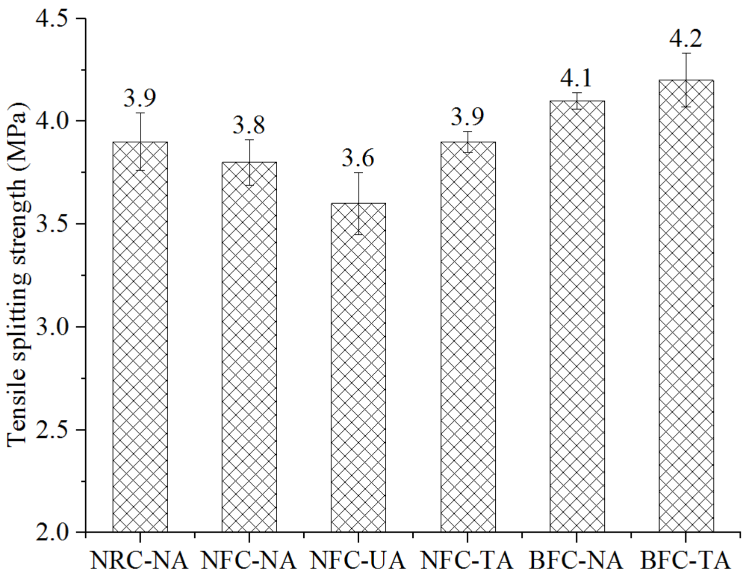
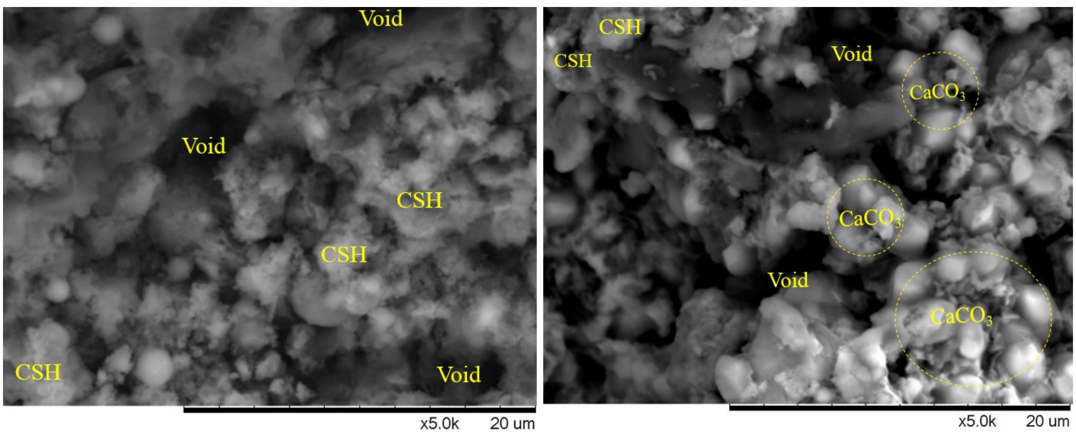


Figure 41 Tensile splitting strength of each group.



(a) (b)

Figure 42. SEM photo of the concrete matrix without bacteria (a) and with bacteria (b).

Section 4.5 Summary

This part of the research explored ureolytic bacteria in FA and RCA concrete together with nutrition and extra calcium ions, respectively. Significant improvements in concrete were found by applying the biomineralisation method adopted herein. Furthermore, an optimized Sp bacterial solution was used in concrete with both FA and RCA and got a sustainable recycled waste concrete with the same mechanical performance as reference concrete. The following points can be drawn:

- The bacterial cells lubricated the fresh concrete, and during the curing phase, bacteria gradually became CC blocks and filled in the voids and pores inside the concrete. Hence, Lf, Bm and Sp used in this research can significantly improve the workability, water permeability resistance, and compressive strength of concrete with FA and RCA. The effectiveness is influenced by numerous factors such as nutrition and additional calcium source, bacteria strain, bacterial concentration, and bacterial culture medium. Especially when culturing ureolytic bacteria in a medium consisting of urea, bacteria with more powerful ureolytic capacity are selected, which will cause a more significant improvement in these properties. Bacteria can create CC, densifying concrete microstructure by filling voids in the cement matrix. CC was proved to be calcite based on SEM and XRD analyses. On the other hand, neither bacteria nor CC created by bacteria can improve low pozzolanic activity, in other words accelerating the hydration of FA particles, which is the critical point contributing to the inadequate mechanical performance and durability of FA concrete at an early age.
- The mechanical properties of concrete with 40% FA and 30% treated RCA enhanced by Sp bacterial solution will be close to normal concrete with the same mixing design.

- Instead of curing concrete in a bacterial agent, the simplified operation of directly mixing bacteria, nutrition and extra calcium ions into mixing water when casting fresh concrete and curing concrete in the air also showed an apparent improvement, which means that this method is feasible in the onsite application.
- Though biomineralisation induced by ureolytic bacteria can improve significantly, it is inevitable to create ammonia gas. Therefore, some substituted strains should be adopted to avoid the creation of ammonium in concrete.

Chapter 5. Result and discussion of denitrification bacteria in recycled waste concrete

Section 5.1 Introduction

Ureolytic bacterial concrete test exposed its drawback that creating ammonia during biomineralisation process is inevitable. Hence, the project should consider some new strains, and this chapter focuses on using denitrification bacteria strains to induce biomineralisation. Due to the lack of existing literature, it is difficult to find enough references about the fundamental parameters of bacteria solution for concrete mixing, including strains, concentration, and nutrition composition. Hence, it is necessary to apply sufficient trial tests in order to optimise the composition of the bacterial solution in cement environments. Though the failure mode of mortar is different from the concrete due to the influence of the bond between the coarse aggregate and matrix; however, the adverse factors for the activity of bacteria, including hydration heat and high alkaline environment in mortar, are similar to concrete. Hence, a bacterial solution composition with significant biomineralisation ability in mortar should also show significant ability in concrete. At the same time, mortar specimens are convenient to produce. Therefore, mortar testing was carried out first to optimise the composition of the bacterial solution. And based on the mortar test results, bacterial and corresponding nutrition and calcium ions were directly added to the mixing water. Denitrification bacterial concrete was designed, and its compressive and tensile splitting strength, elastic modulus, UPV and water absorption/permeability were tested. Furthermore, this chapter also paid attention to testing the freeze-thaw resistance of concrete to fill the gaps in researching the influence of biomineralisation on the durability of concrete. In addition, because neither XRD analysis nor SEM observation can get the quantificational result, TGA was used to acquire the composition of the concrete.

Section 5.2 Mortar test

Section 5.2.1 Mixing design

Three strains, including Pd, Psp and Pf, and three bacterial concentrations, including 10^5 CFU/ml, 10^7 CFU/ml, and 10^9 CFU/ml, were attempted. Glucose which can increase the activity of bacteria was also introduced to concrete in different concentrations. The mixing designs are listed in Table 10.

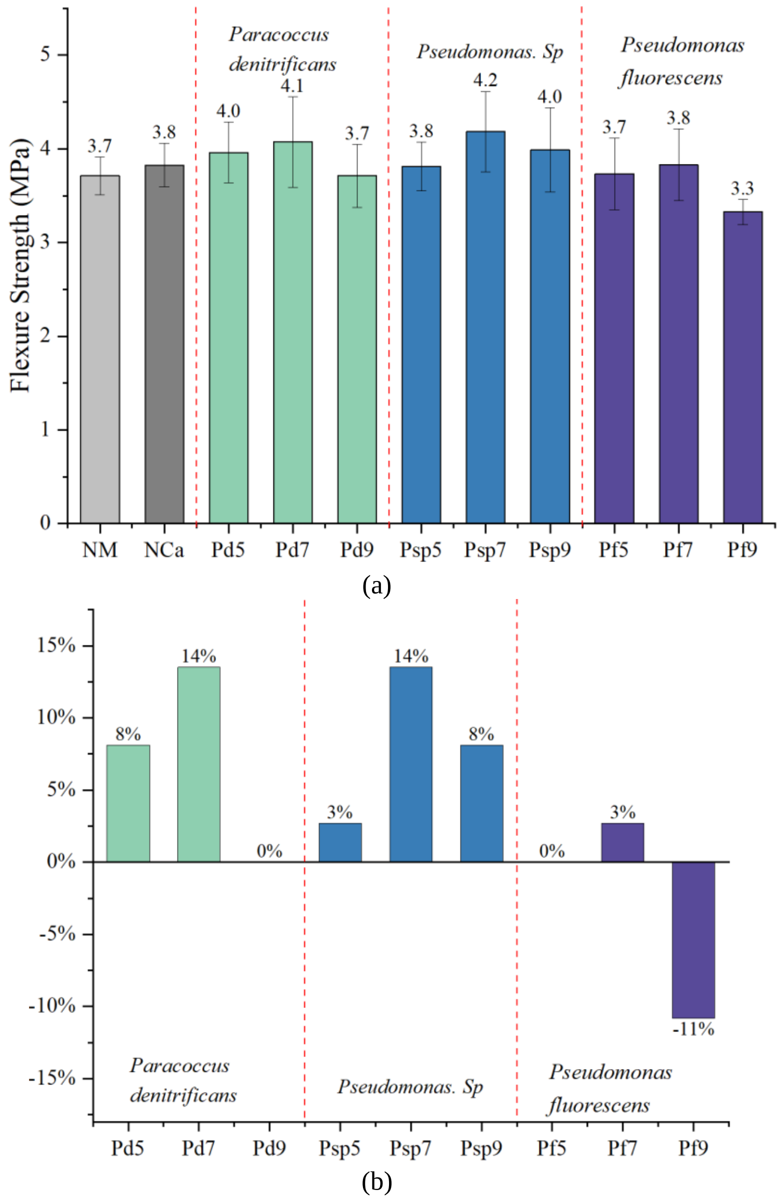
Table 10 Mixing designs of each mortar group.

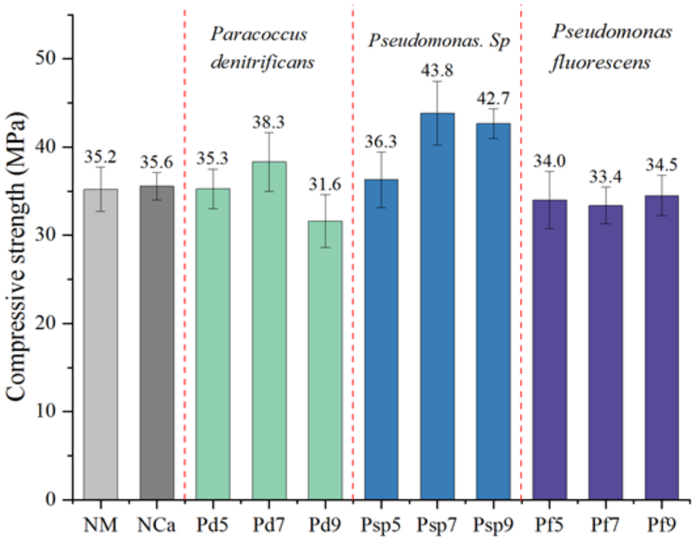
Label	Bacteria strain	Concentration CFU/ml	Colony-forming unit	Cement g	Sand g	Water g	$Ca(NO_3)_2$ g	$Ca(HCOO)_2$ g	Glucose g/L water
NM	None	N/A	N/A	500.0	1500.0	250.0	0.0	0.0	0.0
Nca	None	N/A	N/A	500.0	1500.0	250.0	2.5	7.0	0.0
Pd5	*Pd*	10^5	2.5×10^7	500.0	1500.0	250.0	2.5	7.0	0.0
Pd7	*Pd*	10^7	2.5×10^9	500.0	1500.0	250.0	2.5	7.0	0.0
Pd9	*Pd*	10^9	2.5×10^{11}	500.0	1500.0	250.0	2.5	7.0	0.0
Psp5	*Psp*	10^5	2.5×10^7	500.0	1500.0	250.0	2.5	7.0	0.0
Psp7	*Psp*	10^7	2.5×10^9	500.0	1500.0	250.0	2.5	7.0	0.0
Psp9	*Psp*	10^9	2.5×10^{11}	500.0	1500.0	250.0	2.5	7.0	0.0
Pf5	*Pf*	10^5	2.5×10^7	500.0	1500.0	250.0	2.5	7.0	0.0
Pf7	*Pf*	10^7	2.5×10^9	500.0	1500.0	250.0	2.5	7.0	0.0
Pf9	*Pf*	10^9	2.5×10^{11}	500.0	1500.0	250.0	2.5	7.0	0.0
GPsp0.1	*Psp*	10^7	2.5×10^9	500.0	1500.0	250.0	2.5	7.0	0.1
GPsp0.5	*Psp*	10^7	2.5×10^9	500.0	1500.0	250.0	2.5	7.0	0.5
GPsp1	*Psp*	10^7	2.5×10^9	500.0	1500.0	250.0	2.5	7.0	1.0
GPsp2	*Psp*	10^7	2.5×10^9	500.0	1500.0	250.0	2.5	7.0	2.0
GPsp5	*Psp*	10^7	2.5×10^9	500.0	1500.0	250.0	2.5	7.0	5.0
GPsp10	*Psp*	10^7	2.5×10^9	500.0	1500.0	250.0	2.5	7.0	10.0
GPsp20	*Psp*	10^7	2.5×10^9	500.0	1500.0	250.0	2.5	7.0	20.0

Section 5.2.2 Influence of bacterial strains and concentration on mortar strength

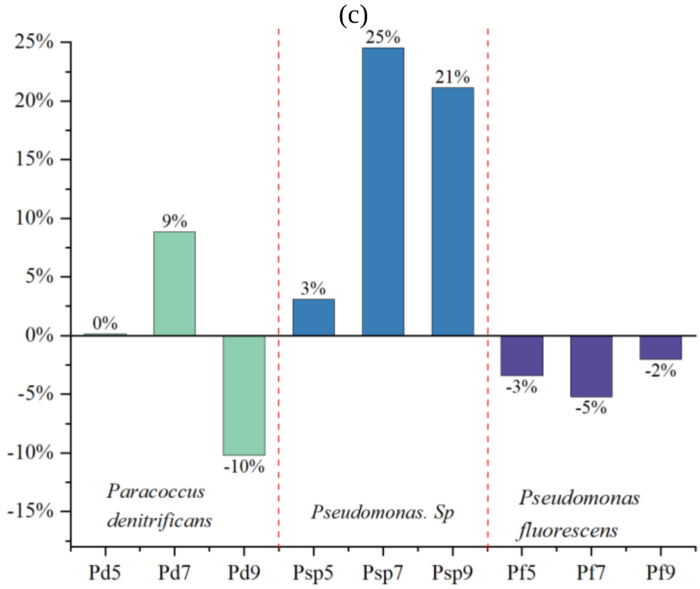
The mortar flexure and compressive strength are presented in Figures 43 (a) and (b). Compared to the NM group, the Psp7 group improved 13.5% and 24.4% of the flexure and compressive strength of mortar, respectively; and the flexure strength and compressive strength of the Psp9 group also increased by 8.1% and 21.4%, respectively. However, relative low improvements were observed in the Pd group;

maximum improvements percentage, 10.8% and 8.8% in flexure and compressive strength, respectively, were found in the pd7 group. Pf even showed a negative influence on compressive strength, which may be due to the metabolism of Pd, and Pf being seriously limited in the environment inside of concrete. If the bacteria cell cannot become a tiny CC crystal, it may delay the hydration because bacterial cells are organic substances. Psp showed high adaptive capacity in mortar. Therefore, 10^7 CFU/ml of Psp was the optimum dosage for the concentration and was adopted in the concrete mix design.





(c)



(d)

Figure 43. Flexure strength (a) and percentage changes against NM (b), compressive strength (c) and percentage changes against NM (d) of mortar at 28 days.

Section 5.2.3 Influence of glucose with different concentrations on mortar strength

Glucose was used to improve the activity of bacteria in a harsh environment; however, as an organic, glucose can slow down the hardening speed of cement. A series of concentration range tests were implemented to explore the suitable glucose concentration. As shown in Figure 44, 0.5 g/L glucose exhibited the peak value of compressive strength. Hence, for directly mixing bacterial solution into concrete, the concentration was selected 0.5g/L.

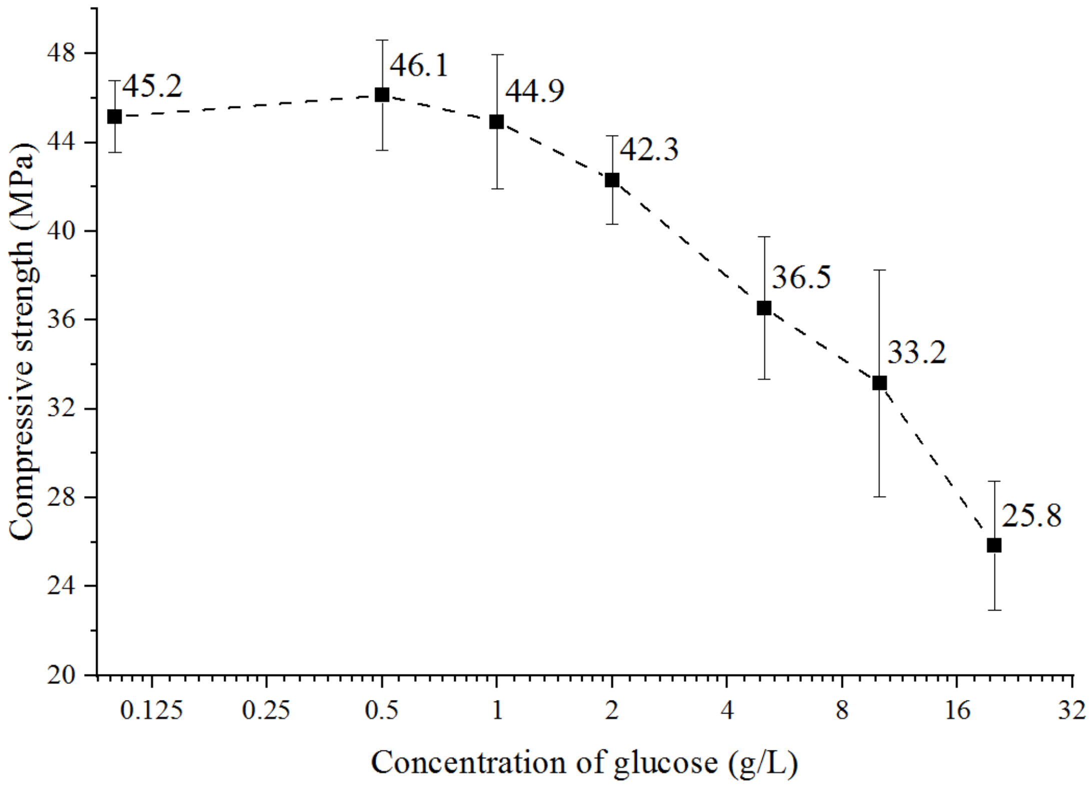


Figure 44. Compressive strength of mortar with different concentrations of glucose.

Section 5.3 Improving the properties of RCA

Section 5.3.1 Treatment process

The composition of the bio-agent for treating RCA is listed in Table 11. Calcium formate ($Ca(HCOO)_2$) and calcium nitrate ($Ca(NO_3)_2$) provided both calcium ions and nutrition for denitrification bacteria. In addition, glucose was added to the bio-agent, which could provide energy for bacteria and increase the activity of bacteria in a harsh environment [229]. The alkaline environment was suitable for CC formation, while the most suitable PH for bacteria is 7~8. Hence, three PH of bacteria solutions were designed. In consequence, a small amount of disodium hydrogen phosphate (Na_2HPO_4) and lime water ($Ca(OH)_2$ solution) was used to adjust the PH of each group.

Figure 45 depicts the complete process of RCA treatment. The RCA were soaked in a flat container. A curing chamber was used to keep the humidity and temperature. The RCA were cleaned and soaked in the bacterial solution for 24 h. Then the calcium salt was supplied for the biomineralisation. The biological research on denitrification and biomineralisation [230] pointed out that the reaction rate of both denitrification and biomineralisation shows the highest value at 30 °C. Hence, the treatment continued for fourteen days at 30°C. Previous results (in Section 4.3) showed that 72-h treatment might not completely develop the improving potential on properties of RCA. This experiment tested the properties at seven days and fourteen days, respectively.

Table 11 Composition of bio-agent.

Method label	Bacteria strain	Glucose	PH.	$Ca(HCOO)_2$	$Ca(NO_3)_2$
		g/L		g/L	g/L
NRC	None	1	7.3 ± 0.1	42	15
PS7-7	*Pseudomonas. Sp*	1	7.3 ± 0.1	42	15
PS7-9	*Pseudomonas. Sp*	1	9.0 ± 0.1	42	15
PS7-11	*Pseudomonas. Sp*	1	11.0 ± 0.1	42	15

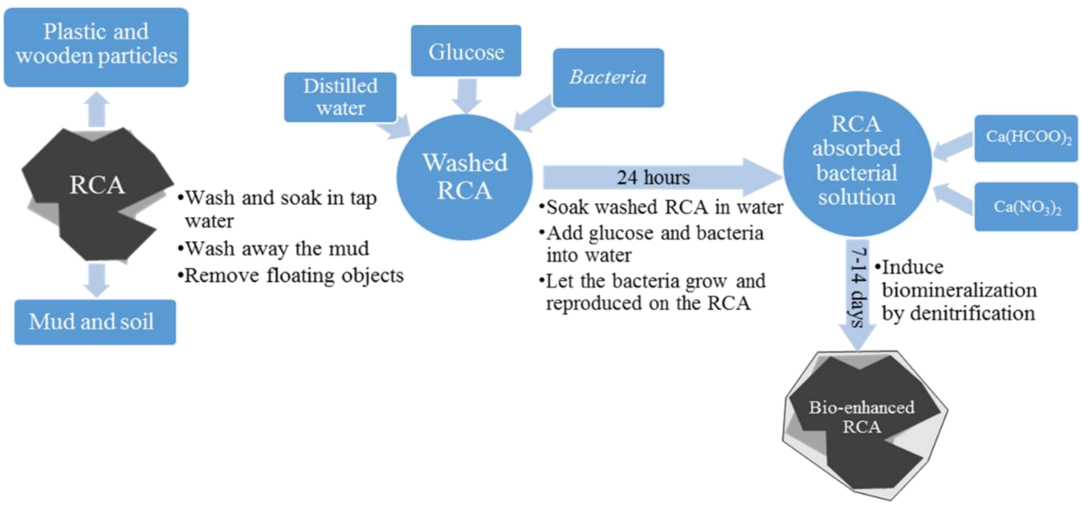
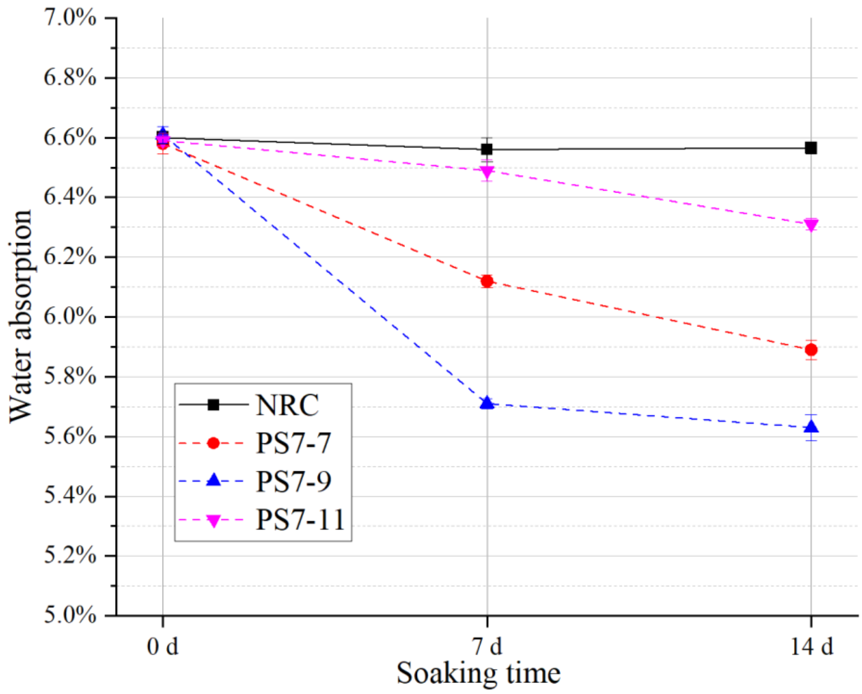


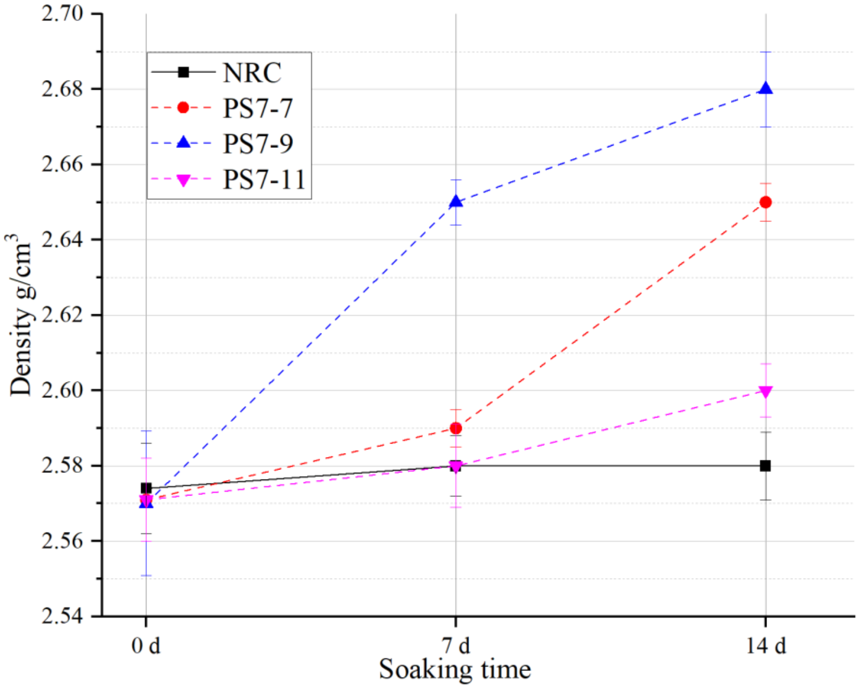
Figure 45. Bio-treatment procedures.

Section 5.3.2 Properties of treated RCA.

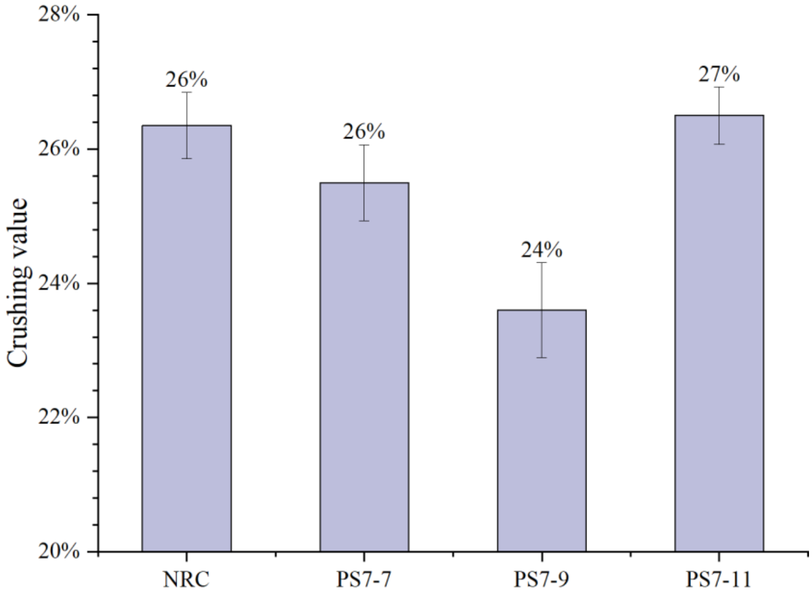
The water absorption, density and crushing value of the RCA are presented in Figure 46 and Table 12. After the treatment, a considerable improvement in properties was found in groups PS7-7 and PS7-9. Compared with NRC, the water absorption at 14 days reduced by 9% and 14%, respectively; the density increased by 3% and 4%, respectively, and the crushing index reduced by 3% and 10%, respectively. PH 9, rather than PH 7, showed the most effective improvement in denitrification bacteria groups, which conform to the previous microbiological kinetic analysis [230,231]. The reaction rate of denitrification is highest in PH 7; however, the rate of biomineralisation is highest in PH 9 because higher pH is beneficial to the precipitation of calcium ions [230,231]. At the same time, no significant improvement was found in the group PS7-11. The reason was that the bacteria were not suited to the high alkaline environment, and therefore, the activity of bacteria was significantly inhibited. In this research, the improving effect of denitrification bacteria was not as well as ureolytic bacteria. However, there was no pungent odour during the treatment rather than harmless nitrogen gas. For the ureolytic bacteria, producing one carbonate ion created two ammonium ions, leading to a pungent odour in an indoor space. Hence, the denitrification method showed a significant advantage from the environmental perspective.



(a)



(b)



(c)

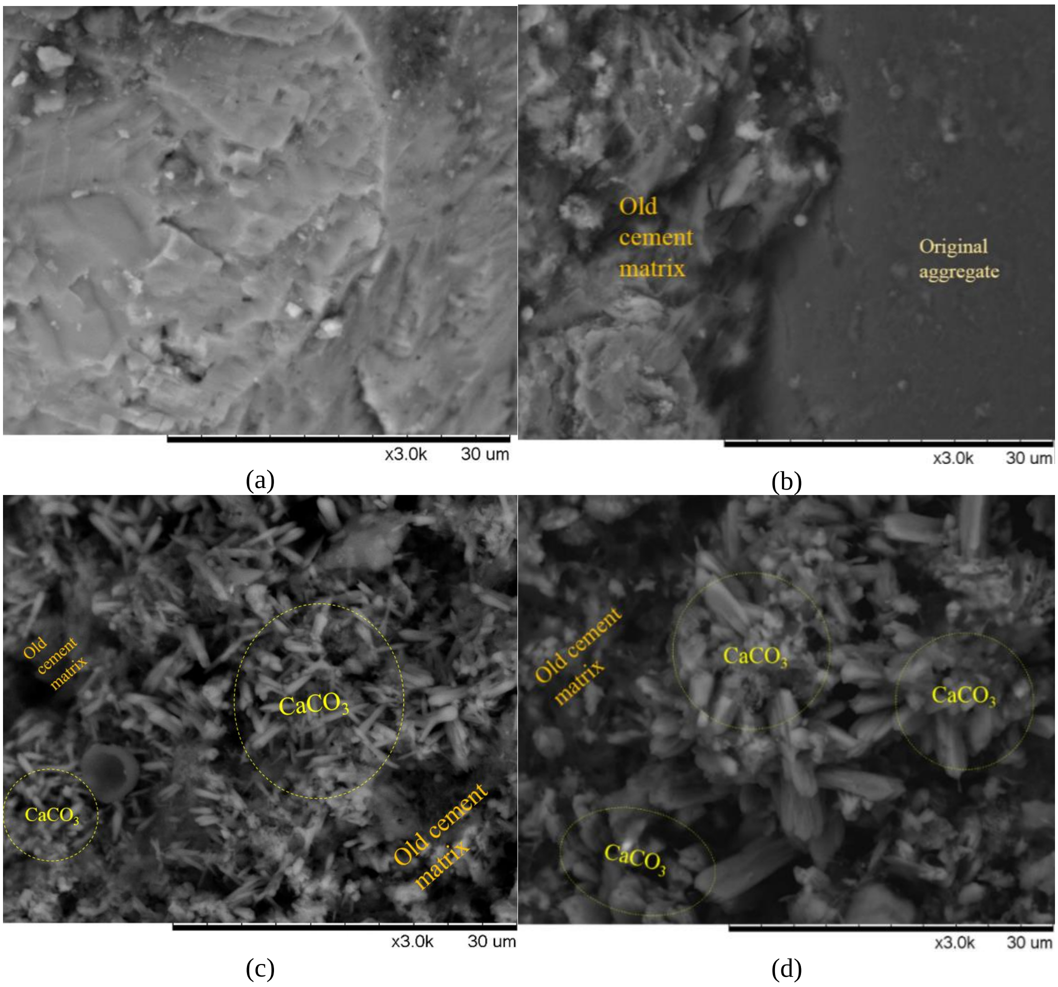
Figure 46. Comparison of each group in water absorption (a), density (b) and crushing value (c).

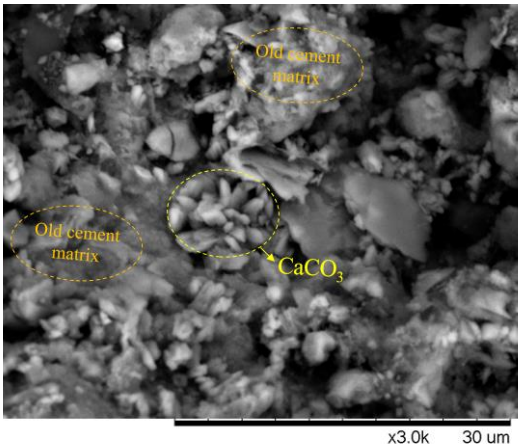
Table 12 Relative value of properties of each group.

Group	Water absorption		Density		Crushing value
	7 days	14 days	7 days	14 days	14 days
NRC	1.00	1.00	1.00	1.00	1.00
PS7-7	0.98	0.91	1.00	1.03	0.97
PS7-9	0.87	0.86	1.02	1.04	0.90
PS7-11	0.99	0.96	1.00	1.01	1.01

Section 5.3.3 SEM analysis of RCA surface

SEM observations detected the CC on the surface of RCA, which was created by biomineralisation. As shown in Figure 47, compared with the NA, the RCA showed two parts: the original aggregate and adhered old cement matrix. CC crystals were observed on the matrix of RCA in groups PS7-7, PS7-9, and BM7-7. However, few crystals in the old matrix were found in group PS9-11. CC crystals existed in various shapes in separate groups; in the denitrification bacteria group, it was a "needle-like" crystal in PS7-7; in group PS7-9 the crystal was coarser.





(e)

Figure 47. SEM photos of NA (a), URCA (b) and RCA treated by PS7-7(c), PS7-9 (d) and RCA treated by PS7-11 (e).

Section 5.4 Mechanical performance RCAC with denitrification bacteria

Section 5.4.1 Mixing design of RCAC with denitrification bacterial

The mixing design of each group is shown in Table 13. Group N-NC was the baseline control group. Based on the mixing design of NC, the NCA were substituted by treated RCA (TRCA) and untreated RCA (RCA) with different percentages (30% and 100% by volume). The TRCA was treated by method PS7-9 (the detail shown in Table 11). Directly mixing bacteria solution in fresh concrete was also explored. *Pseudomonas. Sp* was added to the mixing water until the concentration arrived at 10^7 CFU/ml. Specimens were cured in a chamber at 30°C, and the relative humidity was 95%.

Table 13 Mix designs of each group in this research.

Label	Bacteria strain	OPC	Water	NFA*	NCA*	RCA*	TRCA*	$Ca(HCOO)_2$	Glucose	$Ca(NO_3)_2$
		kg/m^3	kg/m^3	kg/m^3	kg/m^3	kg/m^3	kg/m^3	kg/m^3	g/m^3	kg/m^3
NC	None	433.6	195.1	620.6	1152.5	0.0	0.0	0.0	0.0	0.0
RCAC30	None	433.6	195.1	620.6	806.8	110.2	0.0	0.0	0.0	0.0
BRCAC30	*Psp*	433.6	195.1	620.6	806.8	110.2	0.0	8.2	97.6	2.9
RCAC	None	433.6	195.1	620.6	0.0	1042.9	0.0	0.0	0.0	0.0
TRCAC	None	433.6	195.1	620.6	0.0	0.0	1063.2	0.0	0.0	0.0
BRCAC	*Psp*	433.6	195.1	620.6	0.0	1042.9	0.0	8.2	97.6	2.9
BTRCAC	*Psp*	433.6	195.1	620.6	0.0	0.0	1063.2	8.2	97.6	2.9

* *The mass of aggregate was considered in saturated–surface dry (SSD) condition.*

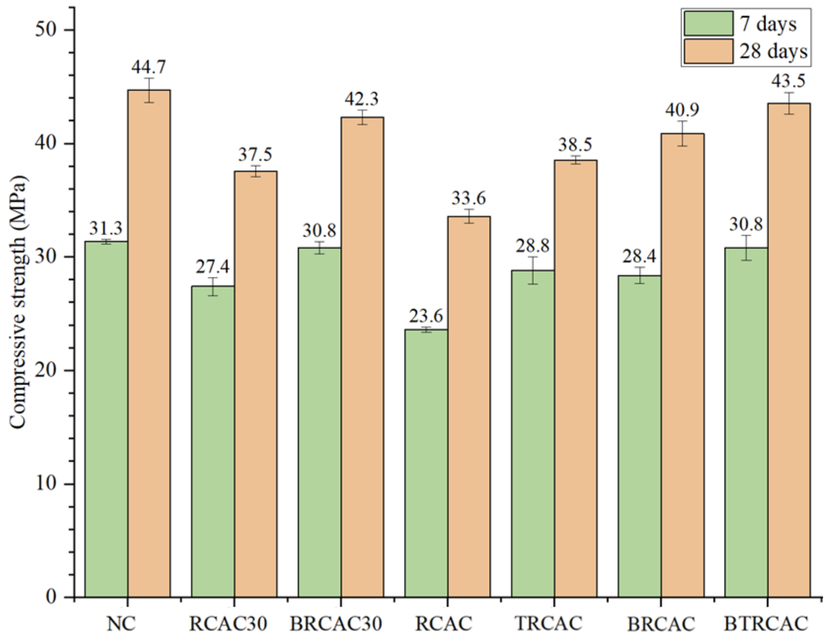
Section 5.4.2 Compressive strength of concrete

The cubic specimens with a size of 150 mm for each group were tested to obtain the compressive strength at 7 and 28 days, and the test results are shown in Figure 48. The results indicated that the biomineralisation method efficiently enhances the compressive strength. The compressive strength of the baseline control group (NC) was 31.3 MPa and 44.7 MPa at 7 days and 28 days, respectively. If 30% of NCA was replaced with RCA, the compressive strength decreased to 27.4 MPa and 37.5 MPa, respectively, at 7 days and 28 days. However, if this substitute were TRCA, the compressive strength could arrive at 30.8 MPa and 42.3 MPa at 7 and 28 days, respectively. In this sense, the application of the bio-treated RCA almost compensated for the strength reduction due to the use of 30% RCA as the supplementary material of NCA.

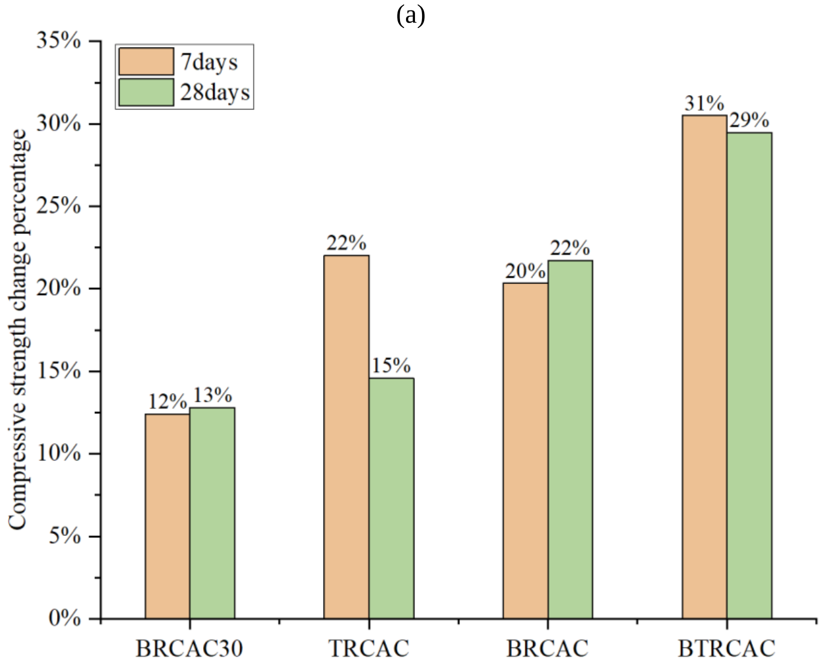
Furthermore, for the 100% replacement ratio, the group with untreated RCA (RCAC) showed a strength of 23.6 MPa and 33.6MPa at 7 days and 28 days, respectively. A sharp fall in compressive strength occurred if the NCA was totally replaced with URCA. Even using TRCA instead of RCA (TRCAC) or applying bacteria into fresh concrete (BRCAC), the compressive strength is still apparently lower than the baseline control group. Hence, using the two methods above to enhance the concrete with 100% RCA should be considered to compromise the decrease in compressive strength. By the joint application of the two methods, the compressive strength of the BTRCAC group improved 30.5% and 25.7% compared with RCAC at 7 days and 28days, respectively. The compressive strength arrived 30.8MPa and 43.5 MPa at 7 days and 28days, respectively. The compressive strength of the BTRCAC 100 group arrived at 96.1% and 93.6% that of the NC groups at 7 days and 28days, respectively. Therefore, by applying the denitrification bacteria-induced biomineralisation methods, RCA can replace 100% NCA without apparently compressive strength decrease.

When ureolytic bacteria were used, Kalhori and Bagherpour [99] observed that improvements of 27.4% and 13.3% in compressive and tensile splitting strengths were achieved, respectively; Nain et al. [96] noted enhancements of 22.6% and 18.3%, respectively. Although the studies on the use of ureolytic bacteria in concrete are limited, the previous results indicate that the strength enhancement ability of this bacterial strain is similar to that of the denitrification bacteria used in the present research.

In addition, twelve cubic specimens with 100 mm of NC, RCAC, BRCAC and BTRCAC groups were used to test the compressive strength change in the long-term, including 28 days, 56 days, 90 days, and 180 days. As shown in Figure 49, compared with that of the RCAC group, the compressive strength of BRCAC increased by 26.8%, 17.9%, 15.7%, and 10.7%, and that of BTRCAC further increased by 30.3%, 23.3%, 17.6%, and 11.2% at 28, 56, 90, and 180 days, respectively. According to the variation in compressive strength with age, the improvement provided by bacteria to the concrete after 28 days decreased with the increase of curing age. The development of compressive strength of both bacterial groups and non-bacterial groups showed a similar growth rate, which illustrated the compressive strength improvement mainly caused by further hardening of cementitious materials. Hence, by directly introducing the bacterial solution, enhancement potential can be developed in 28 days or less when all the bacterial cells become the cores of CC crystals.



(a)



(b)

Figure 48. Compressive strength of concrete in each group (a) and the compressive strength percentage of each bacterial group compared to corresponding control groups (b).

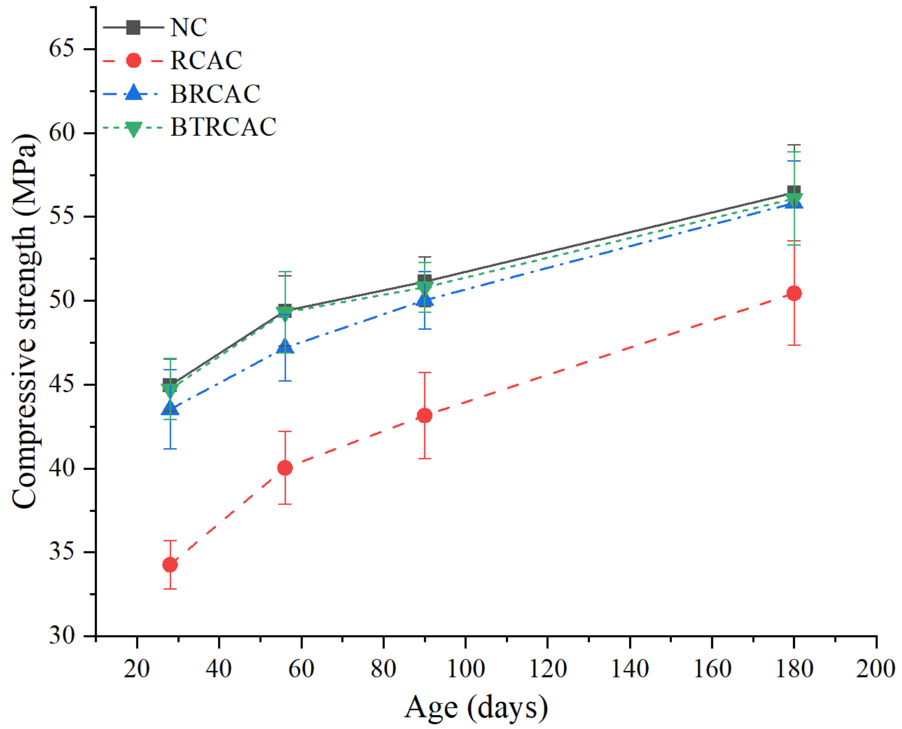


Figure 49 long-term compressive strength of NC, RCAC, BRCAC and BTRCAC groups

Section 5.4.3 Tensile splitting strength

The tensile splitting test results of each group are presented in Figure 50. A considerable improvement in the tensile splitting strength of RCA concrete was realized by directly introducing the bacterial solution to fresh concrete. BRCAC30 improved 11.8% on tensile splitting strength compared with RCAC30, and the improvement observed for BRCAC was 24.1% compared with RCAC. However, using treated RCA to replace untreated RCA did not show considerable improvement in tensile splitting strength. The tensile splitting strength difference between RCAC and TRCAC was only 0.2 MPa, and the difference between BRCAC and BTRCAC is ignoble. Mix viable bacteria into fresh concrete further enhanced the matrix of the concrete.

In contrast, after the treatment, drying the TRCA inactivated the residual bacteria on the surface of RCA, only using TRCA particles in concrete without further supplementing the bacteria solution, biomineralisation cannot occur in the cement matrix. Therefore, the tensile strength of concrete mainly depends on the strength of the cement matrix and the adhesion (bond) between the cement matrix and aggregate [46]. Since few bacteria survived in TRCA after drying, no CC could be further created to enhance the bond between the cement matrix and aggregate. Therefore, only using TRCA may not enhance tensile splitting strength.

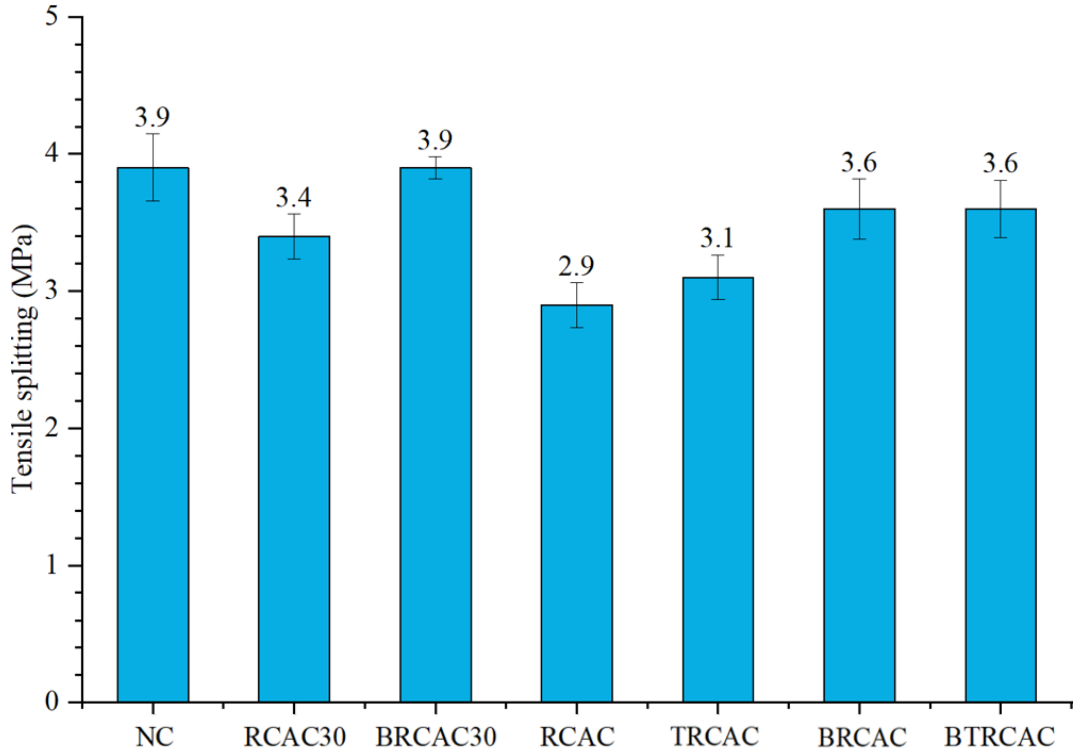


Figure 50. Tensile splitting strength of concrete in each group.

Section 5.4.4 Elastic modulus

The elastic modulus test result shows in Figure 51. In this research, the elastic modulus varied in a similar trend as the compressive strength. Replacing NA with RCA reduced the elastic modulus of concrete; replacement ratios of 30% and 100% caused an 8.9% and a 14.5% reduction in elastic modulus, respectively. The bio-methods can compromise this reduction. Compared with RCAC30, the elastic modulus of UR-PsC30 increased by 6%. Compared with RCAC, the elastic modulus of TRCAC, BRCAC, and BTRCAC increased by 2.9%, 7.4% and 7.5%, respectively.

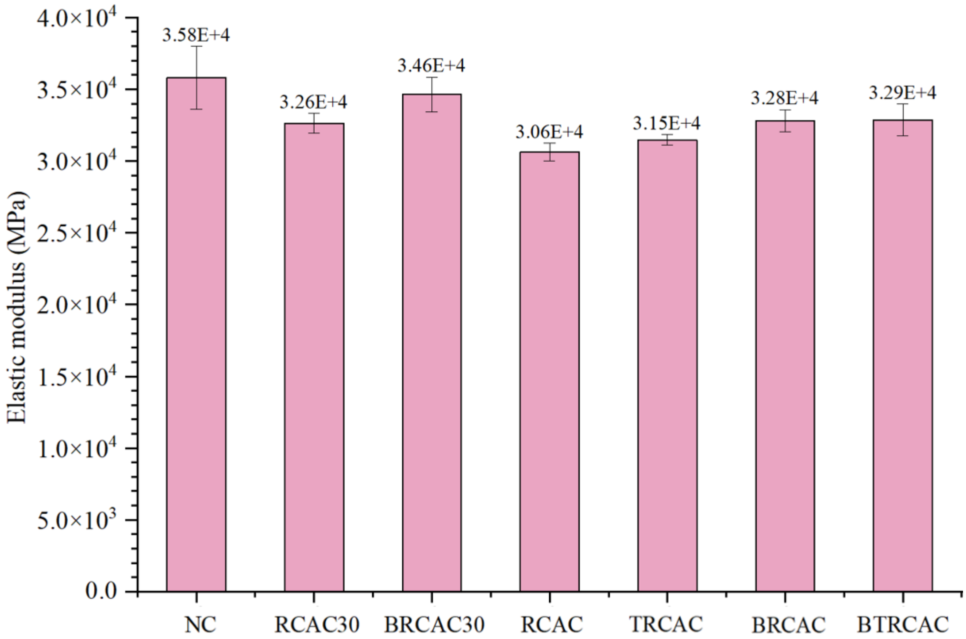


Figure 51. Elastic modulus of concrete in each group.

Section 5.4.5 Ultrasonic pulse velocity test

The velocity of ultrasonic waves passing the specimen was affected by the internal compactness of the concrete. In this sense, fewer voids and cracks inside the concrete provided the ultrasonic pulse with a more unobstructed route leading to a higher velocity of ultrasonic wave transformed through the specimen. According to the test result shown in Figure 52, the velocity of ultrasonic pulse transformed in bacteria-enhanced groups was higher than in the corresponding non-bacteria group. This phenomenon can be attributed to the bacteria-induced biomineralisation, which compacted the concrete by creating CC filling in the void and cracks in the remind matrix of RCA and the new-formed matrix of the concrete. As shown in Figure 53, linear regression analysis between compressive strength of the prism at 28 days and ultrasonic pulse velocity was performed. The function expression of the fitting line is $y=61.05719x+2126.64421$. And the R square value arrives at 0.94248, which indicates that compressive strength has a strong linear positive correlation with ultrasonic pulse velocity. Therefore, it was concluded that the bacteria enhanced concrete compactness, improving the concrete strength.

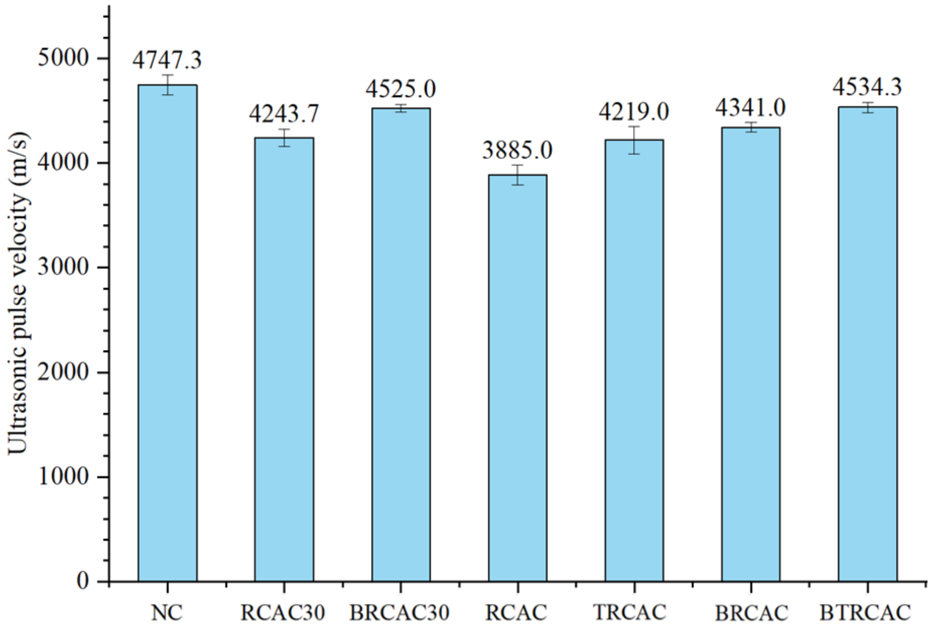


Figure 52. Ultrasonic pulse velocity in each group.

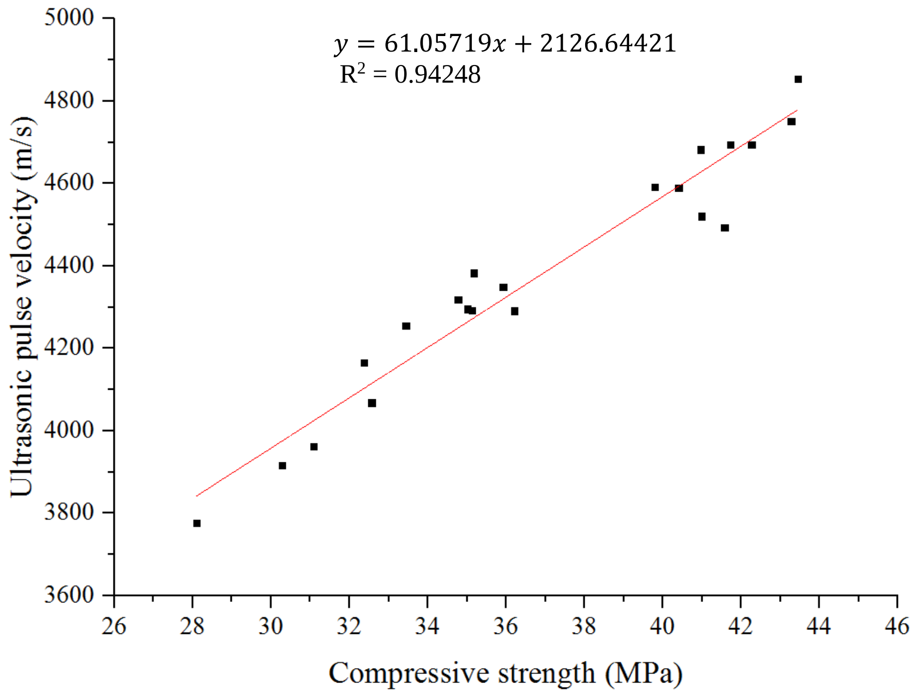


Figure 53. Linear regression analysis curve.

Section 5.5 Durability of RCAC with denitrification bacteria

Section 5.5.1 Mixing design

NC, RCAC, BRCAC and BTRCA groups whose mixing design was shown in Table 13 were used in durability tests. In addition, $Ca(NO_3)_2$ and $Ca(HCOO)_2$ may influence the durability of concrete [114]; hence, RCAC with nutrition (8.2 kg/m^3 of calcium formate and 2.9 kg/m^3 of calcium nitrate) was designed and labelled RCAC+N.

Section 5.5.2 SEM observation

The micro-structures of the cement matrices of RCAC, BRCA, and BTRCAC (magnified 5000 times) are shown in Figure 54. It was found that the matrix of RCAC showed more cracks and looser structure compared with that of BRCAC. CC was observed in the voids of Calcium Silicate Hydrate CSH with further magnification in BRCAC. Figure 55 compares the micro-structures of ITZ of RCAC without bacteria and with bacteria. The CC crystals were observed in the matrix inside the concrete. It was observed that the ITZ of bacterial groups was denser than non-bacterial groups. CH crystals were detected in the new ITZ of RCAC.

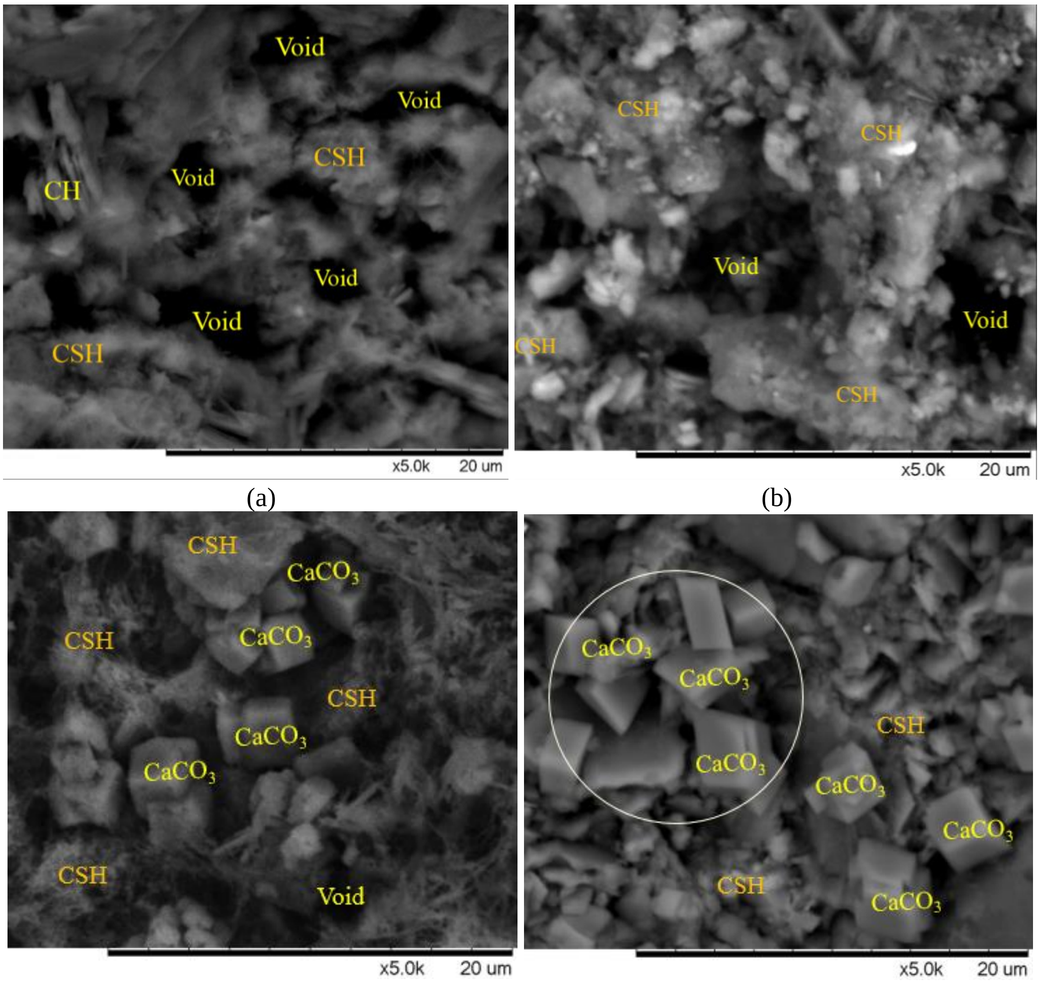


Figure 54. Micro-structures of cement matrices in (a) NC, (b) RCAC , (c) BRCAC, and (d) BTRCAC.

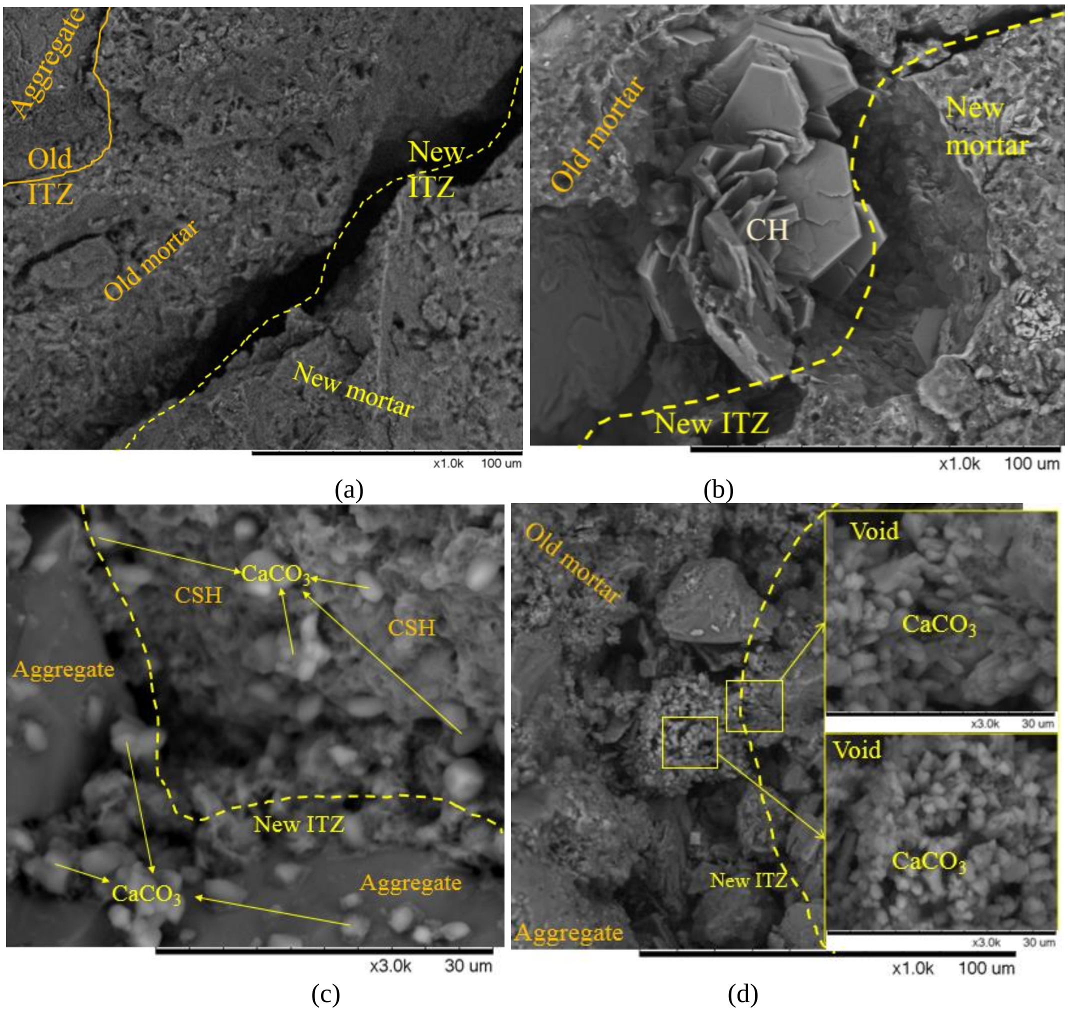


Figure 55. Micro-structures in ITZs of (a) NC, (b) RCAC, (c) BRCAC, and (d) BTRCAC.

Section 5.5.3 Thermogravimetric analysis (TGA) results

The sampling position is shown in Figure 56. The weight loss due to temperature increment for RCAC, BRCAC and BTRCAC are summarised in Figure 57 (a). Their corresponding first derivative of cement matrix from RCAC and BRCAC are depicted in Figure 57 (b) ~ (d), respectively. During the heating, dehydration of CSH, ettringite (Aft) and monosulphate (Afm), de-hydroxylation of CH and decarburization of CC occurred in sequence [63,89,233]. As shown in Figure 58, based on the weight change (%) of this decomposition reaction, the weight proportion of CC and CH were calculated. The weight change (%) of bound water was used to represent the relative weight of other hydration products, including CSH, Aft and Afm, due to there being no fixed chemical formulae of these mixtures. The quantification results showed that the weight proportion of CC in BRCAC and BTRCAC was 23.2% and 28.5%, while in RCAC, it was only 19.6%. The CH weight proportion of BRCAC and BTRCAC were 0.7 and 2.7 percentage points lower than that of RCAC. According to the weight change of bond water, the weight proportion of cement hydration product RCAC was higher than that of BRCAC and BTRCAC. This was verified by the SEM observation of CH crystals in the pores between the new and old mortars in RCAC. Water was absorbed and accumulated in the new ITZ due to the loose structure and high porosity of RCA, leading to the formation of a considerable amount of CH crystals [178,234]. Carbon dioxide was created in the bacterial groups during the denitrification process based on Eq. 10 ~ Eq. 15; CH was transformed into CC by combining with carbon dioxide. Concurrently, bio-mineralisation created CC, contributing to the significant increase in its weight proportion in the bacterial groups.

Figure 56. Sampling position

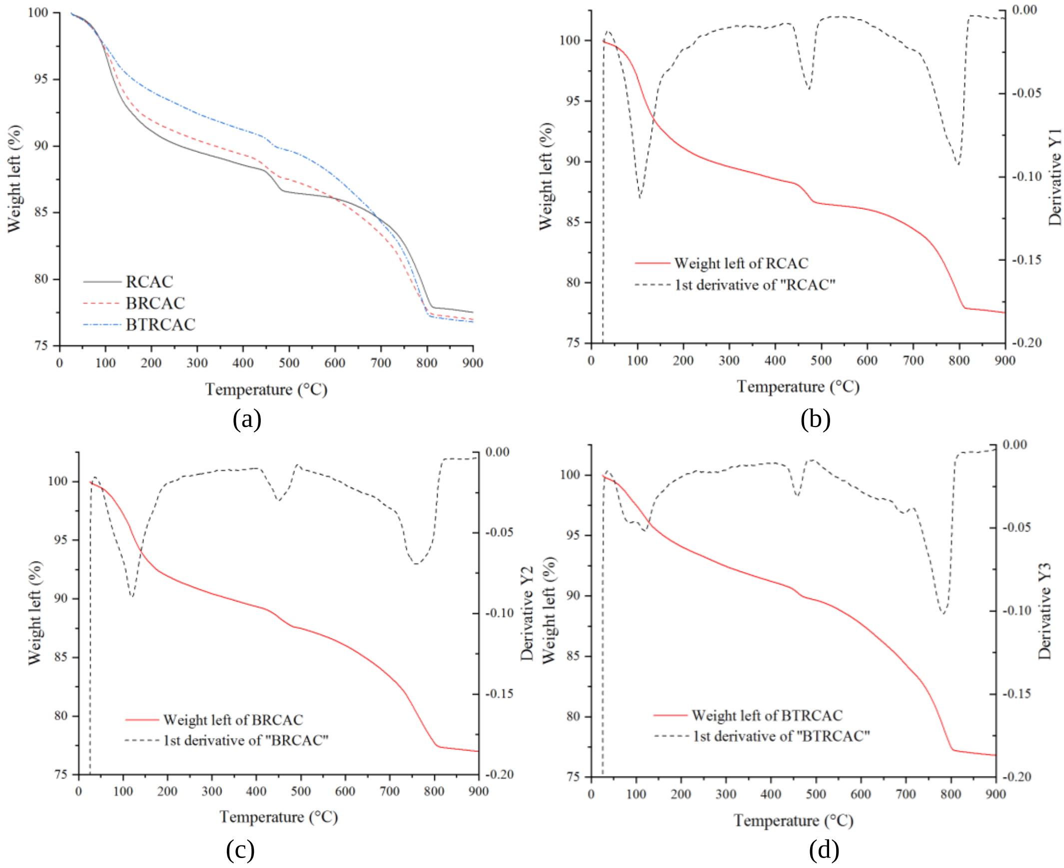


Figure 57. TGA graphs of samples from each group.

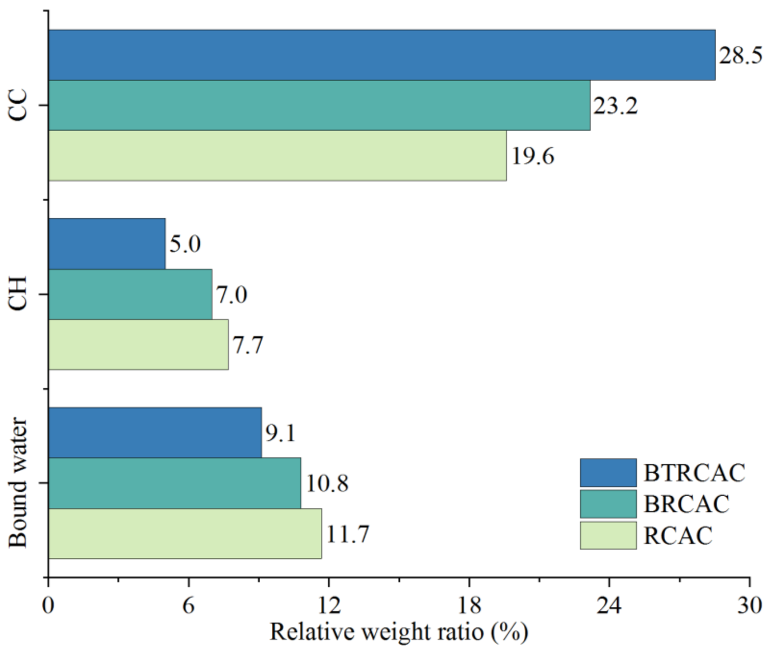
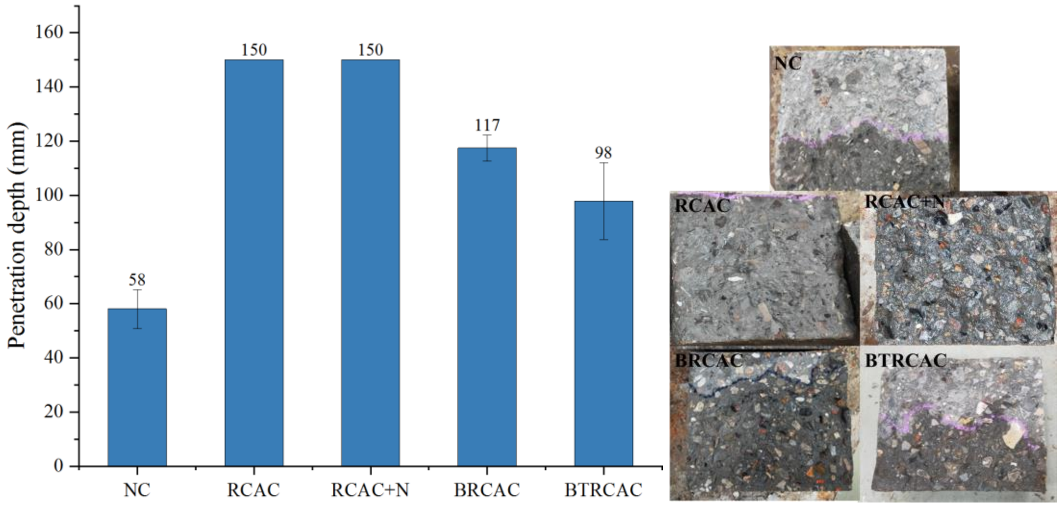


Figure 58. Quantification of the bound water, CH, and CC content.

Section 5.5.4 Depth of penetration of water under pressure and absorption test result

Water penetrating concrete can create cracks during freeze-thaw cycles, significantly reducing concrete durability. Water permeability and water absorption indirectly reflect the freeze-thaw resistance of concrete. The average water penetration depth and water absorption in each concrete group are shown in Figure 59 and Figure 60. The average water penetration depth of NC was 58 mm. Water easily penetrated RCA compared with NCA due to the loose structure of the former. The 100% replacement of NCA by RCA significantly increased the water permeability of concrete. Consequently, all specimens in the RCAC and RCAC+N groups were fully saturated by water after 18–20 h. However, through the enhancement provided by the denitrification bacteria to the BRCAC and BTRCAC groups, water was prevented from penetrating the specimens for 24 h; the water penetration depths were reduced to 117 and 98 mm, respectively. The water absorption test result agreed with that of the water permeability test. The water absorption of BRCAC compared with that of RCAC decreased by 24.7%. Because the water absorption of TRCA is lower than that of RCA, the water absorption of bacterial concrete with TRCA (BTRCAC) compared with that of bacterial concrete with RCA (BRCAC) further decreased by 10.9%. In previous research, 27.0% [98], 22.0% [65], 23.5% [99], and 23.1% [100] reductions in water absorption were also observed when ureolytic bacteria were applied to concrete. Therefore, similar to ureolytic bacteria, denitrification bacteria also efficiently reduced water penetration into the concrete. Moreover, no ammonia was released during concrete mixing, curing, and testing; hence, denitrification bacteria might be a potential strain for enhancing concrete.



(a) (b)

Figure 59. The average depth of water penetration (a) and photos of specimens after being split up (b) (RCAC and RCAC+N were overpassed by water; the valve was the height of the specimen).

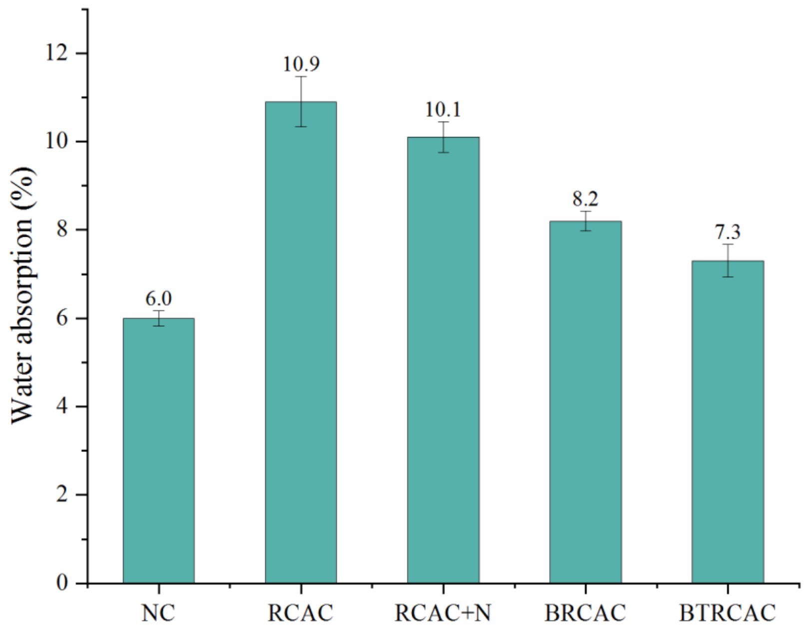


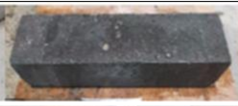
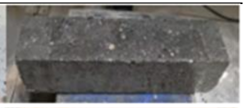
Figure 60. Water absorption of each group.

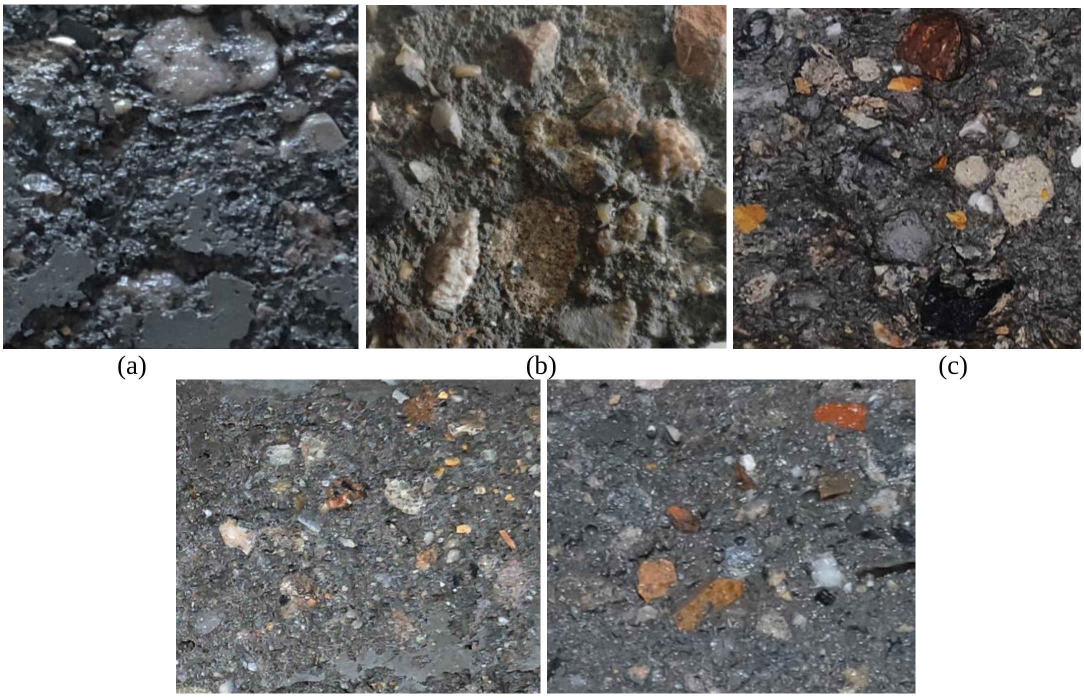
Section 5.5.5 Freeze-thaw resistance

Section 5.5.5.1 Results

The morphology of prism specimens after 0, 25, 100, and 175 freeze-thaw cycles are summarised in Table 14. The morphology remained relatively intact for the NC groups, and few aggregates were exposed. However, the RCA replacement significantly decreased the concrete’s freeze-thaw resistance. Although the addition of nutrients slightly increased the freeze-thaw resistance of RCAC, the mass loss of concrete from both the RCA and RCAC+N groups exceeded 5% after 175 cycles. The surface cement pastes of the specimens in the RCAC and RCAC+N groups completely peeled off, and the aggregates began to be deprived after 100 cycles, resulting in sharp mass loss. The introduction of bacterial solution considerably reduced the mass and RDME loss rates. The BRCAC and BTRCAC groups, compared with the RCAC and RCAC+N groups, endured 50 and 75 more cycles, respectively. By observing the surface of the damaged concrete, a considerable part of the body of RCA in the RCAC and RCAC+N groups is exposed. In contrast, for BRCAC and BTRCAC, only the RCA surface is exposed; in the NC group, some aggregates are still covered by the cement matrix. Moreover, the old mortar of RCA was found to easily disintegrate when exposed due to the bacterial solution, whereas NCA and the original aggregates of RCA were well-preserved. The mass and RDME loss rates of each group are shown in Figure 62 and Figure 63, respectively. The mass and RDME loss rates of the NC group were still under the upper limit specified by GB/T50082-2009 [220] after 300 cycles. The residual compressive strength of bacterial specimens also improves significantly, as shown in Figure 64. The loss rates of BRCAC and BTRCAC were 27.7% and 20.0%, respectively, whereas that of RCAC was recorded as 73.3%. According to GB/T 50028-2009 [220], the freeze-thaw resistance levels of the NC, RCAC, RCAC+N, BRCAC, and BTRCAC groups reached F300, F150, F150, F200, and F225, respectively. Moreover, according to JGJ/T 193-2009 [235], after the introduction of bacteria, both BRCAC and BTRCAC can satisfy the frost resistance requirement for plain concrete in areas with fresh water and low-freezing areas with seawater (average temperature (the coldest month) > −4 °C) in China.

Table 14 Morphologies of prism specimens after 0, 25, 100, and 175 freeze-thaw cycles.

	0 cycles	25 cycles	100 cycles	175 cycles
NC				
RCAC				
RCAC+N				
BRCAC				
BTRCAC				



(a) (b) (c)

(d) (e)

Figure 61. Damaged surfaces of (a) NC, (b) RCAC, (c) RCAC+N, (d) BRCAC, and (e) BTRCAC after 175 cycles.

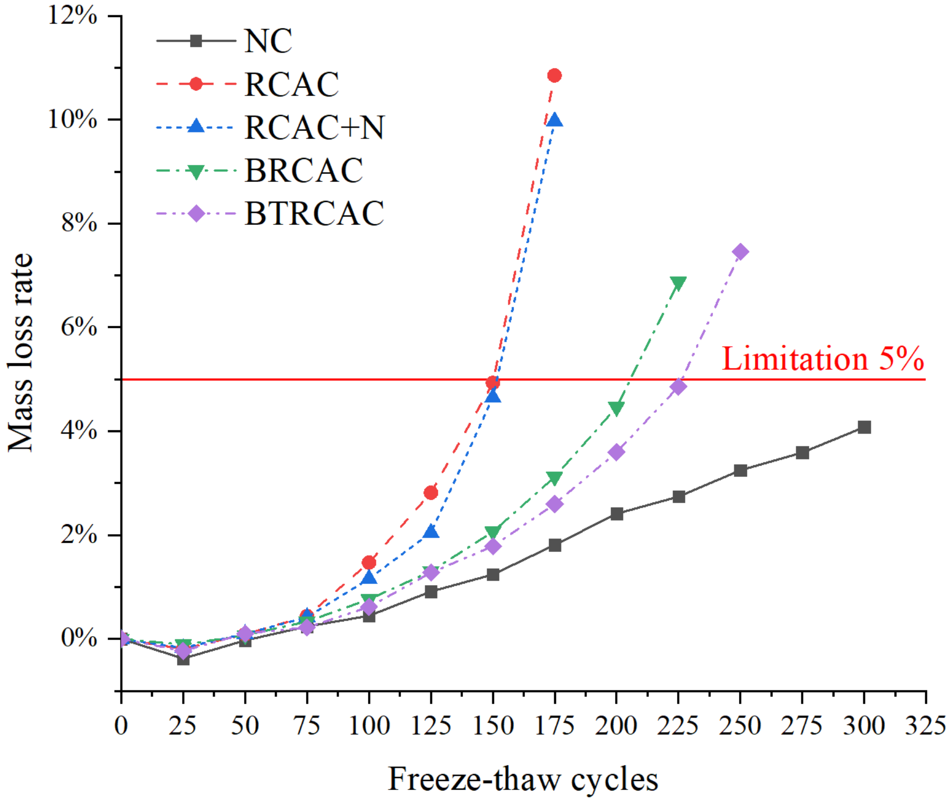


Figure 62. Mass loss rate of each group every 25 cycles.

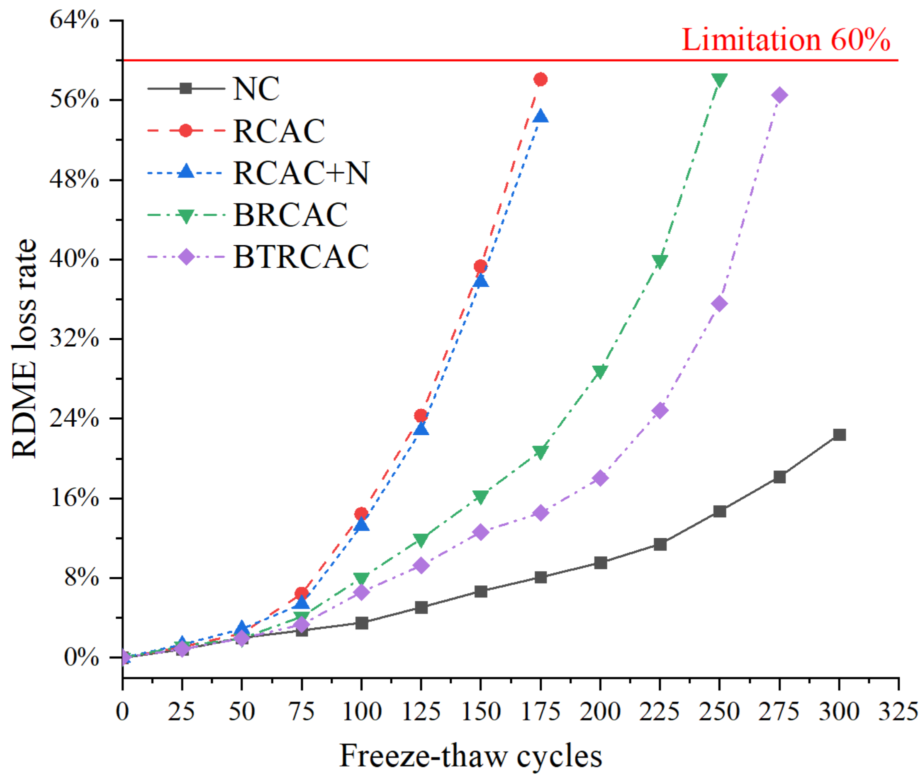


Figure 63. RDME loss rate of each group every 25 cycles.

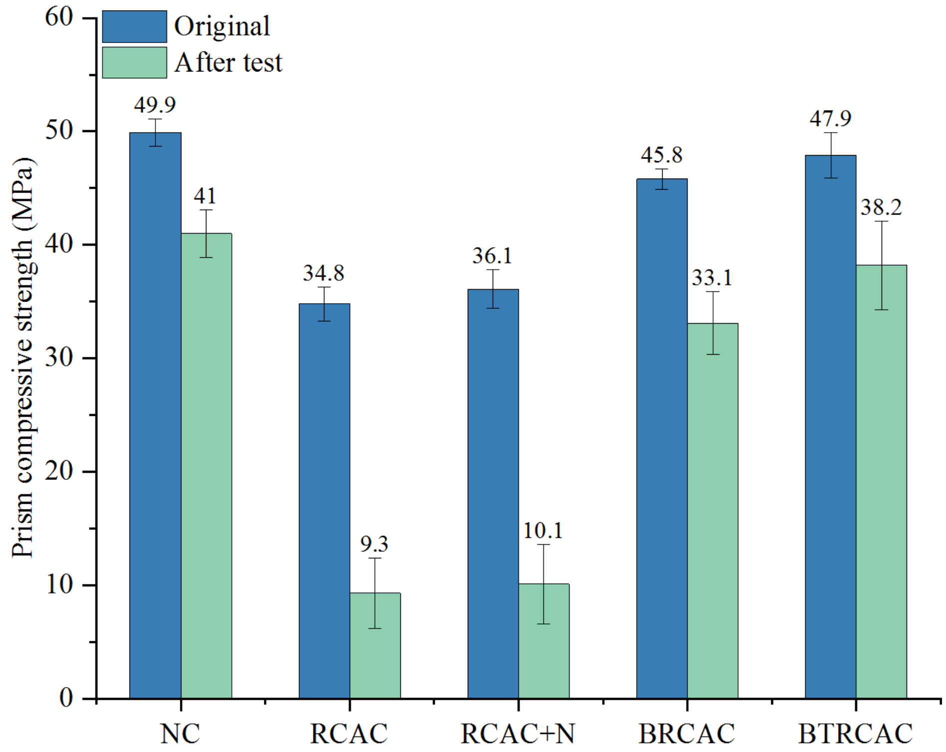


Figure 64. Residual compressive strength of each group.

Section 5.5.5.2 Influence of water absorption on freeze-thaw resistance

As shown in Figure 65, the freeze-thaw resistance represented by the RDME loss rate (after 175 cycles) and water absorption presented a positive relationship. Previous research [166] also indicated a strong positive relationship between freeze-thaw resistance and water absorption. This implies that water absorption has a considerable influence on freeze-thaw resistance. Due to the loose structure and high porosity of the old mortar left in RCA, the complete replacement of NCA by RCA significantly increased the water permeability/absorption and deteriorated the freeze-thaw resistance of concrete. From the macro-perspective, the amount of water in concrete when freezing occurs determines the swelling increment; thus, according to the hydrostatic pressure hypothesis, this affects the degree of damage [236]. The introduced bacteria effectively reduced the free water absorbed inside the concrete and contributed to enhancing freeze-thaw resistance.

Section 5.5.5.3 Influence of microstructure on freeze-thaw resistance

As shown in Figure 65, the freeze-thaw resistance of non-bacterial groups has a linear relationship with water absorption. The function expression of the fitting line is y=10.38334x-54.03577. (R^2 = 0.99392). In contrast, all the points of bacterial groups are below the fitting line, indicating that in addition to decreasing water absorption, bacteria also improved the capacity of concrete to resist water-freezing expansion from the micro-structure perspective; when bacteria were introduced into RCAC, CC crystals formed inside the concrete voids. Bacteria cells, approximately 0.9–1.2 μm in size, have the capacity to move; hence, upon reaching the open pores larger than 1 μm, the bio-mineralisation procedure can occur. Seifan et al.[237] indicated that the CC produced by bacteria exhibited a high affinity to fill the pores inside the concrete. Yousef et al. [62] asserted that the bacteria moving into the voids also plugged the pore connectivity by creating CC. Hence, the introduction of bacteria effectively densified the matrix and increased the capacity of concrete to resist water-freezing expansion. The bacteria cells (0.9–1.2 μm in size) that are unable to fill the pores smaller than 1 μm close the pores; this is also considered a positive factor for freeze-thaw resistance [238].

The ITZs of concrete groups with bacteria compared with that of the RCAC group show a denser structure because the voids and pores of RCA are filled by the CC crystals created by bacteria. Moreover, CC also provides a nucleation site for hydration products, thus rendering a dense connection between the aggregate and matrix [184]. Consequently, the introduction of bacteria into concrete decreased the expansion effect of frozen water in the ITZs. It improved the cohesion of ITZs to withstand the expansion effect, significantly improving the freeze-thaw resistance. In contrast, excess CH has been considered a negative factor for concrete strength and durability

[234,239]. Hence, introducing the bacterial solution into concrete also increased the freeze-thaw resistance of RCAC because bacteria consumed CH [240].

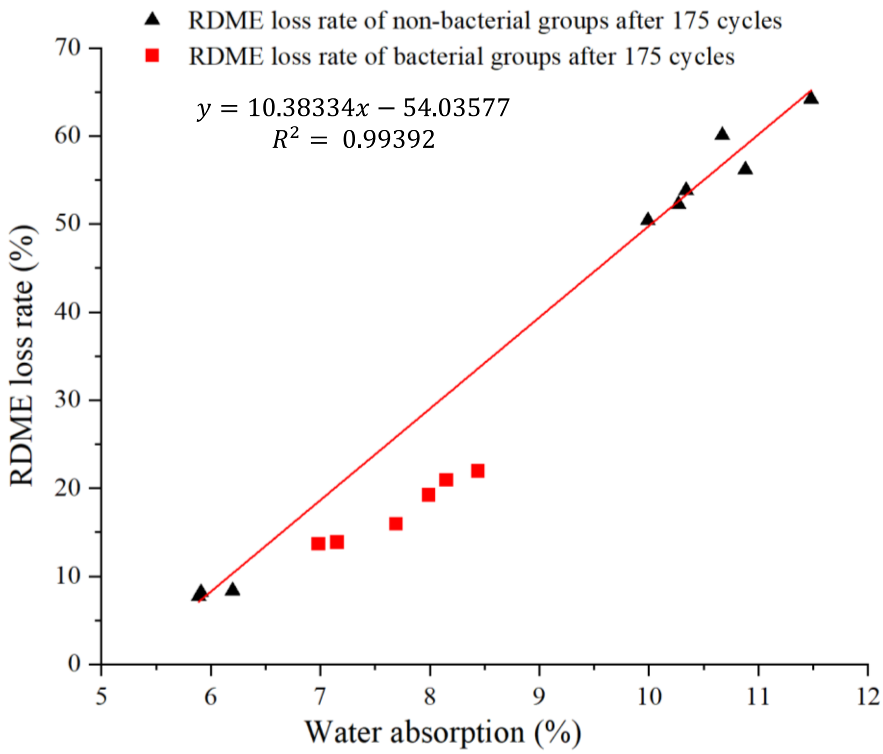


Figure 65. Relationship between the RDME loss rate and water absorption.

Section 5.6 Summary

This chapter explored the effectiveness of using denitrification bacteria to enhance RCA and concrete with RCA. The three strains of denitrification bacteria were attempted. Psp showed its superiority in enhancing mortar specimens. Hence, Psp was applied in treating RCA and fresh concrete mixing; and considerable improvements in this type of concrete were found. The following points can be drawn:

- The mortar testing results showed that 10^7 CFU/ml of Psp bacteria with 0.5 g/ml of glucose was the optimised composition for inducing the biomineralisation process in the cement matrix.
- Replacing NC with RCA significantly decreases the mechanical performance and durability of concrete. Introducing calcium formate and calcium nitrate into

concrete without bacteria shows limited improvement in the durability of RCAC.

- Denitrification bacteria (Psp) can improve the properties of RCA. By soaking RCA in denitrification bacterial solution, a maximum 14% reduction of water absorption, 4% increase of apparent density and 10% reduction of crushing value were detected compared with RCA soaked in the non-bacterial solution.
- Either replacing raw RCA with treated RCA or introducing denitrification bacteria into fresh concrete during mixing can improve the compressive strength of the RCA concrete. Through the joint use of both methods, the compressive improved by 27.5% and 24.6% at 7 days and 28days, respectively. A 7.5% improvement was also found in elastic modulus.
- Introducing denitrification bacteria into fresh concrete can efficiently improve the tensile splitting strength. In contrast, substituting raw RCA with treated RCA is less effective in improving tensile splitting strength because few bacteria survive after drying the RCA. By directly introducing the bacterial solution, the development of enhancement potential can be achieved in 28 days or less when all the bacterial cells become the cores of CC crystals.
- By ultrasonic pulse velocity test and SEM analysis, it can be concluded that the bacteria filled the voids and pores inside concrete by producing CC crystals in microcosmic. The improvement of microstructure correlates well with the property improvement at the macroscopic level.
- From the macro perspective, bacteria can reduce water absorption and permeability, and increase the tensile strength of concrete, thus improving freeze-thaw resistance.
- From the micro-perspective, bacteria directly introduced into fresh concrete can consume CH in ITZs as well as fill pores and voids by creating CC, contributing

to a dense connection between the aggregate and matrix. The pre-treatment of RCA can further improve this effect. Bacteria can reduce the expansion effect of frozen water in ITZs and improve the cohesion of ITZs to withstand the expansion effect, significantly enhancing the freeze-thaw resistance.

- The introduction of bacteria in two stages of concrete production enables the application of RCAC to certain frost areas, further easing the environmental pressure caused by mining and using NCA.
- Overall, denitrification bacteria can substantially increase the strength and durability of concrete. Because no poisonous or polluting substances are produced, this approach is an environmentally friendly method for enhancing the freeze-thaw resistance of RCAC. This can also enable the widespread application of this concrete in cold regions.

Chapter 6. Result and discussion of life cycle assessment (LCA) of bio-based recycled waste concrete

Section 6.1 Goal and scope definition

There is currently a lack of studies focusing on the sustainability of concrete with FA, RCA, and bacteria. To judge whether the introduction of bacterial solution can improve the sustainability of concrete production, this chapter explored the influence of bacteria Sp and Psp on the concrete with FA and RCA and conducted LCA analysis on concrete products in different scenarios. The LCA comparison of this study includes material production processes and transportation processes from the "cradle to gate" stage. A comprehensive evaluation including workability, strength, durability, and EI was conducted on nine scenarios to assess the sustainability of concrete with FA, RCA, and bacteria. Quantified EI was calculated and analysed in this chapter based on the mechanical performance in the previous experiment.

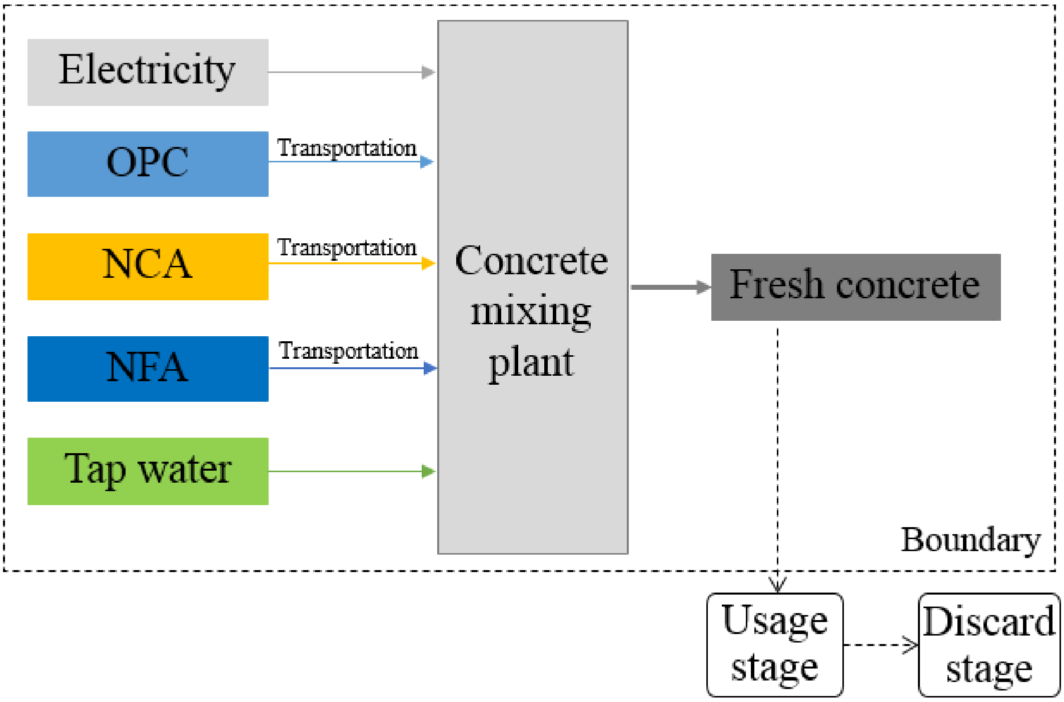
Section 6.1.1 Product System

In this chapter, the concrete produced by the different scenarios was designed and analysed based on the experimental results of previous chapters. Nine mixing designs of concrete were used and summarised in Table 15, six 100 mm cubes of each mixing design were cast, and compressive strength, tensile splitting strength, and water absorption at 28 days were assessed and listed in Table 15. It was assumed that similar EI of each scenario would contribute to the usage and discard stages [51]. Figure 66 shows the system boundary for concrete manufacture of each scenario, and Figure 67 shows the transportation model and distance. After considering the package mass of each product, a parameter of 1.01 was multiplied when calculating the transportation mass.

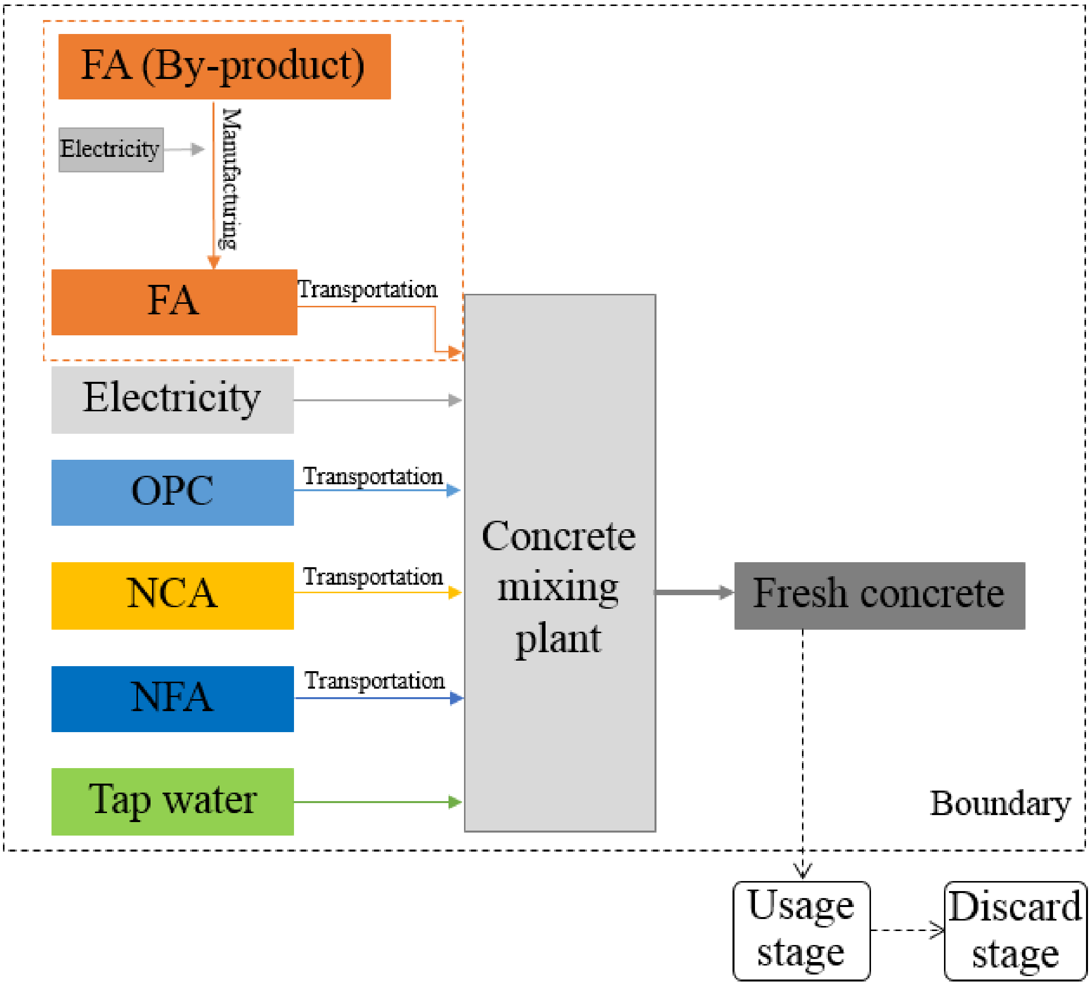
Table 15 Mix proportions of concrete in each concrete.

	Unit	S1	S2	S3	S4	S5	S6	S7	S8	S9
Bacteria	10^{12} cells	N/A	N/A	N/A	Sp	Sp	Sp	Psp	Psp	Psp
Urea	kg/m^3	0.0	0.0	0.0	3.9	3.9	3.9	0.0	0.0	0.0
$Ca(HCOO)_2$	kg/m^3	0.0	0.0	0.0	0.0	0.0	0.0	8.2	8.2	8.2
$Ca(NO_3)_2$	kg/m^3	0.0	0.0	0.0	2.3	2.3	2.3	2.9	2.9	2.9
OPC	kg/m^3	433.6	260.2	260.2	260.2	260.2	260.2	260.2	260.2	260.2
FA	kg/m^3	0.0	173.4	173.4	173.4	173.4	173.4	173.4	173.4	173.4
NFA*	kg/m^3	620.6	620.6	620.6	620.6	620.6	620.6	620.6	620.6	620.6
NCA*	kg/m^3	1152.5	1152.5	0.0	1152.5	0.0	0.0	1152.5	0.0	0.0
RCA*	kg/m^3	0.0	0.0	1042.9	0.0	1042.9	0.0	0.0	1042.9	0.0
TRCA*	kg/m^3	0.0	0.0	0.0	0.0	0.0	1068.5	0.0	0.0	1063.2
Water	kg/m^3	195.1	195.1	195.1	195.1	195.1	195.1	195.1	195.1	195.1
Glucose	g/m^3	0.0	0.0	0.0	0.0	0.0	0.0	97.6	97.6	97.6
Compressive strength (STD)	MPa	44.2 (2.6)	34.2 (2.9)	33.3 (4.1)	40.1 (3.9)	39.8 (4.7)	41.4 (3.7)	42.8 (2.1)	41.9 (3.7)	42.1 (3.9)
Tensile splitting strength (STD)	MPa	4.8 (0.1)	3.9 (0.2)	3.8 (0.3)	4.5 (0.2)	4.3 (0.3)	4.4 (0.3)	4.8 (0.2)	4.4 (0.3)	4.8 (0.3)
Water absorption (STD)	%	5.9 (0.2)	6.0 (0.3)	4.5 (0.3)	7.0 (0.2)	5.4 (0.4)	5.5 (0.4)	7.1 (0.3)	5.4 (0.3)	5.8 (0.4)
Slump (STD)	mm	57.0 (2.1)	73.0 (3.9)	81.0 (4,7)	78.0 (3.1)	82.0 (5.1)	87.0 (6.3)	81.0 (2.9)	85.0 (5.8)	82.0 (6.9)

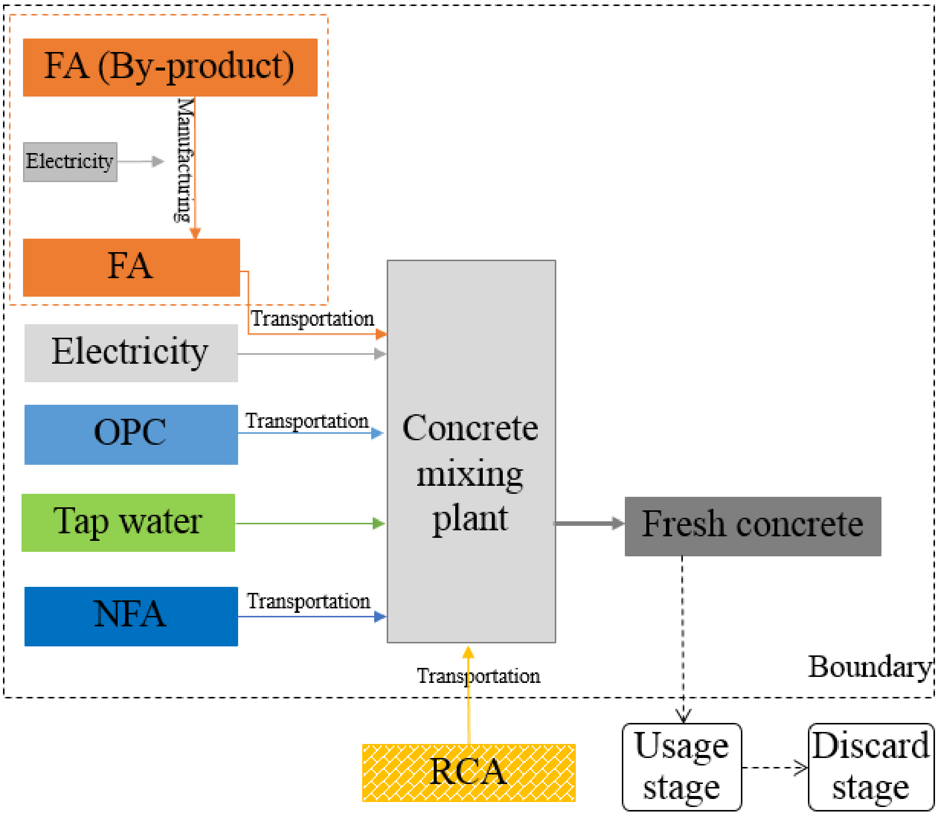
*The mass of aggregate was considered in SSD condition


Electricity
OPC
Transportation
NCA
Transportation
NFA
Transportation
Tap water
Concrete mixing plant
Fresh concrete
Boundary
Usage stage
Discard stage

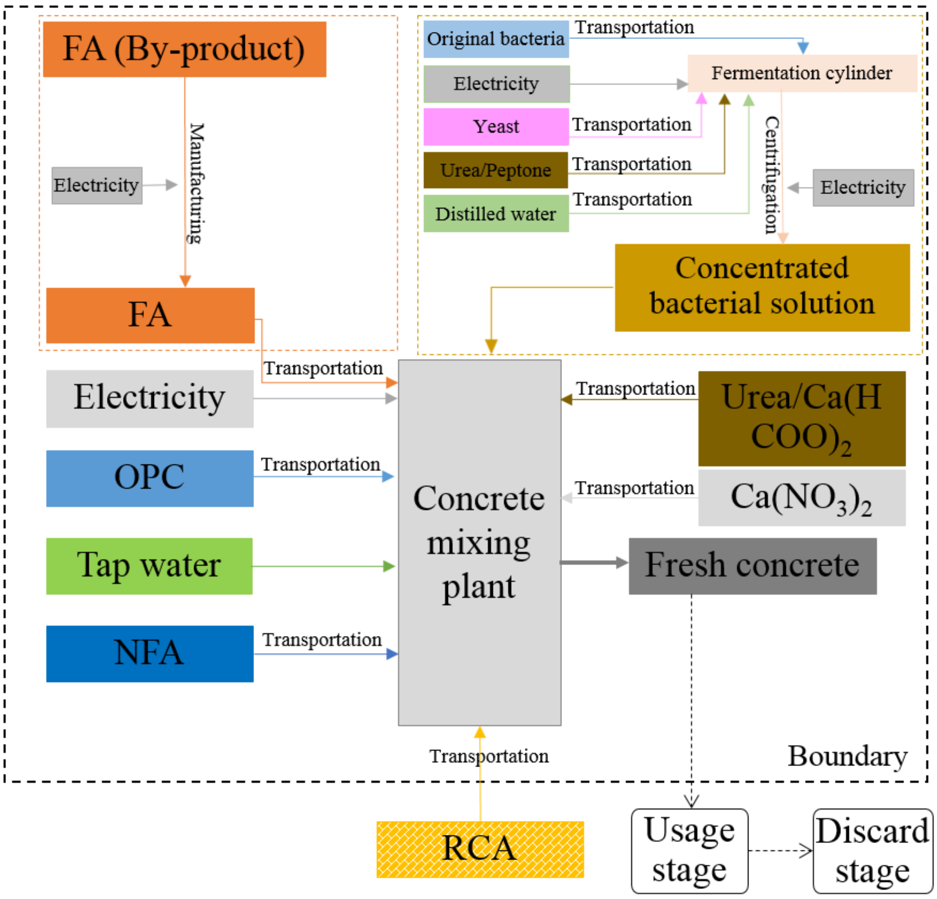
(a)


FA (By-product)
Electricity
Manufacturing
FA
Transportation
Electricity
OPC
Transportation
NCA
Transportation
NFA
Transportation
Tap water
Concrete mixing plant
Fresh concrete
Boundary
Usage stage
Discard stage

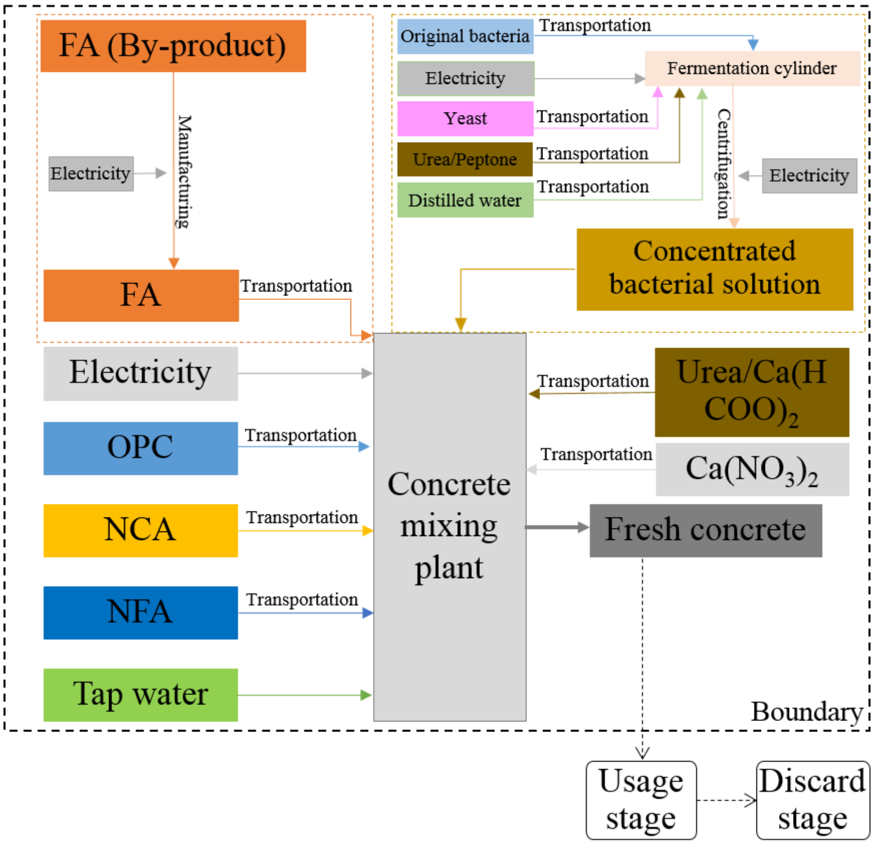
(b)


FA (By-product)
Electricity
Manufacturing
FA
Transportation
Electricity
OPC
Transportation
Concrete mixing plant
Tap water
Fresh concrete
NFA
Transportation
Transportation
Boundary
RCA
Usage stage
Discard stage

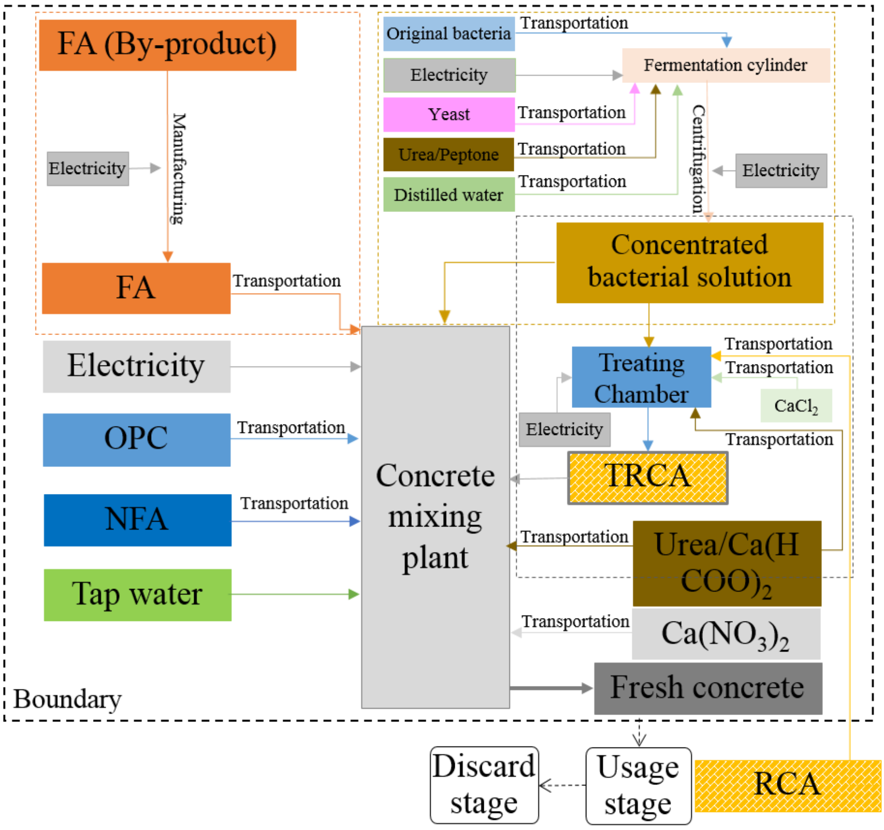
(c)


FA (By-product)
Electricity
Manufacturing
FA
Original bacteria
Transportation
Electricity
Fermentation cylinder
Yeast
Transportation
Urea/Peptone
Transportation
Distilled water
Transportation
Centrifugation
Electricity
Concentrated bacterial solution
Transportation
Electricity
Transportation
Urea/Ca(HCOO)$_2$
OPC
Transportation
Transportation
Ca(NO$_3$)$_2$
Concrete mixing plant
Tap water
Fresh concrete
NFA
Transportation
Transportation
Boundary
RCA
Usage stage
Discard stage

(d)



(e)



(f)

Figure 66. System boundaries of S1(a), S2 (b), S3(c), S4 and S7 (d), S5 and S8 (e), and S6 and S9 (f)

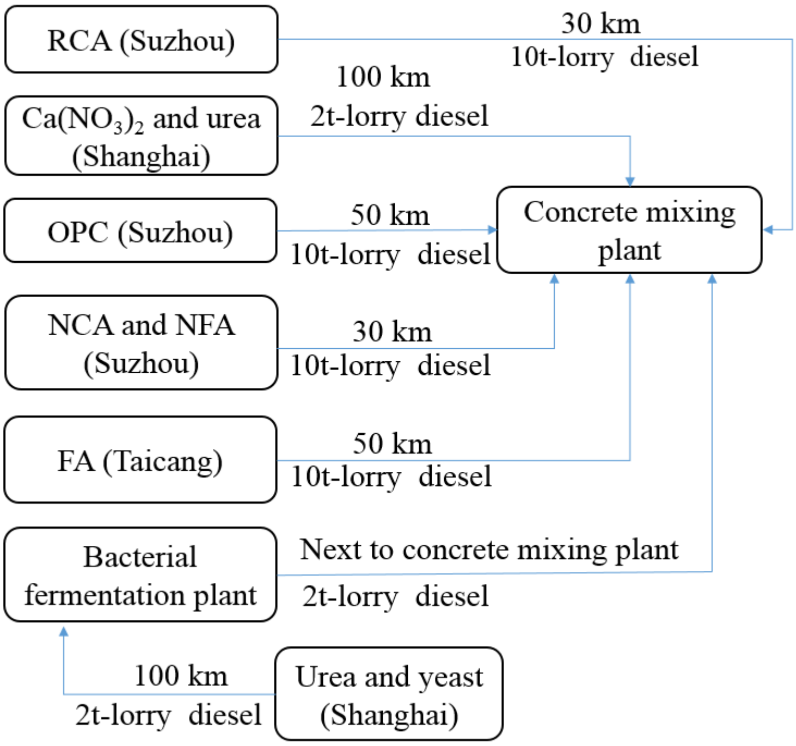


Figure 67. Transportation mode and distance adapted for Suzhou.

Section 6.1.2 System boundary

Generally, cost, labour, and equipment construction are out of the research scope of LCA analysis. Ideally, a comprehensively LCA analysis should contain all phases, including the production phase, usage and maintenance phase, and abandonment and demolition phase, from "cradle to grave". However, the "cradle to gate" was adopted in this project for the following reason:

1). The emissions of concrete in different scenarios during the use and maintenance phase are almost the same [241].

2). It is difficult to obtain the data for the usage and maintenance phase and abandonment and demolition phase. No existed building is constructed with the new construction materials, and the service life of concrete construction may arrive 100 years; hence, there is no data that can be referenced and cannot investigate the data in a short time.

3). “Cradle to gate” also can be used as a system boundary according to the ISO 14040 [48].

Therefore, life cycle stages were “cradle to gate,” which included the production of all raw materials used in the concrete mixing, as well as the EI created by transporting all these materials from the production place to the concrete plant.

Section 6.1.3 Functional unit and reference flow

According to the test results, the compressive strength of each scenario was different; thus, simply using volume or mass as a functional unit may be biased. Due to the compressive strength being the most crucial performance of concrete, the functional unit considered using volume divided by compressive strength and written in one m^3/MPa concrete.

Section 6.2 Life Cycle Inventory (LCI)

Section 6.2.1 Allocation method of FA and RCA

FA and electricity are created by coal fire power stations simultaneously, and both of them can be sold and create economic benefit. Hence, the FA and electricity should share the emissions caused by coal fire power stations. Nevertheless, the economic benefits of FA and electricity are much different. Therefore, though there is no consensus on the allocation method in FA [242,243], the economic allocation method is considered more suitable than the mass allocation method for FA [202,244]. Hence, the allocation ratio was calculated by Eq. 24. The economic value evaluation referenced by *China Electric Power Industry Annual Development Report 2021* [245] are listed in Table 16. The emission of coal power generation is referenced [245–247] and shown in Table 17. 1.39% of these emissions were allocated to FA. In addition, manufacturing FA, equal to the electricity of 0.011 kWh/kg FA, was also involved in the FA production process [7].

Disposal C&DW was considered a burden on the city [248]. Hence, a cut-off rule is commonly recommended to be applied to RCA in predicting the EI of the concrete product [249,250]; thus, the EI contribution caused by their parent concrete production was also ignored. Nevertheless, the contribution of manufacturing the C&DW into RCA was considered in this research. In addition, using RCA can avoid the EI caused by landfilling was also considered [250,251].

Table 16 Amount and prices of FA and coal-fired power

	Amount of generation (m)	Market price (¥)	Allocation ratio by economic method
Coal-fired power	1 kWh	0.795 Yuan/kWh*	98.61%
FA	55.9 g/kWh coal-fired power	200 Yuan/t	1.39%

* Unit kWh price of 380 V industrial electricity in Jiangsu province [252].

$$R = \frac{(¥\times m)_{\text{by-product}}}{(¥\times m)_{\text{main product}} + (¥\times m)_{\text{by-product}}} \times 100\% \quad (24)$$

Where R is the Allocation ratio by economic method; $(¥ \times m)_{\text{by-product}}$ is the total economic cost of by-products and $(¥ \times m)_{\text{main product}}$ is the total economic cost of the main product.

Table 17 Emission of the coal-fired power station for generating 1 kWh electricity FA [246]

Emissions	Emission amount g
CO_2	690.01
SO_2	6.92
No*x*	6.79
CO	1.48
TSP*	13.32
Wastewater	680.96

*TSP: total suspended particulate

Section 6.2.2 Data source

The raw materials of concrete were calculated according to the mixing design, and the electricity of concrete mixing is referred to as the Eco-invent 3.5 concrete production 40 MPa [253]. Bacteria culturing in bio-lab is a complex procedure [254,255] that is not suitable for actual construction. Bacteria can be cultured in a fermentation cylinder and manufactured into powders for sale to a broad market; the raw materials include original bacteria, yeast, distilled water and urea, and electricity heating autoclaving and centrifugation. The bacteria were assumed to be acquired from large-scale culturing in a fermentation cylinder. The background data of materials and energy were collected from the Chinese Life Cycle Database (CLCD) [256] and Eco-invent 3.1 [257], as listed in Table 18.

Table 18 The data source of concrete production.

Item	Unit of measurement	Upstream process
OPC	kg	CLCD
FA	kg	Allocated by economic method
Tap water	kg	CLCD
NFA	kg	CLCD
NCA	kg	CLCD
RCA	kg	N/A
Treating RCA for recycling	kg	Eco-invent 3.5
Disposing and landfilling RCA	kg	Eco-invent 3.5
TRCA(Sp)	kg	Live process data (Table 19)
TRCA(Psp)	kg	Live process data (Table 20)
Sp	Unit*	Live process data (Table 21)
Psp	Unit*	Live process data (Table 22)
Urea	kg	CLCD
$Ca(NO_3)_2$	kg	CLCD
$Ca(HCOO)_2$	kg	Eco-invent 3.5
Glucose	g	Eco-invent 3.5
The electricity of concrete mixing	kWh**	CLCD

*1 unit included around 10^{12} bacteria cells and some nutrition substance weighed 1kg.
**All electricity in this project is considered to acquire from East China Power Network.

Table 19 Live process data of 3kg TRCA(Sp).

Item	Unit of measurement	Amount	Upstream process
RCA	g	980.9	N/A
Electricity (Heating)	Wh	1.1	CLCD
Water	kg	0.3	CLCD
$CaCl_2$	g	22.2	CLCD
Bacterial concentrated solution	Unit	1	Live process data (Table 21)
Urea	g	20.0	CLCD

Table 20 Live process data of 3kg TRCA(Psp).

Item	Unit of measurement	Amount	Upstream process
RCA	g	980.9	N/A
Electricity (Heating)	Wh	1.1	CLCD
Water	kg	0.3	CLCD
$Ca(NO_3)_2$	g	22.2	CLCD
Psp concentrated solution	Unit	1	Live process data (Table 21)
Urea	g	20.0	CLCD

Table 21 Live process data of one-unit (10^{10}cells) Sp concentrated solution.

Item	Unit of measurement	Amount	Upstream process
Electricity (Heating, autoclaving, and centrifugation)	Wh	76.5	CLCD
Yeast	g	1.0	Eco-invent 3.5
Water	kg	0.1	CLCD
Original bacteria	g	1.0	N/A
Peptone	g	2.0	Eco-invent 3.5
Urea	g	2.0	CLCD

Table 22 Live process data of one-unit (10^{10}cells) Psp concentrated solution.

Item	Unit of measurement	Amount	Upstream process
Electricity (Heating, autoclaving, and centrifugation)	Wh	76.5	CLCD
Yeast	g	1.0	Eco-invent 3.5
Water	kg	0.1	CLCD
Original bacteria	g	1.0	N/A
Peptone	g	4.0	Eco-invent 3.5

Section 6.2.3 Selected EI categories

The LCIA analysis was performed using the eFootprint platform developed by IKE Environmental Technology Co. Ltd in this study. It contained CLCD adopted in previous LCA research [203–206]. Six EI categories, including Global Warming Potential (GWP), Acidification Potential (AP), Photochemical Ozone Formation Potential (POFP), Particulate matter (RI), Ionizing radiation (IRP), Primary Energy Demand (PED), were selected to analysis as shown in Table 22.

Table 23 Selected EI categories

Life-Cycle Inventory	Unit
Global Warming Potential (GWP)	kg CO_2 eq.
Ecotoxicity on freshwater (ET-freshwater)	CTUe
Photochemical Ozone Formation Potential (POFP)	kg NMVOC eq.
Particulate matter (RI)	kg PM2.5 eq.
Ionizing radiation (IRP)	kg U235 eq
Primary Energy Demand (PED)	MJ

Section 6.3 Life Cycle Impact Assessment (LCIA)

Section 6.3.1 LCIA results and sensitivity analysis

Table 24 and Table 25 summarise the characterised value of the eight selected EI of the nine scenarios and the relative value shown in Figure 68. Generally, applying FA into concrete decreased all selected EI categories. Further introducing the RCA only decreased the IRP. Introducing ureolytic bacteria into concrete decreased GWP, PED, POFP, IRP and ET-freshwater while raising the contribution to RI. Compared with ureolytic bacteria, denitrification bacteria showed a similar threshold in POFP and ET-freshwater, decreasing the GWP, PED and RI while significantly increasing the IRP. Both kinds of TRCA concrete showed significantly high contribution.

Table 24 EI results to produce 1 m^3 concrete of the nine scenarios

No	GWP	POFP	RI	IRP	ET-freshwater	PED
	kg CO_2 eq	kg NMVOC eq	kg PM2.5 eq	kBq U235 eq	CTUe	MJ
S1	3.42E+02	4.31E-01	2.47E-01	1.25E+00	3.07E+03	2.89E+03
S2	2.25E+02	3.69E-01	2.36E-01	1.06E+00	2.38E+03	2.34E+03
S3	2.21E+02	3.73E-01	2.46E-01	4.13E-01	2.60E+03	2.30E+03
S4	2.33E+02	3.18E-01	2.11E-01	8.90E-01	2.30E+03	2.06E+03
S5	2.28E+02	3.14E-01	2.15E-01	3.75E-01	2.35E+03	1.98E+03
S6	2.71E+02	3.11E-01	2.94E-01	5.77E-01	2.83E+03	2.41E+03
S7	2.42E+02	3.06E-01	2.08E-01	1.56E+00	2.19E+03	2.16E+03
S8	2.37E+02	3.06E-01	2.15E-01	1.06E+00	2.30E+03	2.12E+03
S9	3.20E+02	3.45E-01	3.19E-01	3.65E+00	3.42E+03	3.20E+03

Table 25 EI results to produce one function unit concrete of the nine scenarios

No	GWP	POFP	RI	IRP	ET-freshwater	PED	Normalised threshold
	kg CO_2 eq	kg NMVOC eq	kg PM2.5 eq	kBq U235 eq	CTUe	MJ	
NFs*	2.37E+13	2.80E+11	4.61E+10	4.29E+12	2.94E+14	N/A	
S1	7.74E+00	1.26E-02	7.43E-03	3.11E-02	7.72E+01	6.98E+01	8.03E-13
S2	6.59E+00	1.08E-02	7.09E-03	2.65E-02	5.99E+01	5.66E+01	6.80E-13
S3	6.63E+00	1.09E-02	7.38E-03	1.03E-02	6.53E+01	5.55E+01	7.03E-13
S4	5.81E+00	9.31E-03	6.33E-03	2.22E-02	5.78E+01	4.97E+01	6.17E-13
S5	5.73E+00	9.19E-03	6.47E-03	9.34E-03	5.91E+01	4.79E+01	6.18E-13
S6	6.54E+00	9.10E-03	8.84E-03	1.44E-02	7.10E+01	5.82E+01	7.45E-13
S7	5.65E+00	8.94E-03	6.25E-03	3.90E-02	5.51E+01	5.22E+01	6.02E-13
S8	5.66E+00	8.95E-03	6.47E-03	2.64E-02	5.79E+01	5.12E+01	6.14E-13
S9	7.59E+00	1.01E-02	9.58E-03	9.10E-02	8.60E+01	7.74E+01	8.78E-13

*Normalisation factors(NFS) referenced by EU Environmental Footprint 3.0 (EF 3.0) [258]

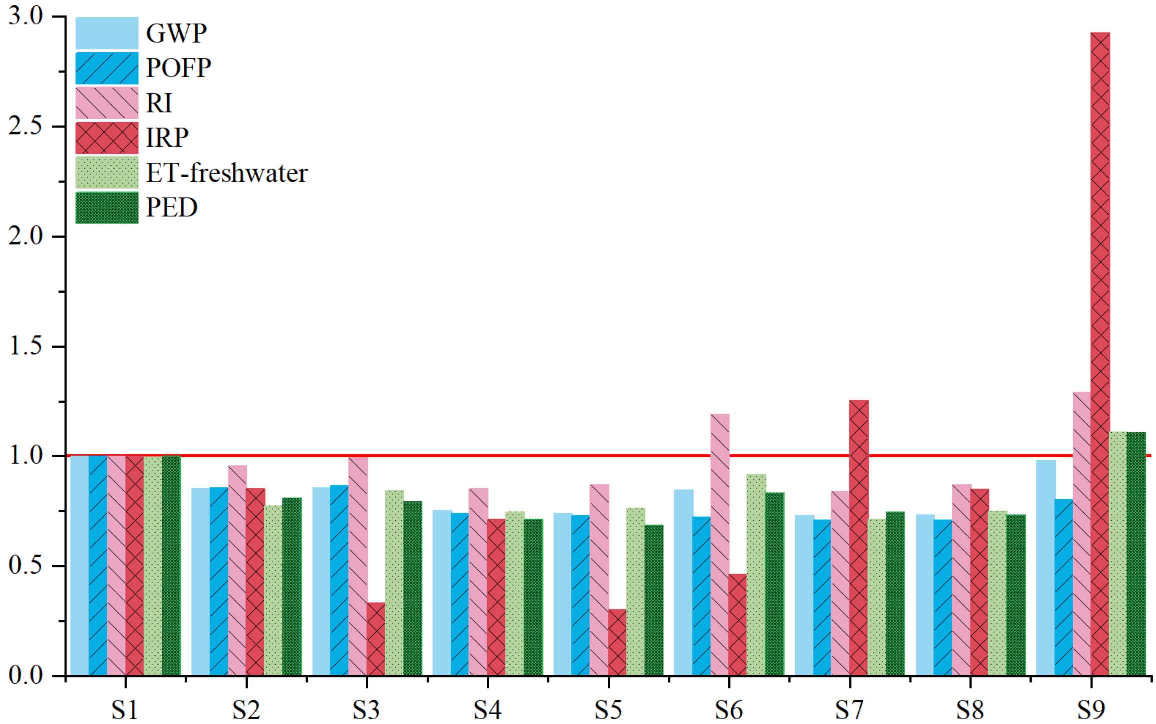


Figure 68. Relative value of the eight selected EI of the nine scenarios

Section 6.3.2 OPC and FA

Figure 69 compares the contribution of the same mass of OPC and FA. It was found that the unit mass EI values of FA were significantly lower than OPC in GWP, PED, POFP, IR, IRP and ET-freshwater, respectively. Hence, substituting the OPC can efficiently decrease the EI of concrete.

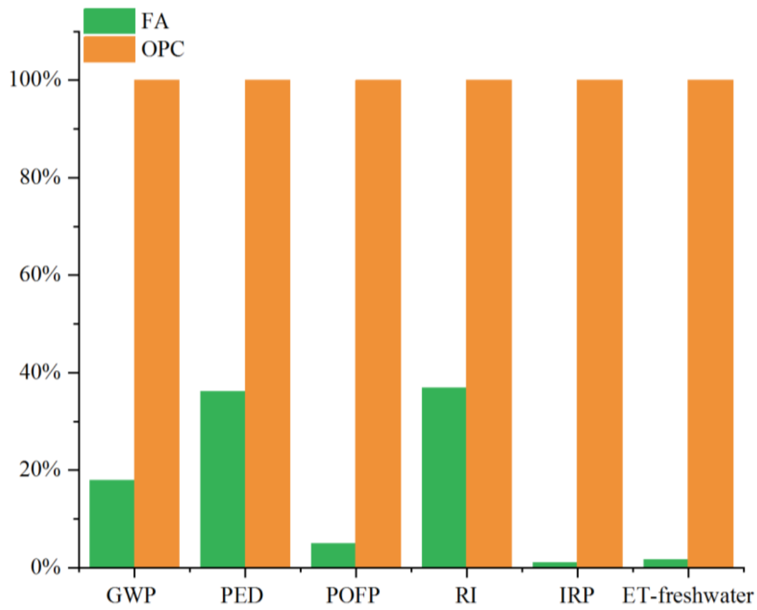


Figure 69. Relative EI contribution of OPC and FA with the same mass.

Section 6.3.3 NCA, RCA, TRCA(Sp) and TRCA(Psp)

As shown in Figure 70, except for RI, the selected EI of RCA was negative. According to Eq. 33, the negative value means that the EI created by manufacturing CD&W to RCA was lower than treating and landfilling, except for RI. The effectiveness of treating RCA by bacteria in improving the properties of RCA was reported [184,185,187,259]; however, the usage of urea, calcium chloride, calcium formate and calcium nitrate showed high EI contribution, as shown in Figure 71 and Figure 72. Moreover, the contribution of NCA was insignificant; hence, the TRCA scenarios (S6 and S9) exhibited extremely high EI. The high EI contribution was because of nutrition and calcium source. In nature, these bacterial strains can use organic, nitrogen or sulphur sewage and also has biomineralisation ability [20]. Hence, by replacing the nutrition and calcium source with some waste substance, the sustainability of TRCA concrete might be increased significantly. For the ureolytic treatment method, urea production was the most sensitive factor. Calcium formate was the most sensitive factor for the denitrification bacterial treatment method. Formate ions as the carbon source were replaceable because the denitrification bacteria can utilize various carbon sources [60,260], including the organic in sewage [261], nitrate ions and calcium ions to induce biomineralisation. According to the LCIA results, the EI values of glucose only occupied 23.3%, 37.1%, 19.0%, 11.1%, 5.5% and 24.1% of formate ions in GWP, PED, POFP, RI, IRP, and ET-freshwater, respectively. If the carbon source was replaced by organic sewage, the EI might be minus due to contaminant reduction. Therefore, the bio-treated RCA still showed potential to improve the sustainability of concrete.

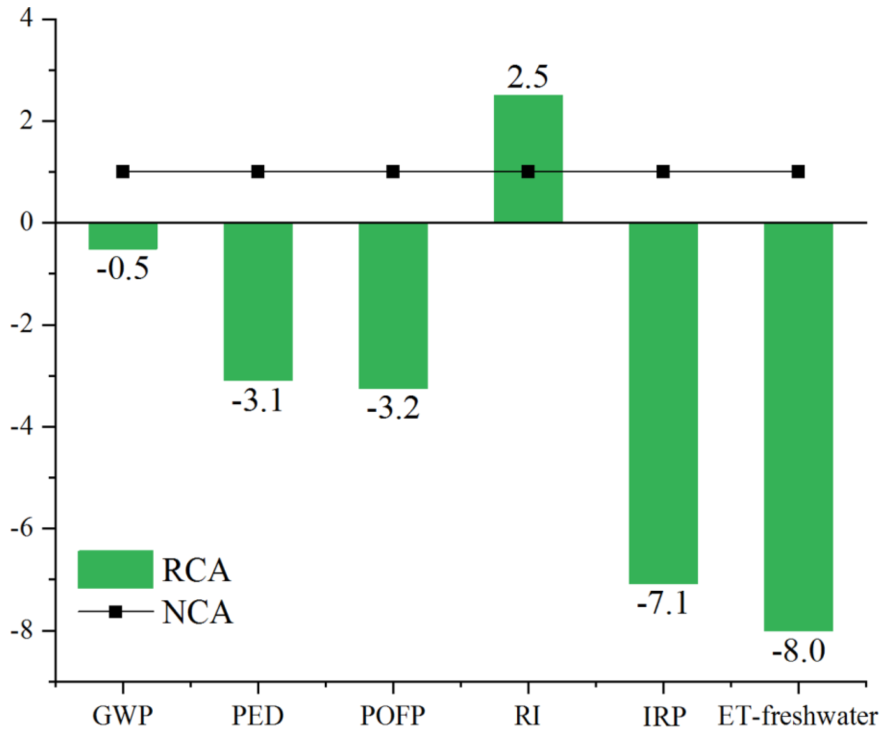


Figure 70. Relative EI contribution of NCA and RCA with the same volume.

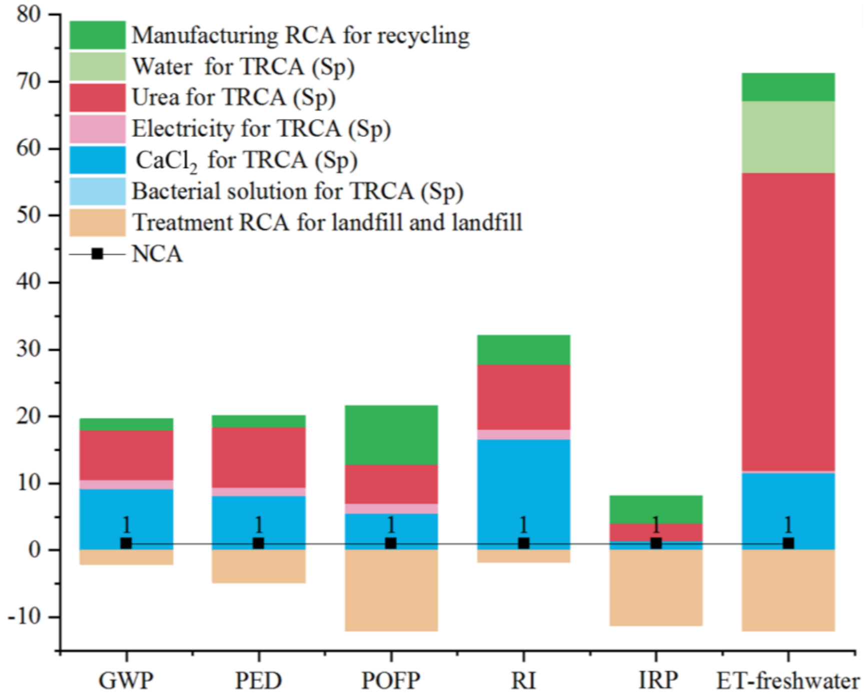


Figure 71. Relative EI contribution of NCA and TRCA (Sp) with the same volume.

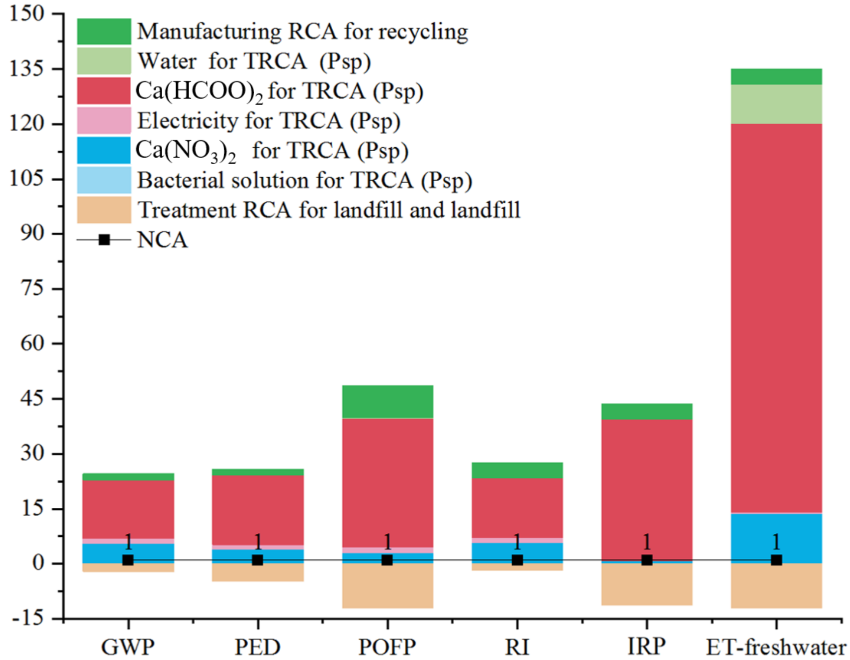


Figure 72. Relative EI contribution of NCA and TRCA with the same volume.

Section 6.3.4 Global warming potential (GWP)

The results of GWP with the unit of CO_2 eq emissions are shown in Figure 73. OPC production was the most sensitive factor for all scenarios. Hence, replacing OPC with FA significantly decreased the GWP. Both bacteria productions showed negligible GWP, while urea, calcium nitrate, and calcium formate as the nutrition and calcium source for biomineralisation contributed considerable CO_2eq emissions. Due to the strength enhancement, S4 and S7 and S5 and S8 showed much lower CO_2eq emissions per functional unit concrete than S2 and S3, respectively.

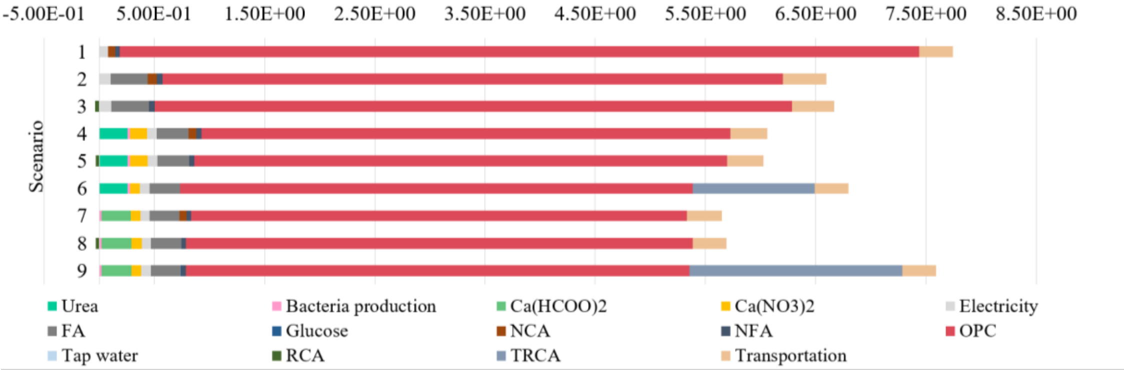


Figure 73. Contribution of each process to GWP.

Section 6.3.5 Photochemical ozone formation potential (POFP)

The results of POFP with the unit of kg NMVOC eq. are shown in Figure 74. OPC contributed the most percentage of POFP, and replacing OPC with FA also significantly decreased the value of this category. Due to the low sensitivity of nutrition, calcium source and bacteria production, the POFP increase caused by the biomineralisation method was mild. All the bacterial scenarios significantly reduced the POFP of concrete production, attributing to their strength enhancement effect.

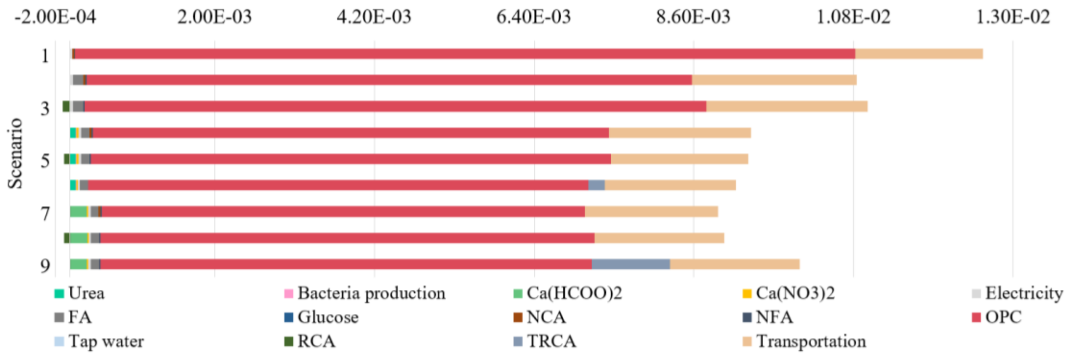


Figure 74. Contribution of each process to POFP.

Section 6.3.6 Particulate matter (RI)

The results of IR with the unit of kg PM2.5 eq. are shown in Figure 75. OPC contributed the most in this category. Usage of FA showed the most limited advantage for reducing RI in all selected EI categories, and RI caused by RCA was much higher than NCA; hence the RI of concrete with FA and RCA (S3) showed higher RI than S1.

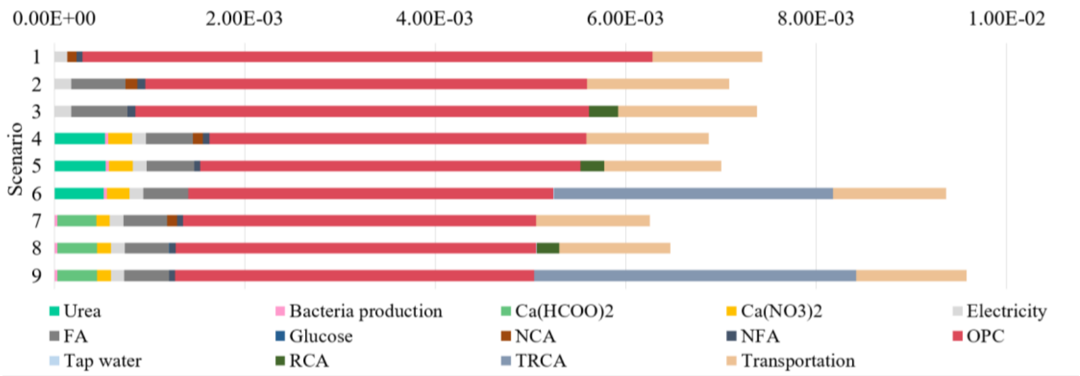


Figure 75. Contribution of each process to RI.

Section 6.3.7 Ionizing radiation potential (IRP)

The results of IR with the unit of kBq U235 eq. are shown in Figure 76. The contribution of OPC was most, except for the TRCA, while that of FA was negligible. Calcium formate showed extremely high sensitivity accounting for the negative influence of all denitrifications bacterial scenarios in reducing IRP and pretreating RCA by Psp bacterial solution consumed much more calcium formate compared to other scenarios; thus, the IRP of S9 was significantly higher than other scenarios. Replacing NCA with RCA significantly decreased the IRP. Fortunately, according to EF 3.0 [258], IRP only occupied a low ratio of total normalised EI. Hence, S9 did not show extremely high normalised EI, as shown in Figure 79.

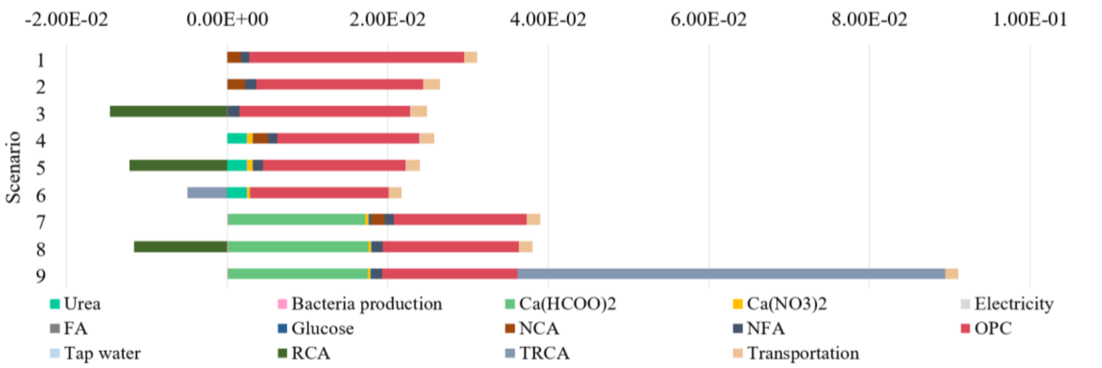


Figure 76. Contribution of each process to IRP.

Section 6.3.8 Ecotoxicity freshwater (EP-freshwater)

The results of EP-freshwater with the unit of CTUe are shown in Figure 77. OPC was still the most sensitive factor, while the sensitivity of FA was not high in this category. Hence, FA scenarios (S2~S5) contribute less than S1. The sensitivity of urea was relatively high, while due to the significant strength enhancement of the biomineralisation method, the EP-freshwater contribution of S3 and S4 was still lower than S2 and S3.

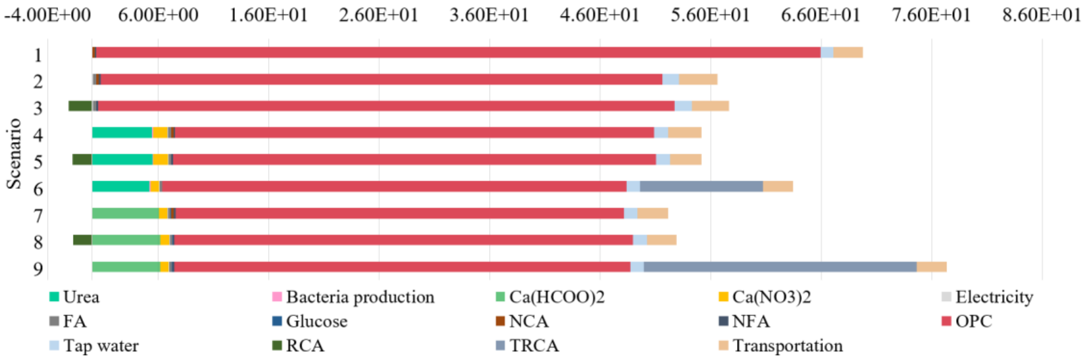


Figure 77. Contribution of each process to EP-freshwater.

Section 6.3.9 Primary energy demand (PED)

The results of PED with the unit of MJ are shown in Figure 78. OPC is still the most sensitive process of concrete production. The FA substitution was also highly influential on PED reduction. Replacing NCA with RCA considerable decreased the PED consumption of unit volume concrete. However, the reduction was not able to compensate for the increase of PED caused by compressive strength lost because the functional unit was m^3 concrete/MPa. The nutrition and calcium sources still contribute to considerable PED consumption; both biomineralisation methods decreased this category.

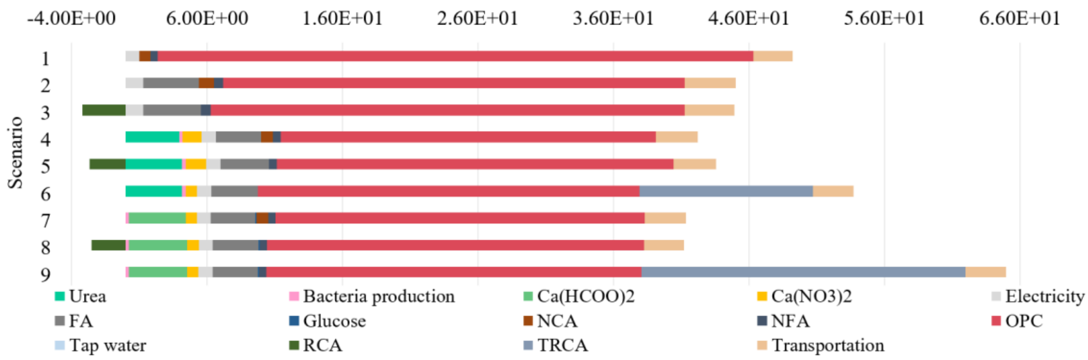


Figure 78. Contribution of each process to PED.

Section 6.3.10 Normalisation

The severity of different EI categories is different. Hence, obtain the total EI by sum the individual value is unreasonable. Hence, a weighted summation method should be adopted to obtain the total EI, which was called normalised EI. According to the EU Environmental Footprint 3.0 (EF 3.0) [258], the normalised threshold of each scenario was summed, as shown in Figure 79. It was found that using FA in concrete decreased the normalised EI, and the enhancement due to the utilisation of bacteria further reduced the EI. Compared with the benchmark scenario (S1), the accumulated normalised EI of S2~S8 decreased by 11.5%, 10.2%, 22.6%, 22.2%, 7.0%, 24.2%, and 22.2%nrespectively. While, due to the massive consumption of calcium formate for pre-treating TRCA(Psp), the accumulated normalised EI of S9 increased by 8.4%. The denitrification bacterial FA concrete (S7) was the scenario with the lowest EI. Though using RCA decreased the EI of the unit volume of concrete due to its adverse effect on strength, using RCA showed limited influence on normalised EI.

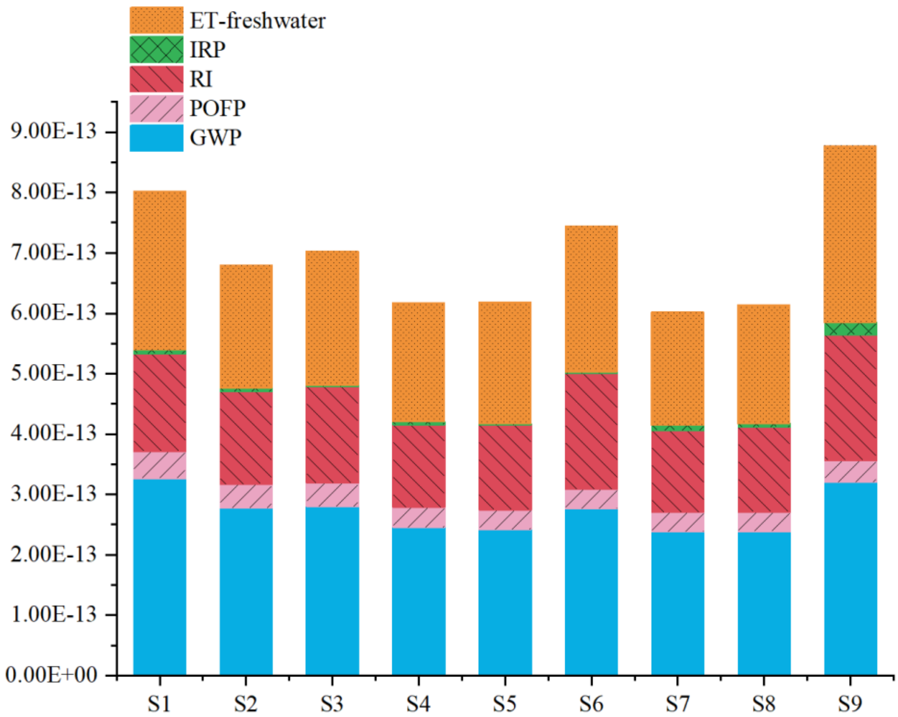


Figure 79. Normalised EI contribution of each scenario.

Section 6.4 Interpretation and comprehensive comparison

To address a comprehensive comparison of the nine scenarios, six dimensions, including environmental protection, energy-saving, workability, compressive strength, tensile strength, and durability, were evaluated by normalised EI threshold, PED, slump, compressive strength testing value, tensile splitting testing value, and water absorption testing value, of each scenario respectively. For the convenience of comparison, S1 was set as the benchmark item, and its six dimensions were marked as one point, respectively. Following a rule that the higher mark means better performance, environment protection, energy saving, compressive strength, tensile strength and durability of other scenarios were marked by the value of themselves divided by corresponding valves in S1; while their marks of workability were the quotient of the slump of S1 divided by their slump respectively, as listed in Table 26 and shown in Figure 80. It was observed that applying FA benefited environment protection, energy-saving, workability, and durability. 100% replacing NCA and RCA showed limited benefits for concrete with FA. The further intruding bacterial solution into FA concrete performed advantageous improvement of all tested items. Pre-treating RCA improved the strength and durability of concrete; nevertheless, it was detrimental to environment protection and energy saving.

Table 26 The relative mark of environmental protection, energy-saving, tensile strength, durability, workability, and compressive strength of each scenario.

	Environment protection	Energy saving	Workability	Compressive strength	Tensile strength	Durability
S1	1.00	1.00	1.00	1.00	1.00	1.00
S2	1.13	1.15	1.28	0.77	0.82	1.01
S3	1.12	1.11	1.42	0.75	0.79	0.76
S4	1.28	1.22	1.37	0.91	0.94	1.19
S5	1.29	1.20	1.44	0.90	0.90	0.91
S6	1.07	0.89	1.53	0.94	0.92	0.94
S7	1.30	1.25	1.42	0.97	1.00	1.21
S8	1.29	1.25	1.49	0.95	0.92	0.92
S9	0.92	0.75	1.44	0.95	0.99	0.98

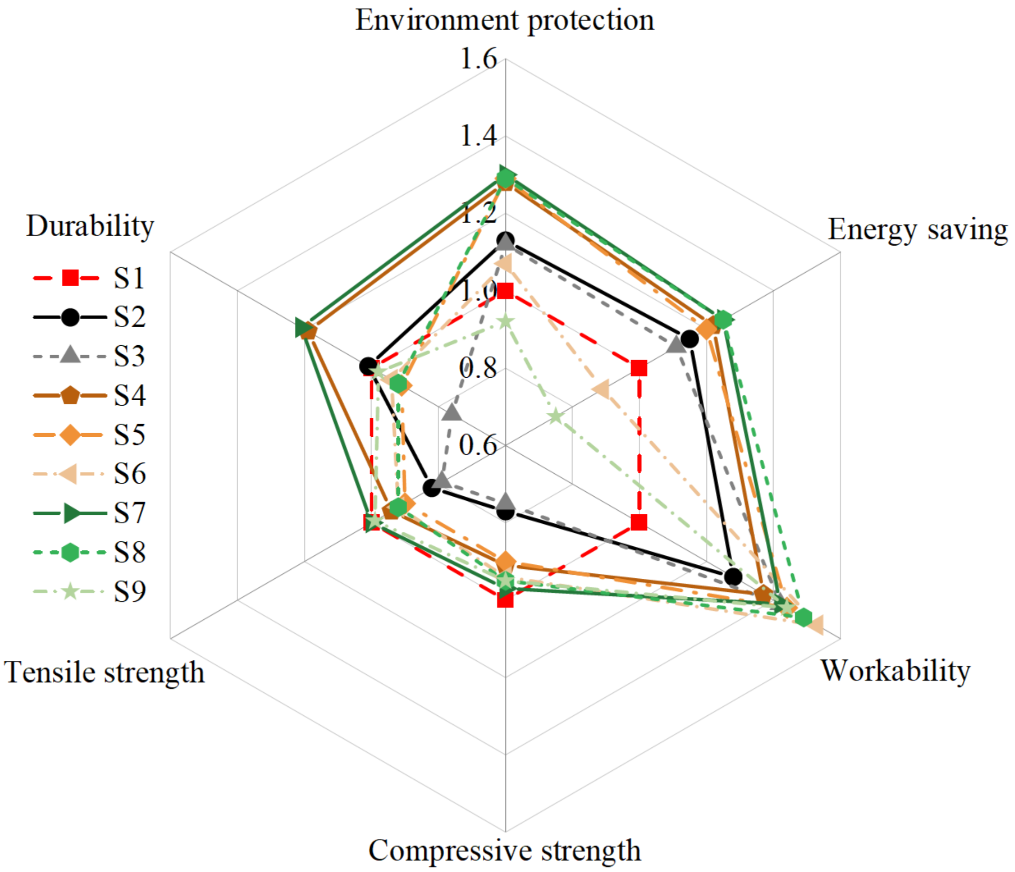


Figure 80. Comprehensive comparison of the nine scenarios

Section 6.5 Summary

OPC production is an exceedingly sensitive factor in concrete production. Replacing it with FA can decrease all the selected EI categories, even though the RI created by coal-fired power plants are allocated to FA by the economic allocation method. Using the biomineralisation method to enhance can further decrease the EI unit strength of concrete. Negligible EI contributions were created by bacterial solution production for the selected EI categories. However, the EI caused by using nutrition and calcium sources is considerable. Especially, calcium formate production is a heavy contamination process; hence, it is necessary to find some new carbon source to replace it. NCA only occupies a low percentage of the total EI contribution of concrete production. Hence, considering the adverse effect on strength, replacing them with RCA only decreases the IRP. Pre-treating the RCA with bacteria can increase the properties of RCA concrete. However, the environmental performance is unsatisfactory due to the high EI-contributed substance used in the bio-treating procedure. Hence, the chemical agent, especially calcium formate, should be replaced by soluble organic wastes and nitrate ions in wastewater.

Chapter 7. Conclusions and suggestions for future work

Section 7.1 Conclusions

This research intends to investigate the effects of the biomineralisation method on the mechanical performance and durability of the concrete with FA and RCA through extensive experimental tests and analysis. The usage of FA and RCA increased the sustainability of concrete. At the same time, the application of the biomineralisation method keeps the recycled waste concrete with qualified performance. The principal conclusions of the whole project can be drawn as follows:

- Ureolytic bacteria can significantly increase workability water permeability resistance, decrease water absorption, and improve the compressive strength of recycled waste concrete. The effectiveness is influenced by factors such as strain and concentration of bacteria and the culture medium. Especially when culturing ureolytic bacteria in a medium consisting of urea, bacteria with more powerful ureolytic capacity are selected, which caused a more significant improvement. However, it is inevitable to create ammonia due to the metabolism of ureolytic bacterial groups. Therefore, some substituted methods should be adopted to avoid the creation of ammonia in concrete.
- The replacement of NCA with RCA degrades the performance of concrete. However, the introduction of bacteria significantly increases the compressive and tensile splitting strengths. It reduces the water absorption and water permeability of RCAC by creating bio-induced CC that fills the voids and pores inside the concrete. The denitrification bacteria have the same enhancing ability as the ureolytic bacteria. More importantly, the biomineralisation method induced by denitrification bacteria can avoid creating polluted or poisonous substances.
- For enhancing the concrete with RCA into concrete, two methods, pretreating the

RCA with bacteria and mixing the bacterial solution into fresh concrete, exhibit significant improvement effects. The pretreatment of RCA creates more CC providing an improvement effect to RCA particles due to a more suitable environment out of concrete. In contrast, directly mixing bacteria into concrete creates less CC because the harsh environment inside the concrete partly deactivates the micro-organisms. However, CC is broadly distributed with fresh concrete mixing, filling the voids in both matrix and ITZ of concrete, as well as providing cores for hydration of cementitious materials.

- Besides improving mechanical performance, denitrification bacteria (Psp) can also improve the freeze-thaw resistance of recycled waste concrete. From the macro-perspective, bacteria can reduce water absorption and permeability, thus improving the freeze-thaw resistance by decreasing the water swelling. From the micro-perspective, bacteria directly introduced into fresh concrete can consume CH in ITZs and fill pores and voids by creating CC, contributing to a dense connection between the aggregate and matrix. The pretreatment of RCA can further improve this effect. Bacteria can reduce the expansion effect of frozen water in ITZs and improve the cohesion of ITZs to withstand the expansion effect, significantly enhancing the freeze-thaw resistance. Hence, this approach is an environmentally friendly method for enhancing the freeze-thaw resistance of RCAC and can also enable the widespread application of this concrete in cold regions.
- From the LCA aspect: OPC production contributes an exceedingly high percentage of total EI in concrete production. Hence, replacing OPC with FA decreases the EI of concrete. The enhancement of the biomineralisation method

further decreased the EI contribution of bio-based FA concrete. Negligible EI contributions were created by bacterial solution production for the selected EI categories. However, The EI caused by the usage of nutrition and calcium sources is considerable. Especially, calcium formate production is a heavy contamination process; hence, it is necessary to find some new carbon source to replace it. NCA only occupies a low percentage of the total EI contribution of concrete production. Hence, considering the adverse effect on strength, replacing them with RCA shows limited environmental benefit.

- In summary, the bio-based recycled waste concrete with acceptable performance and significantly low environmental impacts is a sustainable way to replace the traditional concrete.

Section 7.2 Future Work

- Although it has been practised in this project that uses the biomineralisation method induced by denitrification bacteria to enhance concrete, there are still limited numbers of studies compared with ureolytic bacteria. Hence, more strains of denitrification bacteria should be explored. In addition, specific bacterial strains for concrete usage should be screened or designed by gene modification.
- Although the method proposed in this project has significantly increased mechanical performance and durability, a gap in durability remains between recycled waste concrete and ordinary concrete. Future research may focus on common using another method, taking nanomaterials and fibres, to further enhance the performance of recycled waste concrete.
- The nutrition and calcium ions used in this project were provided by chemical agents, which created extra EI for bio-concrete production. Hence, it is necessary to use some wastes taken from wastewater with organics, calcium ions and nitrate

ions to replace the chemical agents. Moreover, the economic cost must be investigated. The cost of lab-cultured bacteria is unacceptable for actual construction. Hence, using a construction area, a bacterial fermentation technique for producing denitrification bacteria should be attempted.

Bibliography

[1] J. Xu, X. Wang, Self-healing of concrete cracks by use of bacteria-containing low alkali cementitious material, Construction and Building Materials. 167 (2018) 1–14. doi:10.1016/j.conbuildmat.2018.02.020.

[2] M.K. Dixit, J.L. Fernández-Solís, S. Lavy, C.H. Culp, Identification of parameters for embodied energy measurement: A literature review, Energy and Buildings. 42 (2010) 1238–1247. doi:10.1016/j.enbuild.2010.02.016.

[3] G. Habert, S.A. Miller, V.M. John, J.L. Provis, A. Favier, A. Horvath, K.L. Scrivener, Environmental impacts and decarbonization strategies in the cement and concrete industries, Nature Reviews Earth & Environment. 1 (2020) 559–573. doi:10.1038/s43017-020-0093-3.

[4] I.N. York, I. Europe, Concrete needs to lose its colossal carbon footprint, Nature. 597 (2021) 593–594. doi:10.1038/d41586-021-02612-5.

[5] E.M. Gartner, D.E. MacPhee, A physico-chemical basis for novel cementitious binders, Cement and Concrete Research. 41 (2011) 736–749. doi:10.1016/j.cemconres.2011.03.006.

[6] C. Peng, Calculation of a building's life cycle carbon emissions based on Ecotect and building information modeling, Journal of Cleaner Production. 112 (2016) 453–465. doi:10.1016/j.jclepro.2015.08.078.

[7] M. Sandanayake, C. Gunasekara, D. Law, G. Zhang, S. Setunge, Greenhouse gas emissions of different fly ash based geopolymer concretes in building construction, Journal of Cleaner Production. 204 (2018) 399–408. doi:10.1016/j.jclepro.2018.08.311.

[8] L. Tosti, A. van Zomeren, J.R. Pels, A. Damgaard, R.N.J. Comans, Life cycle assessment of the reuse of fly ash from biomass combustion as secondary cementitious material in cement products, Journal of Cleaner Production. 245 (2020). doi:10.1016/j.jclepro.2019.118937.

[9] H. Hafez, R. Kurda, W.M. Cheung, B. Nagaratnam, Comparative life cycle assessment between imported and recovered fly ash for blended cement concrete in the UK, Journal of Cleaner Production. 244 (2020) 118722. doi:10.1016/j.jclepro.2019.118722.

[10] X. Chen, H. Wang, H. Najm, G. Venkiteela, J. Hencken, Evaluating engineering properties and environmental impact of pervious concrete with fly

ash and slag, Journal of Cleaner Production. 237 (2019) 117714. doi:10.1016/j.jclepro.2019.117714.

[11] J. Li, W. Zhang, C. Li, P.J.M. Monteiro, Eco-friendly mortar with high-volume diatomite and fly ash: Performance and life-cycle assessment with regional variability, Journal of Cleaner Production. 261 (2020) 121224. doi:10.1016/j.jclepro.2020.121224.

[12] R.M. Andrew, Global CO2 emissions from cement production Robbie, Earth System Science Data. 10 (2018) 195–217. doi:10.5194/essd-10-195-2018.

[13] V. Subathra Devi, B.K. Gnanavel, Properties of concrete manufactured using steel slag, Procedia Engineering. 97 (2014) 95–104. doi:10.1016/j.proeng.2014.12.229.

[14] R. Kurda, J. de Brito, J.D. Silvestre, Influence of recycled aggregates and high contents of fly ash on concrete fresh properties, Cement and Concrete Composites. 84 (2017) 198–213. doi:10.1016/j.cemconcomp.2017.09.009.

[15] N. Tošić, S. Marinković, T. Dašić, M. Stanić, Multicriteria optimization of natural and recycled aggregate concrete for structural use, Journal of Cleaner Production. 87 (2015) 766–776. doi:10.1016/j.jclepro.2014.10.070.

[16] K. Vijay, M. Murmu, S. V Deo, Bacteria based self healing concrete – A review, Construction and Building Materials. 152 (2017) 1008–1014. doi:10.1016/j.conbuildmat.2017.07.040.

[17] P.Y. Chen, J. McKittrick, M.A. Meyers, Biological materials: Functional adaptations and bioinspired designs, Progress in Materials Science. 57 (2012) 1492–1704. doi:10.1016/j.pmatsci.2012.03.001.

[18] V. Sharma, A. Srinivasan, A. Roychoudhury, K. Rani, M. Tyagi, K. Dev, F. Nikolajeff, S. Kumar, Characterization of protein extracts from different types of human teeth and insight in biomineralization, Scientific Reports. 9 (2019). doi:10.1038/s41598-019-44268-2.

[19] V. Sharma, A. Srinivasan, F. Nikolajeff, S. Kumar, Biomineralization process in hard tissues: The interaction complexity within protein and inorganic counterparts, Acta Biomaterialia. 120 (2021) 20–37. doi:10.1016/j.actbio.2020.04.049.

[20] E. Boquet, A. Boronat, A. Ramos-Cormenzana, Production of calcite

(Calcium carbonate) crystals by soil bacteria is a general phenomenon, Nature. 246 (1973) 527–529. doi:10.1038/246527a0.

[21] U.K. Gollapudi, C.L. Knutson, S.S. Bang, M.R. Islam, A new method for controlling leaching through permeable channels, Chemosphere. 30 (1995) 695–705. doi:10.1016/0045-6535(94)00435-W.

[22] S.S. Ramachandran, Santosh K., Ramakrishnan, V., Bang, Remediation of Concrete using Micro-Organism, Aci Materials Journal. 98 (2001) 3–9. doi:10.14359/10154.

[23] A. Naqi, S. Siddique, H.K. Kim, J.G. Jang, Examining the potential of calcined oyster shell waste as additive in high volume slag cement, Construction and Building Materials. 230 (2020) 116973. doi:10.1016/j.conbuildmat.2019.116973.

[24] J.H. Seo, S.M. Park, B.J. Yang, J.G. Jang, Calcined oyster shell powder as an expansive additive in cement mortar, Materials. 12 (2019). doi:10.3390/ma12081322.

[25] J. Xiao, W. Li, Y. Fan, X. Huang, An overview of study on recycled aggregate concrete in China (1996-2011), Construction and Building Materials. 31 (2012) 364–383. doi:10.1016/j.conbuildmat.2011.12.074.

[26] Z. Deng, Y. Wang, J. Sheng, X. Hu, Strength and deformation of recycled aggregate concrete under triaxial compression, Construction and Building Materials. 156 (2017) 1043–1052. doi:10.1016/j.conbuildmat.2017.08.189.

[27] R. Siddique, T.R. Naik, Properties of concrete containing scrap-tire rubber - An overview, Waste Management. 24 (2004) 563–569. doi:10.1016/j.wasman.2004.01.006.

[28] W. Shen, L. Shan, T. Zhang, H. Ma, Z. Cai, H. Shi, Investigation on polymer-rubber aggregate modified porous concrete, Construction and Building Materials. 38 (2013) 667–674. doi:10.1016/j.conbuildmat.2012.09.006.

[29] S. Kabir, A. Al-Shayeb, I.M. Khan, Recycled Construction Debris as Concrete Aggregate for Sustainable Construction Materials, Procedia Engineering. 145 (2016) 1518–1525. doi:10.1016/j.proeng.2016.04.191.

[30] V.M. MALHOTRA, P.K. MEHTA, Pozzolanic and cementitious materials, Advances in concrete technology, Gordon and Breach Publishers, NewYork, 1996. https://books.google.com/books?hl=en&lr=&id=IZs6zne_pAUC&pgis=1.

[31] China Power Industry Annual Development Report 2021(in Chinese), Beijing, China, 2021.

[32] S. Wang, Y. Zhang, Y. Gu, J. Wang, Z. liu, Y. Zhang, Y. Cao, C.E. Romero, W. ping Pan, Using modified fly ash for mercury emissions control for coal-fired power plant applications in China, Fuel. 181 (2016) 1230–1237. doi:10.1016/j.fuel.2016.02.043.

[33] Y. Yang, Z. Yang, G. Xu, N. Wang, Situation and prospect of energy consumption for China's thermal power generation, 2013.

[34] K.P. Verian, W. Ashraf, Y. Cao, Properties of recycled concrete aggregate and their influence in new concrete production, Resources, Conservation and Recycling. 133 (2018) 30–49. doi:10.1016/j.resconrec.2018.02.005.

[35] C. Herath, C. Gunasekara, D.W. Law, S. Setunge, Performance of high volume fly ash concrete incorporating additives: A systematic literature review, Construction and Building Materials. 258 (2020) 120606. doi:10.1016/j.conbuildmat.2020.120606.

[36] P. Jagannathan, K.S. Satya Narayanan, K.D. Arunachalam, S.K. Annamalai, Studies on the mechanical properties of bacterial concrete with two bacterial species, Materials Today: Proceedings. 5 (2018) 8875–8879. doi:10.1016/j.matpr.2017.12.320.

[37] G. Xu, X. Shi, Characteristics and applications of fly ash as a sustainable construction material: A state-of-the-art review, Resources, Conservation and Recycling. 136 (2018) 95–109. doi:10.1016/j.resconrec.2018.04.010.

[38] C.Y. Lee, H.K. Lee, K.M. Lee, Strength and microstructural characteristics of chemically activated fly ash-cement systems, Cement and Concrete Research. 33 (2003) 425–431. doi:10.1016/S0008-8846(02)00973-0.

[39] T.Y. Huang, P.T. Chiueh, S.L. Lo, Life-cycle environmental and cost impacts of reusing fly ash, Resources, Conservation and Recycling. 123 (2017) 255–260. doi:10.1016/j.resconrec.2016.07.001.

[40] S. Pradhan, B.R. Tiwari, S. Kumar, S. V. Barai, Comparative LCA of recycled and natural aggregate concrete using Particle Packing Method and conventional method of design mix, Journal of Cleaner Production. 228 (2019) 679–691. doi:10.1016/j.jclepro.2019.04.328.

[41] J. Dang, Experimental Study on the Properties of Recycled Mortat and Concrete with Waste Clay Bricks as Fine Aggregate, Zhengzhou University, 2019.

[42] S.N. Chinnu, S.N. Minnu, A. Bahurudeen, R. Senthilkumar, Recycling of industrial and agricultural wastes as alternative coarse aggregates: A step towards cleaner production of concrete, Construction and Building Materials. 287 (2021) 123056. doi:10.1016/j.conbuildmat.2021.123056.

[43] A.Y. Nayana, S. Kavitha, Evaluation of C02 emissions for green concrete with high volume slag, recycled aggregate, recycled water to build eco environment, International Journal of Civil Engineering and Technology. 8 (2017) 703–708.

[44] V.W.Y. Tam, M. Soomro, A.C.J. Evangelista, A review of recycled aggregate in concrete applications (2000–2017), Construction and Building Materials. 172 (2018) 272–292. doi:10.1016/j.conbuildmat.2018.03.240.

[45] A. Hosan, F.U.A. Shaikh, Compressive strength development and durability properties of high volume slag and slag-fly ash blended concretes containing nano-CaCO3, Journal of Materials Research and Technology. 10 (2021) 1310–1322. doi:10.1016/j.jmrt.2021.01.001.

[46] K.E. Seto, C.J. Churchill, D.K. Panesar, Influence of fly ash allocation approaches on the life cycle assessment of cement-based materials, Journal of Cleaner Production. 157 (2017) 65–75. doi:10.1016/j.jclepro.2017.04.093.

[47] G. Rebitzer, T. Ekvall, R. Frischknecht, D. Hunkeler, G. Norris, T. Rydberg, W.-P. Schmidt, S. Suh, B.P. Weidema, D.W. Pennington, Life cycle assessment: Part 1: Framework, goal and scope definition, inventory analysis, and applications, Environment International. 30 (2004) 701–720. doi:https://doi.org/10.1016/j.envint.2003.11.005.

[48] International Organization for Standardization ISO, BS EN ISO 14040:2006 Environmental Management: Life Cycle Assessment; Principles and Framework, ISO, 2006.

[49] International Organization for Standardization ISO, Environmental management: life cycle assessment; requirements and guidelines, ISO Geneva, Switzerland, 2006.

[50] Y. Zhang, W. Luo, J. Wang, Y. Wang, Y. Xu, J. Xiao, A review of life cycle assessment of recycled aggregate concrete, Construction and Building

Materials. 209 (2019) 115–125. doi:10.1016/j.conbuildmat.2019.03.078.
[51] R. Kurda, J.D. Silvestre, J. de Brito, Life cycle assessment of concrete made with high volume of recycled concrete aggregates and fly ash, Resources, Conservation and Recycling. 139 (2018) 407–417. doi:10.1016/j.resconrec.2018.07.004.
[52] V. Achal, A. Mukherjee, D. Kumari, Q. Zhang, Biomineralization for sustainable construction - A review of processes and applications, Earth-Science Reviews. 148 (2015) 1–17. doi:10.1016/j.earscirev.2015.05.008.
[53] R. Siddique, N.K. Chahal, Effect of ureolytic bacteria on concrete properties, Construction and Building Materials. 25 (2011) 3791–3801. doi:10.1016/j.conbuildmat.2011.04.010.
[54] H.M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, E. Schlangen, Application of bacteria as self-healing agent for the development of sustainable concrete, Ecological Engineering. 36 (2010) 230–235. doi:10.1016/j.ecoleng.2008.12.036.
[55] L.S. Wong, Microbial cementation of ureolytic bacteria from the genus Bacillus: A review of the bacterial application on cement-based materials for cleaner production, Journal of Cleaner Production. 93 (2015) 5–17. doi:10.1016/j.jclepro.2015.01.019.
[56] S. Castanier, G. Le Métayer-Levrel, J.P. Perthuisot, Ca-carbonates precipitation and limestone genesis - the microbiogeologist point of view, Sedimentary Geology. 126 (1999) 9–23. doi:10.1016/S0037-0738(99)00028-7.
[57] V. Achal, A. Mukherjee, D. Kumari, Q. Zhang, Biomineralization for sustainable construction - A review of processes and applications, Earth-Science Reviews. 148 (2015) 1–17. doi:10.1016/j.earscirev.2015.05.008.
[58] L.A. van Paassen, C.M. Daza, M. Staal, D.Y. Sorokin, W. van der Zon, M.C.M. van Loosdrecht, Potential soil reinforcement by biological denitrification, Ecological Engineering. 36 (2010) 168–175. doi:10.1016/j.ecoleng.2009.03.026.
[59] Y.Ç. Erşan, N. Boon, N. De Belie, Microbial self-healing concrete: Denitrification as an enhanced and environment-friendly approach, 5th

International Conference on Self-Healing Materials. (2015).

[60] Y.Ç. Erşan, H. Verbruggen, I. De Graeve, W. Verstraete, N. De Belie, N. Boon, Nitrate reducing CaCO3 precipitating bacteria survive in mortar and inhibit steel corrosion, Cement and Concrete Research. 83 (2016) 19–30. doi:10.1016/j.cemconres.2016.01.009.

[61] F. Hammes, N. Boon, J. De Villiers, W. Verstraete, S.D. Siciliano, Strain-specific ureolytic microbial calcium carbonate precipitation, Applied and Environmental Microbiology. 69 (2003) 4901–4909. doi:10.1128/AEM.69.8.4901-4909.2003.

[62] Y. Al-Salloum, S. Hadi, H. Abbas, T. Almusallam, M.A. Moslem, Bio-induction and bioremediation of cementitious composites using microbial mineral precipitation – A review, Construction and Building Materials. 154 (2017) 857–876. doi:10.1016/j.conbuildmat.2017.07.203.

[63] K. Van Tittelboom, N. De Belie, W. De Muynck, W. Verstraete, Use of bacteria to repair cracks in concrete, Cement and Concrete Research. 40 (2010) 157–166. doi:10.1016/j.cemconres.2009.08.025.

[64] L.P. Singh, V. Bisht, M.S. Aswathy, L. Chaurasia, S. Gupta, Studies on performance enhancement of recycled aggregate by incorporating bio and nano materials, Construction and Building Materials. 181 (2018) 217–226. doi:10.1016/j.conbuildmat.2018.05.248.

[65] L.P. Singh, V. Bisht, M.S. Aswathy, L. Chaurasia, S. Gupta, Studies on performance enhancement of recycled aggregate by incorporating bio and nano materials, Construction and Building Materials. 181 (2018) 217–226. doi:10.1016/j.conbuildmat.2018.05.248.

[66] L. Chaurasia, V. Bisht, L.P. Singh, S. Gupta, A novel approach of biomineralization for improving micro and macro-properties of concrete, Construction and Building Materials. 195 (2019) 340–351. doi:10.1016/j.conbuildmat.2018.11.031.

[67] H.K. Kim, S.J. Park, J.I. Han, H.K. Lee, Microbially mediated calcium carbonate precipitation on normal and lightweight concrete, Construction and Building Materials. 38 (2013) 1073–1082. doi:10.1016/j.conbuildmat.2012.07.040.

[68] F. Ameri, P. Shoaei, N. Bahrami, M. Vaezi, T. Ozbakkaloglu, Optimum rice husk ash content and bacterial concentration in self-compacting concrete,

Construction and Building Materials. 222 (2019) 796–813. doi:10.1016/j.conbuildmat.2019.06.190.

[69] V. Achal, X. Pan, Influence of calcium sources on microbially induced calcium carbonate precipitation by Bacillus sp. CR2, Applied Biochemistry and Biotechnology. 173 (2014) 307–317. doi:10.1007/s12010-014-0842-1.

[70] V. Achal, X. Pan, N. Özyurt, Improved strength and durability of fly ash-amended concrete by microbial calcite precipitation, Ecological Engineering. 37 (2011) 554–559. doi:10.1016/j.ecoleng.2010.11.009.

[71] G.T. Zhou, J.C. Yu, X.C. Wang, L.Z. Zhang, Sonochemical synthesis of aragonite-type calcium carbonate with different morphologies, New Journal of Chemistry. 28 (2004) 1027–1031. doi:10.1039/b315198k.

[72] U.U. Jadhav, M. Lahoti, Z. Chen, J. Qiu, B. Cao, E.H. Yang, Viability of bacterial spores and crack healing in bacteria-containing geopolymer, Construction and Building Materials. 169 (2018) 716–723. doi:10.1016/j.conbuildmat.2018.03.039.

[73] L. Wang, J. Wang, X. Qian, P. Chen, Y. Xu, J. Guo, An environmentally friendly method to improve the quality of recycled concrete aggregates, Construction and Building Materials. 144 (2017) 432–441. doi:10.1016/j.conbuildmat.2017.03.191.

[74] S. Amidi, J. Wang, Surface treatment of concrete bricks using calcium carbonate precipitation, Construction and Building Materials. 80 (2015) 273–278. doi:10.1016/j.conbuildmat.2015.02.001.

[75] H. Chen, C. Qian, H. Huang, Self-healing cementitious materials based on bacteria and nutrients immobilized respectively, Construction and Building Materials. 126 (2016) 297–303. doi:10.1016/j.conbuildmat.2016.09.023.

[76] Kunal, R. Siddique, A. Rajor, Influence of bacterial treated cement kiln dust on the properties of concrete, Construction and Building Materials. 52 (2014) 42–51. doi:10.1016/j.conbuildmat.2013.11.034.

[77] F. Hammes, W. Verstraete*, W. Verstraete, Key roles of pH and calcium metabolism in microbial carbonate precipitation, Re/Views in Environmental Science & Bio/Technology. 1 (2002) 3–7. doi:10.1023/A:1015135629155.

[78] S. Saxena, A.R. Tembhurkar, Developing biotechnological technique for reuse

of wastewater and steel slag in bio-concrete, Journal of Cleaner Production. 229 (2019) 193–202. doi:10.1016/j.jclepro.2019.04.363.

[79] R. Ševčík, P. Šašek, A. Viani, Physical and nanomechanical properties of the synthetic anhydrous crystalline CaCO3 polymorphs: vaterite, aragonite and calcite, Journal of Materials Science. 53 (2018) 4022–4033. doi:10.1007/s10853-017-1884-x.

[80] N.H. De Leeuw, S.C. Parker, Surface structure and morphology of calcium carbonate polymorphs calcite, aragonite, and vaterite: An atomistic approach, Journal of Physical Chemistry B. 102 (1998) 2914–2922. doi:10.1021/jp973210f.

[81] H. Nebel, M. Epple, Continuous preparation of calcite, aragonite and vaterite, and of magnesium-substituted amorphous calcium carbonate (Mg-ACC), Zeitschrift Fur Anorganische Und Allgemeine Chemie. 634 (2008) 1439–1443. doi:10.1002/zaac.200800134.

[82] W. De Muynck, K. Cox, N. De Belie, W. Verstraete, Bacterial carbonate precipitation as an alternative surface treatment for concrete, Construction and Building Materials. 22 (2008) 875–885. doi:10.1016/j.conbuildmat.2006.12.011.

[83] S. Stocks-Fischer, J.K. Galinat, S.S. Bang, Microbiological precipitation of CaCO3, Soil Biology and Biochemistry. 31 (1999) 1563–1571. doi:10.1016/S0038-0717(99)00082-6.

[84] S. Jena, B. Basa, K.C. Panda, N.K. Sahoo, Impact of Bacillus subtilis bacterium on the properties of concrete, Materials Today: Proceedings. 32 (2020) 651–656. doi:10.1016/j.matpr.2020.03.129.

[85] Y. Zhang, H.X. Guo, X.H. Cheng, Role of calcium sources in the strength and microstructure of microbial mortar, Construction and Building Materials. 77 (2015) 160–167. doi:10.1016/j.conbuildmat.2014.12.040.

[86] J.Y. Wang, D. Snoeck, S. Van Vlierberghe, W. Verstraete, N. De Belie, Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete, Construction and Building Materials. 68 (2014) 110–119. doi:10.1016/j.conbuildmat.2014.06.018.

[87] S.K. Nath, S. Maitra, S. Mukherjee, S. Kumar, Microstructural and morphological evolution of fly ash based geopolymers, Construction and Building Materials. 111 (2016) 758–765.

doi:10.1016/j.conbuildmat.2016.02.106.
[88] D.W. Hobbs, M.G. Taylor, Nature of the thaumasite sulfate attack mechanism in field concrete, Cement and Concrete Research. 30 (2000) 529–533. doi:10.1016/S0008-8846(99)00255-0.
[89] J. Wu, J. Wei, H. Huang, J. Hu, Q. Yu, Effect of multiple ions on the degradation in concrete subjected to sulfate attack, Construction and Building Materials. 259 (2020) 119846. doi:10.1016/j.conbuildmat.2020.119846.
[90] F. Albareda, S. Valls, E. Va, Durability of concrete with addition of dry sludge from waste water treatment plants, 35 (2005) 1064–1073. doi:10.1016/j.cemconres.2004.07.043.
[91] V. Ramakrishnan, S.S. Bang, S. Neelamegam, R.K. Panchalan, DURABILITY OF CEMENT MORTAR MADE WITH DIFFERENT CONCENTRATIONS OF BACTERIA, in: Int. Cement Microscopy Ass. Meetings, 2003: pp. 1–16.
[92] F.G. Ferris, V. Phoenix, Y. Fujita, R.W. Smith, Kinetics of calcite precipitation induced by ureolytic bacteria at 10 to 20°C in artificial groundwater, Geochimica et Cosmochimica Acta. 68 (2004) 1701–1710. doi:10.1016/S0016-7037(03)00503-9.
[93] T. Shanmuga Priya, N. Ramesh, A. Agarwal, S. Bhusnur, K. Chaudhary, Strength and durability characteristics of concrete made by micronized biomass silica and Bacteria-Bacillus sphaericus, Construction and Building Materials. 226 (2019) 827–838. doi:10.1016/j.conbuildmat.2019.07.172.
[94] N. Karimi, D. Mostofinejad, Bacillus subtilis bacteria used in fiber reinforced concrete and their effects on concrete penetrability, Construction and Building Materials. 230 (2020) 117051. doi:10.1016/j.conbuildmat.2019.117051.
[95] S. Mondal, A. (Dey) Ghosh, Investigation into the optimal bacterial concentration for compressive strength enhancement of microbial concrete, Construction and Building Materials. 183 (2018) 202–214. doi:10.1016/j.conbuildmat.2018.06.176.
[96] N. Nain, R. Surabhi, N. V. Yathish, V. Krishnamurthy, T. Deepa, S. Tharannum, Enhancement in strength parameters of concrete by application of Bacillus bacteria, Construction and Building Materials. 202 (2019) 904–908.

doi:10.1016/j.conbuildmat.2019.01.059.

[97] S.A. Kadapure, G.S. Kulkarni, K.B. Prakash, Study on properties of bacteria-embedded fly ash concrete, Asian Journal of Civil Engineering. 20 (2019) 627–636. doi:10.1007/s42107-019-00127-z.

[98] R. Andalib, M. Zaimi, A. Majid, M. Warid, M. Ponraj, A. Keyvanfar, J. Mirza, H. Lee, Optimum concentration of Bacillus megaterium for strengthening structural concrete, Construction and Building Materials. 118 (2016) 180–193. doi:10.1016/j.conbuildmat.2016.04.142.

[99] H. Kalhori, R. Bagherpour, Application of carbonate precipitating bacteria for improving properties and repairing cracks of shotcrete, Construction and Building Materials. 148 (2017) 249–260. doi:10.1016/j.conbuildmat.2017.05.074.

[100] R. Siddique, V. Nanda, Kunal, E.H. Kadri, M. Iqbal Khan, M. Singh, A. Rajor, Influence of bacteria on compressive strength and permeation properties of concrete made with cement baghouse filter dust, Construction and Building Materials. 106 (2016) 461–469. doi:10.1016/j.conbuildmat.2015.12.112.

[101] N. Chahal, R. Siddique, Permeation properties of concrete made with fly ash and silica fume: Influence of ureolytic bacteria, Construction and Building Materials. 49 (2013) 161–174. doi:10.1016/j.conbuildmat.2013.08.023.

[102] A.U. Charpe, M. V. Latkar, T. Chakrabarti, Biocementation: An eco-friendly approach to strengthen concrete, Proceedings of the Institution of Civil Engineers: Engineering Sustainability. 172 (2019) 438–449. doi:10.1680/jensu.18.00019.

[103] N. Parastegari, D. Mostofinejad, D. Poursina, Use of bacteria to improve electrical resistivity and chloride penetration of air-entrained concrete, Construction and Building Materials. 210 (2019) 588–595. doi:10.1016/j.conbuildmat.2019.03.150.

[104] V. Achal, A. Mukherjee, M.S. Reddy, Microbial concrete: Way to enhance the durability of building structures, Journal of Materials in Civil Engineering. 23 (2011) 730–734. doi:10.1061/(ASCE)MT.1943-5533.0000159.

[105] J.A. Bogas, J. De Brito, D. Ramos, Freeze-thaw resistance of concrete produced with fine recycled concrete aggregates, Journal of Cleaner Production. 115 (2016) 294–306. doi:10.1016/j.jclepro.2015.12.065.

[106] S.B. Huda, M. Shahria Alam, Mechanical and freeze-thaw durability properties of recycled aggregate concrete made with recycled coarse aggregate, Journal of Materials in Civil Engineering. 27 (2015). doi:10.1061/(ASCE)MT.1943-5533.0001237.

[107] Y. Zhao, L. Peng, W. Zeng, C. sun Poon, Z. Lu, Improvement in properties of concrete with modified RCA by microbial induced carbonate precipitation, Cement and Concrete Composites. 124 (2021) 104251. doi:10.1016/j.cemconcomp.2021.104251.

[108] Z. Gyurkó, A. Szijártó, R. Nemes, Increasing Freeze-thaw Resistance of Concrete by Additions of Powdered Cellular Concrete and Clay Bricks, Procedia Engineering. 193 (2017) 11–18. doi:10.1016/j.proeng.2017.06.180.

[109] Y.C. Ersan, J.Y. Wang, N. Boon, N. De Belie, Ureolysis and denitrification based microbial strategies for self-healing concrete, Concrete Solutions - Proceedings of Concrete Solutions, 5th International Conference on Concrete Repair. (2014) 59–64. doi:10.1201/b17394-10.

[110] J. Luo, X. Chen, J. Crump, H. Zhou, D.G. Davies, G. Zhou, N. Zhang, C. Jin, Interactions of fungi with concrete: Significant importance for bio-based self-healing concrete, Construction and Building Materials. 164 (2018) 275–285. doi:10.1016/j.conbuildmat.2017.12.233.

[111] A. Wasley, A. Heal, F. Harvey, Ammonia pollution damaging more than 60% of UK land – report, The Guardian. (2019). https://www.theguardian.com/environment/2019/jun/18/ammonia-pollution-damaging-uk-land-report (accessed August 6, 2020).

[112] R. Ewing, British government mulls ban on farmers using urea under air pollution crackdown, Independent Commodity Intelligence Services(ICIS). (2020). https://www.icis.com/explore/resources/news/2020/11/03/10570689/british-government-mulls-ban-on-farmers-using-urea-under-air-pollution-crackdown/ (accessed December 8, 2021).

[113] Y.Ç. Erşan, E. Hernandez-Sanabria, N. Boon, N. De Belie, Enhanced crack closure performance of microbial mortar through nitrate reduction, Cement and Concrete Composites. 70 (2016) 159–170.

doi:10.1016/j.cemconcomp.2016.04.001.
[114] M. Heikal, Effect of calcium formate as an accelerator on the physicochemical and mechanical properties of pozzolanic cement pastes, Cement and Concrete Research. 34 (2004) 1051–1056. doi:10.1016/j.cemconres.2003.11.015.
[115] F. Karagöl, R. Demirboğa, M.A. Kaygusuz, M.M. Yadollahi, R. Polat, The influence of calcium nitrate as antifreeze admixture on the compressive strength of concrete exposed to low temperatures, Cold Regions Science and Technology. 89 (2013) 30–35. doi:10.1016/j.coldregions.2013.02.001.
[116] Z. Xu, X. Dai, X. Chai, Effect of influent pH on biological denitrification using biodegradable PHBV/PLA blends as electron donor, Biochemical Engineering Journal. 131 (2018) 24–30. doi:10.1016/j.bej.2017.12.008.
[117] M. Alquier, C. Kassim, A. Bertron, Y. Rafrafi, C. Sablayrolles, A. Albrecht, B. Erable, Nitrate reducing bacterial activity in concrete cells of nuclear waste disposal, EPJ Web of Conferences. 56 (2013). doi:10.1051/epjconf/20135601003.
[118] Y.Ç. Erşan, N. de Belie, N. Boon, Microbially induced CaCO3 precipitation through denitrification: An optimization study in minimal nutrient environment, Biochemical Engineering Journal. 101 (2015) 108–118. doi:10.1016/j.bej.2015.05.006.
[119] Y.Ç. Erşan, F.B. Da Silva, N. Boon, W. Verstraete, N. De Belie, Screening of bacteria and concrete compatible protection materials, Construction and Building Materials. 88 (2015) 196–203. doi:10.1016/j.conbuildmat.2015.04.027.
[120] J. Iribarne, A. Iribarne, J. Blondin, E.J. Anthony, Hydration of combustion ashes - a chemical and physical study, Fuel. 80 (2001) 773–784. doi:10.1016/S0016-2361(00)00158-7.
[121] A. Durán-Herrera, C.A. Juárez, P. Valdez, D.P. Bentz, Evaluation of sustainable high-volume fly ash concretes, Cement and Concrete Composites. 33 (2011) 39–45. doi:10.1016/j.cemconcomp.2010.09.020.
[122] A.S.M. Akid, S. Hossain, M.I.U. Munshi, M.M.A. Elahi, M.H.R. Sobuz, V.W.Y. Tam, M.S. Islam, Assessing the influence of fly ash and polypropylene fiber on fresh, mechanical and durability properties of concrete, Journal of King Saud University - Engineering Sciences. (2021). doi:10.1016/j.jksues.2021.06.005.

[123] P. Torkittikul, A. Chaipanich, Utilization of ceramic waste as fine aggregate within Portland cement and fly ash concretes, Cement and Concrete Composites. 32 (2010) 440–449. doi:10.1016/j.cemconcomp.2010.02.004.

[124] Y. Kocak, S. Nas, The effect of using fly ash on the strength and hydration characteristics of blended cements, Construction and Building Materials. 73 (2014) 25–32. doi:10.1016/j.conbuildmat.2014.09.048.

[125] Z. Zhang, Q. Zhang, Self-healing ability of Engineered Cementitious Composites (ECC) under different exposure environments, Construction and Building Materials. 156 (2017) 142–151. doi:10.1016/j.conbuildmat.2017.08.166.

[126] J. Dragaš, I. Ignjatović, N. Tošić, S. Marinković, Mechanical and time-dependent properties of high-volume fly ash concrete for structural use, Magazine of Concrete Research. 68 (2016) 632–645. doi:10.1680/jmacr.15.00384.

[127] H. Zhu, C. Li, Y. Cheng, Z. Jiang, K. Wu, Pozzolanicity of fly ash modified by fluidized bed reactor–vapor deposition, Construction and Building Materials. 156 (2017) 719–727. doi:10.1016/j.conbuildmat.2017.09.034.

[128] P.R. de Matos, M. Foiato, L.R. Prudêncio, Ecological, fresh state and long-term mechanical properties of high-volume fly ash high-performance self-compacting concrete, Construction and Building Materials. 203 (2019) 282–293. doi:10.1016/j.conbuildmat.2019.01.074.

[129] X.Y. Zhuang, L. Chen, S. Komarneni, C.H. Zhou, D.S. Tong, H.M. Yang, W.H. Yu, H. Wang, Fly ash-based geopolymer: Clean production, properties and applications, Journal of Cleaner Production. 125 (2016) 253–267. doi:10.1016/j.jclepro.2016.03.019.

[130] P. Topark-Ngarm, P. Chindaprasirt, V. Sata, Setting Time, Strength, and Bond of High-Calcium Fly Ash Geopolymer Concrete, Journal of Materials in Civil Engineering. 27 (2015) 04014198. doi:10.1061/(asce)mt.1943-5533.0001157.

[131] N.P. Rajamane, M.C. Nataraja, J.K. Dattatreya, N. Lakshmanan, D. Sabitha, Sulphate resistance and eco-friendliness of geopolymer concretes, Indian Concrete Journal. 86 (2012) 13–22.

[132] C. feng Lu, W. Wang, Q. tao Li, M. Hao, Y. Xu, Effects of micro-

environmental climate on the carbonation depth and the pH value in fly ash concrete, Journal of Cleaner Production. 181 (2018) 309–317. doi:10.1016/j.jclepro.2018.01.155.

[133] X. Hu, C. Shi, Z. Shi, B. Tong, D. Wang, Early age shrinkage and heat of hydration of cement-fly ash-slag ternary blends, Construction and Building Materials. 153 (2017) 857–865. doi:10.1016/j.conbuildmat.2017.07.138.

[134] M.H. Shehata, M.D.A. Thomas, Use of ternary blends containing silica fume and fly ash to suppress expansion due to alkali-silica reaction in concrete, Cement and Concrete Research. 32 (2002) 341–349. doi:10.1016/S0008-8846(01)00680-9.

[135] British Standards Institution BSI, BS EN 933-11:2009. Tests for geometrical properties of aggregates Part 11: Classification test for the constituents of coarse recycled aggregate, BSI, London, 2009.

[136] R. Kurad, J.D. Silvestre, J. de Brito, H. Ahmed, Effect of incorporation of high volume of recycled concrete aggregates and fly ash on the strength and global warming potential of concrete, Journal of Cleaner Production. 166 (2017) 485–502. doi:10.1016/j.jclepro.2017.07.236.

[137] A.I. Kareem, H. Nikraz, H. Asadi, Application of Double-Coated Recycled Concrete Aggregates for Hot-Mix Asphalt, Journal of Materials in Civil Engineering. 31 (2019) 1–9. doi:10.1061/(ASCE)MT.1943-5533.0002670.

[138] M. Behera, S.K. Bhattacharyya, A.K. Minocha, R. Deoliya, S. Maiti, Recycled aggregate from C&D waste & its use in concrete - A breakthrough towards sustainability in construction sector: A review, Construction and Building Materials. 68 (2014) 501–516. doi:10.1016/j.conbuildmat.2014.07.003.

[139] J. Xiao, W. Li, C. Poon, Recent studies on mechanical properties of recycled aggregate concrete in China-A review, Science China Technological Sciences. 55 (2012) 1463–1480. doi:10.1007/s11431-012-4786-9.

[140] S.C. Kou, C.S. Poon, Long-term mechanical and durability properties of recycled aggregate concrete prepared with the incorporation of fly ash, Cement and Concrete Composites. 37 (2013) 12–19. doi:10.1016/j.cemconcomp.2012.12.011.

[141] J. Qiu, D.Q.S. Tng, E.H. Yang, Surface treatment of recycled concrete aggregates through microbial carbonate precipitation, Construction and Building Materials. 57 (2014) 144–150.

doi:10.1016/j.conbuildmat.2014.01.085.
[142] N.S. Amorim Júnior, G.A.O. Silva, D. V. Ribeiro, Effects of the incorporation of recycled aggregate in the durability of the concrete submitted to freeze-thaw cycles, Construction and Building Materials. 161 (2018) 723–730. doi:10.1016/j.conbuildmat.2017.12.076.
[143] D.X. H, Study on effect of compressive strength of recycled aggregate concrete with water cement ratio (in Chinese), Concrete. 2 (2005) 46–48.
[144] C.S. Poon, Z.H. Shui, L. Lam, H. Fok, S.C. Kou, Influence of moisture states of natural and recycled aggregates on the slump and compressive strength of concrete, Cement and Concrete Research. 34 (2004) 31–36. doi:10.1016/S0008-8846(03)00186-8.
[145] A.S. Brand, J.R. Roesler, A. Salas, Initial moisture and mixing effects on higher quality recycled coarse aggregate concrete, Construction and Building Materials. 79 (2015) 83–89. doi:10.1016/j.conbuildmat.2015.01.047.
[146] S. Hou, Z. Duan, J. Xiao, L. Li, Y. Bai, Effect of moisture condition and brick content in recycled coarse aggregate on rheological properties of fresh concrete, Journal of Building Engineering. 35 (2021) 102075. doi:10.1016/j.jobe.2020.102075.
[147] N. Kisku, H. Joshi, M. Ansari, S.K. Panda, S. Nayak, S.C. Dutta, A critical review and assessment for usage of recycled aggregate as sustainable construction material, Construction and Building Materials. 131 (2017) 721–740. doi:10.1016/j.conbuildmat.2016.11.029.
[148] C.R. Wu, Z.Q. Hong, J.L. Zhang, S.C. Kou, Pore size distribution and ITZ performance of mortars prepared with different bio-deposition approaches for the treatment of recycled concrete aggregate, Cement and Concrete Composites. 111 (2020) 103631. doi:10.1016/j.cemconcomp.2020.103631.
[149] S.K. Kirthika, S.K. Singh, Durability studies on recycled fine aggregate concrete, Construction and Building Materials. 250 (2020) 118850. doi:10.1016/j.conbuildmat.2020.118850.
[150] J.M.V. Gómez-Soberón, Porosity of recycled concrete with substitution of recycled concrete aggregate: An experimental study, Cement and Concrete Research. 32 (2002) 1301–1311. doi:10.1016/S0008-8846(02)00795-0.

[151] R. Wang, N. Yu, Y. Li, Methods for improving the microstructure of recycled concrete aggregate: A review, Construction and Building Materials. 242 (2020) 118164. doi:10.1016/j.conbuildmat.2020.118164.

[152] L. Zhu, C. Zhao, J. Dai, Prediction of compressive strength of recycled aggregate concrete based on gray correlation analysis, Construction and Building Materials. 273 (2021) 121750. doi:10.1016/j.conbuildmat.2020.121750.

[153] A. Abd Elhakam, A.E. Mohamed, E. Awad, Influence of self-healing, mixing method and adding silica fume on mechanical properties of recycled aggregates concrete, Construction and Building Materials. 35 (2012) 421–427. doi:10.1016/j.conbuildmat.2012.04.013.

[154] S.B. Huda, M. Shahria Alam, Mechanical and Freeze-Thaw Durability Properties of Recycled Aggregate Concrete Made with Recycled Coarse Aggregate, Journal of Materials in Civil Engineering. 27 (2015) 04015003. doi:10.1061/(asce)mt.1943-5533.0001237.

[155] B.A. Harish, N.V. Ramana, K. Gnaneswar, Materials Today : Proceedings Experimental and analytical studies on recycled coarse aggregate concrete, Materials Today: Proceedings. 46 (2021) 294–300. doi:10.1016/j.matpr.2020.08.041.

[156] E. Vázquez, M. Barra, D. Aponte, C. Jiménez, S. Valls, Improvement of the durability of concrete with recycled aggregates in chloride exposed environment, Construction and Building Materials. 67 (2014) 61–67. doi:10.1016/j.conbuildmat.2013.11.028.

[157] J. Xiao, J. Li, C. Zhang, Mechanical properties of recycled aggregate concrete under uniaxial loading, Cement and Concrete Research. 35 (2005) 1187–1194. doi:10.1016/j.cemconres.2004.09.020.

[158] S.C. Kou, C.S. Poon, Enhancing the durability properties of concrete prepared with coarse recycled aggregate, Construction and Building Materials. 35 (2012) 69–76. doi:10.1016/j.conbuildmat.2012.02.032.

[159] J.Z. Xiao, B. Lei, C.Z. Zhang, On carbonation behavior of recycled aggregate concrete, Science China Technological Sciences. 55 (2012) 2609–2616. doi:10.1007/s11431-012-4798-5.

[160] B. Wang, L. Yan, Q. Fu, B. Kasal, A Comprehensive Review on Recycled Aggregate and Recycled Aggregate Concrete, Resources, Conservation and

Recycling. 171 (2021) 105565. doi:10.1016/j.resconrec.2021.105565.
[161] M. Rao, P. Rao, V.V. Muniswamy, Delivery performance measurement in an integrated supply chain management: Case study in batteries manufacturing firm, Serbian Journal of Management. 6 (2011) 205–220. doi:10.5937/sjm1102205m.
[162] C. Thomas, J. Setién, J.A. Polanco, P. Alaejos, M. Sánchez De Juan, Durability of recycled aggregate concrete, Construction and Building Materials. 40 (2013) 1054–1065. doi:10.1016/j.conbuildmat.2012.11.106.
[163] M. Bravo, J. De Brito, J. Pontes, L. Evangelista, Durability performance of concrete with recycled aggregates from construction and demolition waste plants, Construction and Building Materials. 77 (2015) 357–369. doi:10.1016/j.conbuildmat.2014.12.103.
[164] S.M.S. Kazmi, M.J. Munir, Y.F. Wu, I. Patnaikuni, Y. Zhou, F. Xing, Effect of recycled aggregate treatment techniques on the durability of concrete: A comparative evaluation, Construction and Building Materials. 264 (2020) 120284. doi:10.1016/j.conbuildmat.2020.120284.
[165] R. Zaharieva, F. Buyle-Bodin, E. Wirquin, Frost resistance of recycled aggregate concrete, Cement and Concrete Research. 34 (2004) 1927–1932. doi:10.1016/j.cemconres.2004.02.025.
[166] M. Tuyan, A. Mardani-Aghabaglou, K. Ramyar, Freeze-thaw resistance, mechanical and transport properties of self-consolidating concrete incorporating coarse recycled concrete aggregate, Materials and Design. 53 (2014) 983–991. doi:10.1016/j.matdes.2013.07.100.
[167] S.M.S. Kazmi, M.J. Munir, Y.F. Wu, I. Patnaikuni, Y. Zhou, F. Xing, Effect of different aggregate treatment techniques on the freeze-thaw and sulfate resistance of recycled aggregate concrete, Cold Regions Science and Technology. 178 (2020) 103126. doi:10.1016/j.coldregions.2020.103126.
[168] H. Luan, J. Wu, J. Pan, Saline water absorption behavior and critical saturation degree of recycled aggregate concrete during freeze-thaw cycles, Construction and Building Materials. 258 (2020) 119640. doi:10.1016/j.conbuildmat.2020.119640.
[169] P.K. Kolay, S.O. Sulaiman, S. Kumar, Freeze-thaw durability of concrete with

natural and recycled concrete aggregates using air-entraining admixture, Advances in Civil Engineering Materials. 7 (2018). doi:10.1520/ACEM20170079.

[170] A. Gokce, S. Nagataki, T. Saeki, M. Hisada, Freezing and thawing resistance of air-entrained concrete incorporating recycled coarse aggregate: The role of air content in demolished concrete, Cement and Concrete Research. 34 (2004) 799–806. doi:10.1016/j.cemconres.2003.09.014.

[171] S.T. Yildirim, C. Meyer, S. Herfellner, Effects of internal curing on the strength, drying shrinkage and freeze-thaw resistance of concrete containing recycled concrete aggregates, Construction and Building Materials. 91 (2015) 288–296. doi:10.1016/j.conbuildmat.2015.05.045.

[172] J. Wu, X. Jing, Z. Wang, Uni-axial compressive stress-strain relation of recycled coarse aggregate concrete after freezing and thawing cycles, Construction and Building Materials. 134 (2017) 210–219. doi:10.1016/j.conbuildmat.2016.12.142.

[173] L. Hao, Y. Liu, J. Xiao, Durability of recycled aggregate thermal insulation concrete under combined flexural loading and freeze–thaw cycles, Construction and Building Materials. 272 (2021) 121652. doi:10.1016/j.conbuildmat.2020.121652.

[174] A. Katz, Treatments for the Improvement of Recycled Aggregate, Journal of Materials in Civil Engineering. 16 (2004) 597–603. doi:10.1061/(ASCE)0899-1561(2004)16:6(597).

[175] S. Ismail, M. Ramli, Mechanical strength and drying shrinkage properties of concrete containing treated coarse recycled concrete aggregates, Construction and Building Materials. 68 (2014) 726–739. doi:10.1016/j.conbuildmat.2014.06.058.

[176] H. Zhang, T. Ji, H. Liu, S. Su, Modifying recycled aggregate concrete by aggregate surface treatment using sulphoaluminate cement and basalt powder, Construction and Building Materials. 192 (2018) 526–537. doi:10.1016/j.conbuildmat.2018.10.160.

[177] K. Bru, S. Touzé, F. Bourgeois, N. Lippiatt, Y. Ménard, Assessment of a microwave-assisted recycling process for the recovery of high-quality aggregates from concrete waste, International Journal of Mineral Processing. 126 (2014) 90–98. doi:10.1016/j.minpro.2013.11.009.

[178] D. Kong, T. Lei, J. Zheng, C. Ma, J. Jiang, J. Jiang, Effect and mechanism of surface-coating pozzalanics materials around aggregate on properties and ITZ microstructure of recycled aggregate concrete, Construction and Building Materials. 24 (2010) 701–708. doi:10.1016/j.conbuildmat.2009.10.038.

[179] Q. Li, Q. Li, G. Yue, S. Gao, Determination of Recycled Coarse Aggregate Mortar Content and Research on Aggregate Lifting Technology, Bulletin of the Chinese Ceramic Society. 36 (2017) 3422–3425.

[180] H.K.A. Al-Bayati, P.K. Das, S.L. Tighe, H. Baaj, Evaluation of various treatment methods for enhancing the physical and morphological properties of coarse recycled concrete aggregate, Construction and Building Materials. 112 (2016) 284–298. doi:10.1016/j.conbuildmat.2016.02.176.

[181] J. Wang, J. Zhang, D. Cao, H. Dang, B. Ding, Comparison of recycled aggregate treatment methods on the performance for recycled concrete, Construction and Building Materials. 234 (2020) 117366. doi:10.1016/j.conbuildmat.2019.117366.

[182] A.M. Grabiec, J. Klama, D. Zawal, D. Krupa, Modification of recycled concrete aggregate by calcium carbonate biodeposition, Construction and Building Materials. 34 (2012) 145–150. doi:10.1016/j.conbuildmat.2012.02.027.

[183] J. Wang, B. Vandevyvere, S. Vanhessche, J. Schoon, N. Boon, N. De Belie, Microbial carbonate precipitation for the improvement of quality of recycled aggregates, Journal of Cleaner Production. 156 (2017) 355–366. doi:10.1016/j.jclepro.2017.04.051.

[184] C.R. Wu, Y.G. Zhu, X.T. Zhang, S.C. Kou, Improving the properties of recycled concrete aggregate with bio-deposition approach, Cement and Concrete Composites. 94 (2018) 248–254. doi:10.1016/j.cemconcomp.2018.09.012.

[185] M. Liu, J. Xia, C. Seong Chin, Z. Liu, Improving the properties of recycled aggregate pervious pavement blocks through bio-mineralization, Construction and Building Materials. 262 (2020) 120065. doi:10.1016/j.conbuildmat.2020.120065.

[186] L. Skevi, B.J. Reeksting, T.D. Hoffmann, S. Gebhard, K. Paine, Incorporation

of bacteria in concrete: The case against MICP as a means for strength improvement, Cement and Concrete Composites. 120 (2021) 104056. doi:10.1016/j.cemconcomp.2021.104056.
[187] K.K. Sahoo, M. Arakha, P. Sarkar, D.P. Robin, S. Jha, Enhancement of properties of recycled coarse aggregate concrete using bacteria, International Journal of Smart and Nano Materials. 7 (2016) 22–38. doi:10.1080/19475411.2016.1152322.
[188] J. García-González, D. Rodríguez-Robles, J. Wang, N. De Belie, J.M. Morán-del Pozo, M.I. Guerra-Romero, A. Juan-Valdés, Quality improvement of mixed and ceramic recycled aggregates by biodeposition of calcium carbonate, Construction and Building Materials. 154 (2017) 1015–1023. doi:10.1016/j.conbuildmat.2017.08.039.
[189] S. Marinković, J. Dragaš, I. Ignjatović, N. Tošić, Environmental assessment of green concretes for structural use, Journal of Cleaner Production. 154 (2017) 633–649. doi:10.1016/j.jclepro.2017.04.015.
[190] P. Van Den Heede, N. De Belie, Environmental impact and life cycle assessment (LCA) of traditional and “green” concretes: Literature review and theoretical calculations, Cement and Concrete Composites. 34 (2012) 431–442. doi:10.1016/j.cemconcomp.2012.01.004.
[191] M. De Schepper, P. Van den Heede, I. Van Driessche, N. De Belie, Life Cycle Assessment of Completely Recyclable Concrete, Materials. 7 (2014) 6010–6027. doi:10.3390/ma7086010.
[192] A. Yazdanbakhsh, L.C. Bank, T. Baez, I. Wernick, Comparative LCA of concrete with natural and recycled coarse aggregate in the New York City area, International Journal of Life Cycle Assessment. 23 (2018) 1163–1173. doi:10.1007/s11367-017-1360-5.
[193] W. Xing, V.W. Tam, K.N. Le, J.L. Hao, J. Wang, Life cycle assessment of recycled aggregate concrete on its environmental impacts: A critical review, Construction and Building Materials. 317 (2022) 125950. doi:10.1016/j.conbuildmat.2021.125950.
[194] D.R. Vieira, J.L. Calmon, F.Z. Coelho, Life cycle assessment (LCA) applied to the manufacturing of common and ecological concrete: A review, Construction and Building Materials. 124 (2016) 656–666. doi:10.1016/j.conbuildmat.2016.07.125.

[195] P. Wu, B. Xia, X. Zhao, The importance of use and end-of-life phases to the life cycle greenhouse gas (GHG) emissions of concrete - A review, Renewable and Sustainable Energy Reviews. 37 (2014) 360–369. doi:10.1016/j.rser.2014.04.070.

[196] J. Oliver-Solà, A. Josa, J. Rieradevall, X. Gabarrell, Environmental optimization of concrete sidewalks in urban areas, International Journal of Life Cycle Assessment. 14 (2009) 302–312. doi:10.1007/s11367-009-0083-7.

[197] C.I. Council, Carbon labelling scheme for construction products: assessment guide: Portland cement, Construction Industry Council, Hong Kong. (2013).

[198] Athena, Cement and Structural Concrete Products: Life Cycle Inventory Update# 2, (2005).

[199] G. Habert, J.B. D'Espinose De Lacaillerie, N. Roussel, An environmental evaluation of geopolymer based concrete production: Reviewing current research trends, Journal of Cleaner Production. 19 (2011) 1229–1238. doi:10.1016/j.jclepro.2011.03.012.

[200] Z. ZHAO, L. COURARD, S. GROSLAMBERT, T. JEHIN, A. LÉONARD, J. XIAO, Use of recycled concrete aggregates from precast block for the production of new building blocks: An industrial scale study, Resources, Conservation and Recycling. 157 (2020) 104786. doi:10.1016/j.resconrec.2020.104786.

[201] B.L. Damineli, F.M. Kemeid, P.S. Aguiar, V.M. John, Measuring the eco-efficiency of cement use, Cement and Concrete Composites. 32 (2010) 555–562. doi:10.1016/j.cemconcomp.2010.07.009.

[202] C. Chen, G. Habert, Y. Bouzidi, A. Jullien, A. Ventura, LCA allocation procedure used as an incitative method for waste recycling: An application to mineral additions in concrete, Resources, Conservation and Recycling. 54 (2010) 1231–1240. doi:10.1016/j.resconrec.2010.04.001.

[203] H. long Zhao, F. Liu, H.Q. Liu, L. Wang, R. Zhang, Y. Hao, Comparative life cycle assessment of two ceramsite production technologies for reusing municipal solid waste incinerator fly ash in China, Waste Management. 113 (2020) 447–455. doi:10.1016/j.wasman.2020.06.016.

[204] J. Jiao, J. Li, Y. Bai, Uncertainty analysis in the life cycle assessment of

cassava ethanol in China, Journal of Cleaner Production. 206 (2019) 438–451. doi:10.1016/j.jclepro.2018.09.199.

[205] Z. Mi, L. Kunliang, P. Feng, Whole Life Cycle Comprehensive Environment Impact Assessment of Hydraulic Power Chuck, 2019 2nd Asia Conference on Energy and Environment Engineering, ACEEE 2019. (2019) 35–39. doi:10.1109/ACEEE.2019.8816876.

[206] X.J. Li, Y. dan Zheng, Using LCA to research carbon footprint for precast concrete piles during the building construction stage: A China study, Journal of Cleaner Production. 245 (2020) 118754. doi:10.1016/j.jclepro.2019.118754.

[207] BSI Standards Publication, BS EN 196-1:2016. Methods of testing cement Part 1: Determination of strength, London, 2016.

[208] H.C.E. Teychenné, R E Franklin, Design of normal concrete mixes, Second, 1997.

[209] British Standards Institution BSI, BSI 1881-125:2013. Testing concrete — Part 125 : Methods for mixing and sampling fresh concrete in the laboratory, British Standards Institution, London, 2013. doi:Construction Standard, CS1:2010.

[210] British Standards Institution BSI, BS EN 1097-6:2013. Tests for mechanical and physical properties of aggregates Part 6 : Determination of particle density and water absorption, BSI, London, 2013.

[211] BS EN 12350-1:2009, Testing fresh concrete. Slump-test, European Norms. (2009) 5–8. https://www.en-standard.eu/din-en-12350-2-testing-fresh-concrete-part-2-slump-test/%0Ahttps://bsol.bsigroup.com/Home.

[212] BSI, BSI Standards Publication Methods of test for masonry units Part 1 : Determination of compressive strength, BSI, 2011.

[213] British Standards Institution BSI, BS EN 12390-6. Testing hardened concrete Part 6: Tensile splitting strength of test specimens, BSI, London, 2009.

[214] BSI, BS EN 12390-13:2013 Testing hardened concrete Part 13: Determination of secant modulus of elasticity in compression, BSI, London, 2013. doi:10.3403/30259410.

[215] L. Qixian, J.H. Bungey, Using compression wave ultrasonic transducers to measure the velocity of surface waves and hence determine dynamic modulus of elasticity for concrete, Construction and Building Materials. 10 (1996) 237–

242. doi:10.1016/0950-0618(96)00003-7.
[216] G. Singh, R. Siddique, Effect of waste foundry sand (WFS) as partial replacement of sand on the strength, ultrasonic pulse velocity and permeability of concrete, Construction and Building Materials. 26 (2012) 416–422. doi:10.1016/j.conbuildmat.2011.06.041.
[217] W. Yan, Z. Wu, F. Niu, T. Wan, H. Zheng, Study on the service life prediction of freeze–thaw damaged concrete with high permeability and inorganic crystal waterproof agent additions based on ultrasonic velocity, Construction and Building Materials. 259 (2020) 120405. doi:10.1016/j.conbuildmat.2020.120405.
[218] B.S.I. BSI, BS EN 12504-4:2004. Testing concrete — Part 4: Determination of ultrasonic pulse velocity, BSI, London, 2004.
[219] British Standards Institution BSI, BS EN 12390-8. Testing hardened concrete Part 8: Depth of penetration of water under pressure, BSI, London, 2009.
[220] Ministry of Housing and Urban-Rural Development of the People's Republic of China, GB/T 50082-2009. Standard for test methods of long-term performance and durability of ordinary concrete, China Construction Industry Press, Beijing, China, 2009.
[221] G. Sua-iam, N. Makul, Incorporation of high-volume fly ash waste and high-volume recycled alumina waste in the production of self-consolidating concrete, Journal of Cleaner Production. 159 (2017) 194–206. doi:10.1016/j.jclepro.2017.05.075.
[222] J. Nicklin, K. Graeme-Cook, R.A. Killington, Microbiology., 2nd ed., BIOS Scientific, 2002. https://liverpool.idm.oclc.org/login?url=https://search.ebscohost.com/login.aspx?direct=true&db=cat00003a&AN=lvp.b1908952&site=eds-live&scope=site.
[223] V. Saraswathy, S. Muralidharan, K. Thangavel, S. Srinivasan, Influence of activated fly ash on corrosion-resistance and strength of concrete, 25 (2003) 673–680. doi:10.1016/S0958-9465(02)00068-9.
[224] J.Y. Wang, N. De Belie, W. Verstraete, Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete, Journal of Industrial Microbiology and Biotechnology. 39 (2012) 567–577. doi:10.1007/s10295-

011-1037-1.
[225] R. Maddalena, C. Hall, A. Hamilton, Effect of silica particle size on the formation of calcium silicate hydrate [C-S-H] using thermal analysis, Thermochimica Acta. 672 (2019) 142–149. doi:10.1016/j.tca.2018.09.003.
[226] H. Aldarmaki, M. Diab, Proceedings of the Second Workshop on Arabic Natural Language Processing, 2015. http://oma-project.com/pub/Deep Learning Models for Sentiment Analysis in Arabic.pdf#page=185.
[227] E. Tajuelo Rodriguez, K. Garbev, D. Merz, L. Black, I.G. Richardson, Thermal stability of C-S-H phases and applicability of Richardson and Groves' and Richardson C-(A)-S-H(I) models to synthetic C-S-H, Cement and Concrete Research. 93 (2017) 45–56. doi:10.1016/j.cemconres.2016.12.005.
[228] V. Achal, A. Mukherjee, M.S. Reddy, Microbial Concrete: Way to Enhance the Durability of Building Structures, Journal of Materials in Civil Engineering. 23 (2011) 730–734. doi:10.1061/(ASCE)MT.1943-5533.0000159.
[229] S. Jiang, L. Jianzhang, Y. Yuexiang, Y. Zhiying, L. Yinzhang, Study on isolation and identification of heterotrophic nitrifying bacteria and optimal denitrification conditions, Transactions of the Chinese Society of Agricultural Engineering. 34 (2013) 35–41. doi:10.11975/j.issn.1002-6819.2018.z.006.
[230] J. feng Su, J. xin Shi, T. lin Huang, F. Ma, Kinetic analysis of simultaneous denitrification and biomineralization of novel Acinetobacter sp. CN86, Marine Pollution Bulletin. 109 (2016) 87–94. doi:10.1016/j.marpolbul.2016.06.017.
[231] M. Dittrich, S. Sibler, Cell surface groups of two picocyanobacteria strains studied by zeta potential investigations, potentiometric titration, and infrared spectroscopy, Journal of Colloid and Interface Science. 286 (2005) 487–495. doi:10.1016/j.jcis.2005.01.029.
[232] H.W. Reinhardt, Factors affecting the tensile properties of concrete, Woodhead Publishing Limited, 2013. doi:10.1533/9780857097538.1.19.
[233] J. Wang, K. Van Tittelboom, N. De Belie, W. Verstraete, Use of silica gel or polyurethane immobilized bacteria for self-healing concrete, Construction and Building Materials. 26 (2012) 532–540. doi:10.1016/j.conbuildmat.2011.06.054.
[234] D. Bonen, Calcium Hydroxide Deposition in the Near Interfacial Zone in Plain Concrete, Journal of the American Ceramic Society. 77 (1994) 193–196.

doi:10.1111/j.1151-2916.1994.tb06976.x.
[235] Ministry of Housing and Urban-Rural Development of the People's Republic of China, JGJT 193-2009. Standard for inspection and assessment of concrete durability, China Construction Industry Press, Beijing, China, 2009.
[236] T.C. Powers, A working hypothesis for further studies of frost resistance of concrete, Journal of the American Concrete Institute. 16 (1945) 245–272.
[237] M. Seifan, A. Ebrahiminezhad, Y. Ghasemi, A. Berenjian, Microbial calcium carbonate precipitation with high affinity to fill the concrete pore space: nanobiotechnological approach, Bioprocess and Biosystems Engineering. 42 (2019) 37–46. doi:10.1007/s00449-018-2011-3.
[238] W. Jin, Y. Zhao, Durability of concrete structures, Second Edi, Ke Xue Chu Ban She, Beijing, 2014. hrrp://www.sciencecep.com.
[239] V.M. Malhotra, P.K. Mehta, Pozzolanic and cementitious materials, CRC Press, 2014.
[240] W. Li, J. Xiao, Z. Sun, S. Kawashima, S.P. Shah, Interfacial transition zones in recycled aggregate concrete with different mixing approaches, Construction and Building Materials. 35 (2012) 1045–1055. doi:10.1016/j.conbuildmat.2012.06.022.
[241] J. Dahmen, J. Kim, C.M. Ouellet-Plamondon, Life cycle assessment of emergent masonry blocks, Journal of Cleaner Production. 171 (2018) 1622–1637. doi:10.1016/j.jclepro.2017.10.044.
[242] H. Hafez, R. Kurda, W.M. Cheung, B. Nagaratnam, Comparative life cycle assessment between imported and recovered fly ash for blended cement concrete in the UK, Journal of Cleaner Production. 244 (2020). doi:10.1016/j.jclepro.2019.118722.
[243] J. Mohammadi, W. South, Life cycle assessment (LCA) of benchmark concrete products in Australia, International Journal of Life Cycle Assessment. 22 (2017) 1588–1608. doi:10.1007/s11367-017-1266-2.
[244] I. Bianco, B. Ap Dafydd Tomos, R. Vinai, Analysis of the environmental impacts of alkali-activated concrete produced with waste glass-derived silicate activator – A LCA study, Journal of Cleaner Production. 316 (2021) 128383. doi:10.1016/j.jclepro.2021.128383.

[245] China Electricity Council, China Electric Power Industry Annual Development Report 2021, Beijing, 2021.
[246] H. Wang, K. Wang, Comparative study on comprehensive benefits of biomass and coal power generation project, Journal of Arid Land Resources and Environment. 36 (2020) 56–61. doi:10. 13448/j. cnki. jalre. 2020. 15.
[247] X.H. Di, Z.R. Nie, T.Y. Zuo, Life cycle emission inventories for the fuels consumed by thermal power in China, Zhongguo Huanjing Kexue/China Environmental Science. 25 (2005) 632–635.
[248] J. Hao, F. Di Maria, Z. Chen, S. Yu, W. Ma, L. Di Sarno, Comparative study of construction and demolition waste management in China and the european union, Detritus. 13 (2020) 114–121. doi:10.31025/2611-4135/2020.14029.
[249] D. Schrijvers, P. Loubet, G. Sonnemann, Consistent allocation using archetypes of LCA Goal and Scope definitions, Sustainability. 12 (2020) 2–3.
[250] W. Xing, V.W. Tam, K.N. Le, J.L. Hao, J. Wang, Life cycle assessment of recycled aggregate concrete on its environmental impacts: A critical review, Construction and Building Materials. 317 (2022) 125950. doi:10.1016/j.conbuildmat.2021.125950.
[251] T. Ding, J. Xiao, V.W.Y. Tam, A closed-loop life cycle assessment of recycled aggregate concrete utilization in China, Waste Management. 56 (2016) 367–375. doi:10.1016/j.wasman.2016.05.031.
[252] Jiangsu Price Bureau, Notice of Jiangsu Price Bureau on adjusting electricity price, Nanjing, 2016. http://www.wuxi.gov.cn/doc/2016/01/08/1205145.shtml.
[253] T. Levova, T. Levova, B. Weidema, E.M. Ruiz, Ecoinvent 3.6 dataset documentation, 2012.
[254] M. Sharma, N. Satyam, K.R. Reddy, Effect of freeze-thaw cycles on engineering properties of biocemented sand under different treatment conditions, Engineering Geology. 284 (2021) 106022. doi:10.1016/j.enggeo.2021.106022.
[255] Y. Su, J. Feng, P. Jin, C. Qian, Influence of bacterial self-healing agent on early age performance of cement-based materials, Construction and Building Materials. 218 (2019) 224–234. doi:10.1016/j.conbuildmat.2019.05.077.
[256] L. Xialu, W. Hongtao, C. Jian, H. Qin, Z. Hao, J. Rui, C. Xuexue, H. Ping, Method and basic model for development of Chinese reference life cycle database, ACTA SCIENTIAE CIRCUMSTANTIAE. 30 (2010) 2136–2144.

http://www.wanfangdata.com.cn/details/detail.do?_type=perio&id=hjkxxb201010030.

[257] G. Wernet, C. Bauer, B. Steubing, J. Reinhard, E. Moreno-Ruiz, B. Weidema, The ecoinvent database version 3 (part I): overview and methodology, International Journal of Life Cycle Assessment. 21 (2016) 1218–1230. doi:10.1007/s11367-016-1087-8.

[258] E. Crenna, M. Secchi, L. Benini, S. Sala, Global environmental impacts: data sources and methodological choices for calculating normalization factors for LCA, International Journal of Life Cycle Assessment. 24 (2019) 1851–1877. doi:10.1007/s11367-019-01604-y.

[259] M. Zhan, M. Fu, G. Pan, X. Lu, Y. Wang, Recycled aggregate mortar enhanced by microbial calcite precipitation, Magazine of Concrete Research. 72 (2020) 622–633. doi:10.1680/jmacr.18.00417.

[260] N. De Belie, Application of bacteria in concrete: a critical evaluation of the current status, RILEM Technical Letters. 1 (2016) 56. doi:10.21809/rilemtechlett.2016.14.

[261] Z. Zhu, Y. Guo, Y. Zhao, R. Zhang, Y. Yu, M. Zhang, T. Zhou, Sewage denitrification performance and sludge properties variation with the addition of liquid from perishable organic anaerobic fermentation, Bioresource Technology. 341 (2021). doi:10.1016/j.biortech.2021.125821.