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Microbial Biomineralization: Creating Wealth from Waste

Ayush Aggarwal, Ishneet Kaur, Vaneet Kaur, Jasmeen Kaur, Mayank Tyagi,
Daljeet Singh Dhanjal, Reena Singh and Chirag Chopra*
School of Bioengineering and Biosciences,
Lovely Professional University, Phagwara, Punjab-144411, India.

(Corresponding author: Chirag Chopra)*

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ABSTRACT: Biomineralization is the formation of inorganic substances by microbes. Microorganisms can initiate and even control the mineralisation process through its various mechanisms. Biomineralization plays a vital role in building construction and its preservation. Concrete is a significant material used in constructing buildings, and *E. coli*, a bacterial species, increase the concrete strength by repairing the cracks that appear within it over time. *Citrobacter* sp., a bacteria, help remove toxic wastes from the environment via bioaccumulation. *Bacillus spharecius* decomposes urea for the formation of carbonate ions. These carbonate ions bind to calcium ions to form calcium carbonate ($CaCO_3$), a mineral that serves many purposes, such as building construction, cement, and many more. Concrete is one of the most used materials globally for building construction. Biomineralization and self-repairing in concrete can address the problem of deterioration within it. Microbial induction in cement by following proper procedure and steps in obtaining self-healing concrete helps reduce the maintenance expenses and reduces the stress on the environment by eliminating conventional toxic methodologies engaged in repairing the cracks in the cement. This review focuses on a few species such as *Bacillus mucilaginous, Bacillus spharecius, Bacillus subtilis, Ascomycota, Purpureocillium, Illicium, Bacillus pasteuri, Sporosarcina pasteuri,* etc. and their role in processes such as calcite precipitation, bio-calcification and bacterially induced biomineralization etc.

Keywords: Biomineralization, Bacteria, Self-healing, Concrete, Environment friendly.

INTRODUCTION

Biomineralization is a combination of two words, "Bio" and "Mineralization", where "Bio" refers to biological elements such as microorganisms, and "mineralisation" refers to the process of producing minerals. Together, it refers to mineral production by microorganisms such as bacteria, fungi, algae, etc. A biologically controlled mineralisation is stepwise produce by which the living entities help to form minerals. The process is done to soften or harden the existing tissue. Through the process of biomineralization, the organisms form mineral materials via heterogeneous accumulations. According to IUPAC, biomineralization is causing by mineralisation by cell-mediated phenomena (Vert *et al.*, 2012). Biomineralization is quite a standard process amongst bacteria, as, during regular interaction of bacteria activity and the environment, a mineral is formed as a secondary product. Biomineralization can help in the removal of water polluted bodies and soils. Here are some bacteria and minerals produce by them via their mechanisms.

Our environment is surrounded by active processes that involve microorganisms exhibiting mineralisation. According to reports, microorganisms from soil and aquatic stream tend to induce calcium carbonate precipitation, which has portrayed the importance of microbial activity in the formation of carbonate (Peckmann *et al.*, 1999; Rivadeneyra *et al.*, 1998; 1994; 1993; Chafetz, 1986; Chafetz and Folk, 1984; Morita, 1980; Krumbein and Giele, 1979).

Table 1: Microorganism using different mechanism to produce minerals.

Mechanism	Minerals	Microorganism Example
Soluble Biopolymers	Manganese (Mn) /Ferric oxyhydroxide FeOOH	*Leptothrix pedomicrobium*
Spore coats	Manganese oxide-hydroxide (MnOOH)	*Bacillus*
Surface layer protein	Ferric oxyhydroxide (FeOOH)	*Lepotothrix*

Biogenic processes, especially in an aquatic environment, have effectively precipitated carbonates (Mann, 2001; Peckmann *et al.*, 1999). In the past few decades, most studies have been more inclined to get insights into the development of calcification processes in marine species (Peckmann *et al.*, 1999; Lowenstam and Weiner, 1989; Novitsky, 1981). A report showed that calcite formation through soil bacteria is a generalised and common phenomenon (Boquet *et al.*, 1973). The study involving carbonate precipitation via soil bacteria is of enormous importance as it helps in knowing deep about terrestrial carbon sink (Lal *et al.*, 1999). Mortar plays a very significant role in it.

Significant advances in industrialisation and urbanisation, be it constructing buildings, dams, roads, tunnels, bridges, highways, there is always a high demand for it. But these structures, due to extreme and aggressive environmental conditions or harsh physical, chemical or biological conditions, tend to lose their strength, their porosity increases, durability decreases, resulting in these structures fail to fulfil their design life services. So, these structures often require intensive repair and maintenance throughout the whole design life, which leads to substantial financial and environmental expenses. This brings us to the point that there is a strong need to adopt and advance the applications of bio-mineralisation as it is a very promising and sustainable repair methodology utilising the application of microorganisms (Park *et al.,* 2012).

Bacterially induced bio-mineralisation occurs via two mechanisms. The first is biologically controlled (BCM) in which, microorganisms control the growth and nucleation of organic-inorganic mineral and synthesise mineral in such a form that it is specific to that species only and is not dependent on the environmental conditions (Weiner and Dove, 2003). Furthermore, the other one is biologically induced (BIM), dependent on environmental conditions and no specific structure or mechanism (Park *et al.,* 2012). The prime methodology is bio-calcification, sometimes referred to as MICP or microbiologically induced calcite precipitation. It is seen that a considerable number of soil microorganisms such as *Bacillus mucilaginous*, *Bacillus spharecius* etc., exhibit urease producing ability and have the potential to improve the durability of concrete. It is generally a process of converting organic compounds to inorganic compounds (Vempada *et al.,* 2011).

A significant example is *Bacillus spharecius* which produce some amount of $CaCO_3$ and urease. Their combination results in the decomposition of urea to form a carbonate ion. This carbonate ion reaches calcium ion in the environment to create a residue and fill in the cracks.

Table 2: Difference between Biologically Controlled Mineralisation (BCM) and Biologically Induced Mineralisation (BIM).

BCM	**BIM**
Controlled by biotic factors	Generally, depending on abiotic environmental factors
Organisms involved have extensive control over the process	Organisms have minor or no control over the process
Microorganisms control the growth and nucleation of organic-inorganic minerals	Environmental factors control it
It is a specific process	No specific structure or mechanism is involved
Usually results in well-ordered mineral structures	Results in heterogenous mineral compositions
Structures formed have a minute or minor variation	Significant size variations are involved in result structures
Do not have poorly defined crystal morphology	Have poorly defined crystal morphology
Microscopic impurity inclusions	Have inclusion of impurities
Example—shells of invertebrates.	For example—ureolytic calcite forming bacteria like *B. sphaericus*

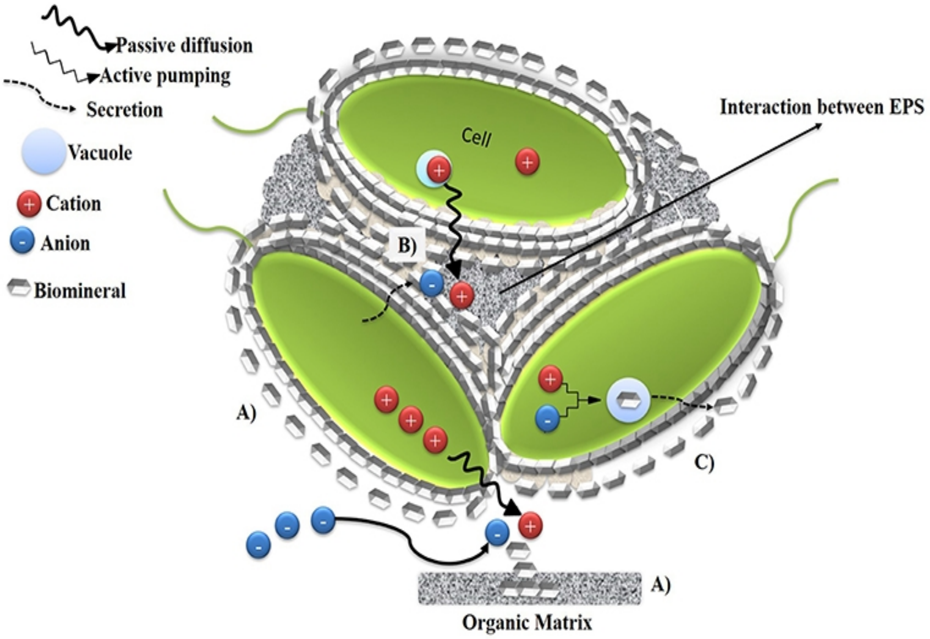

Fig. 1. Schematics of BCM.

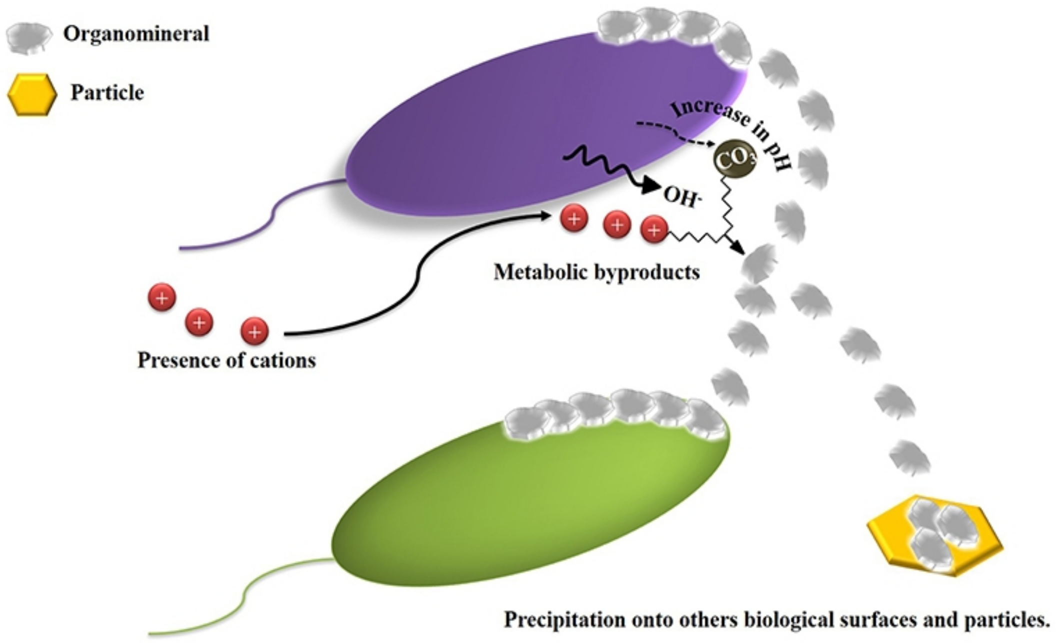

Fig. 2. Schematics of BIM.

Majorly two conventional MICP processes exist. The first process includes the urease system, in which hydrolysis of urea by the bacteria takes place. In this process, the enzyme urease (urea amino hydrolase) as a catalyst is utilised. In the urea-based MICP process, the hydrolysis reaction of urea leads to the production of ammonia and carbonate ions. In the presence of $CaCl_{2,}$ which acts as a source of Ca^{2+}, high pH content bacteria results in crystal precipitation of $CaCO_3$ from the solution? Usually, bacterial cell walls are embedded with various negatively charged ions. They primarily attract cations Ca^{2+} present around the cell wall, and these react with CO_3^{2-} and precipitation of $CaCO_3$ around the cell takes place (Qureshi and Al-Tabbaa, 2020). The second process is the one which is the calcium lactate ($CaC_6H_{10}O_6$) based MICP: calcium lactate is a crystalline salt, and it is produced as an outcome of the reaction between lactic acid and calcium carbonate or hydroxide, it can also be used as a substitute for urea-$CaCl_2$, and precedes bacterial metabolism in concrete to prevent the production of ammonia in hydrolysis reactions. Metabolic absorption and breakdown of calcium lactate with bacteria causes or results in precipitation of $CaCO_3$ (Qureshi and Al-Tabbaa, 2020). The principle behind self-healing concrete is to add carbonate producing bacteria during the process of blending and merging of components of mortar so that at the time when cracking takes place, bacteria will tend to get activated and then cause precipitation of $CaCO_{3,}$ which will fill in the cracks and will increase durability. However, researchers favour spores instead of vegetative cells to increase the life span (De Belie, 2016).

The studies on achieving strength improvements via adding bacteria into concrete mix show variability in results. Moreover, many researchers have researched and mentioned the rise in 28 d compressive strength, which was initially 9% and rose to 25% due to mixing bacterial cells into a standard mixture of concrete or mortar. There is variability in results determined by the bacterial strain used, cell concentration of strain used, or concrete age (De Belie, 2016). Bio-mineralisation technique can compete in the future with other traditional treatments to decrease water absorption and increase the strength of infrastructures constructed from concrete.

MICP – Microbial Induced Calcite Precipitation or Microbial mineralisation converts the organic compounds into inorganic ones using bacteria such as *Bacillus spharecius* and *Bacillus Mucilaginous*. *B. spharecius* produces some amount of calcium carbonate and urease, which, when combined with the latter, produce its actions, i.e., decomposition of urea to form carbonate ion. This carbonate ion goes to calcium ion present in the environment to form calcium carbonate. *Bacillus mucilaginous* is added to the culture solution of *Bacillus spharecius*, which enhanced the yield and growth of *B. sphaecicus*. For the proper growth of *B. spharecicus*, the things that need to be taken care of are the optimum pH value, i.e., 7 -8, the urea concentration, i.e. 0.5 mol/L, the Ca^{2+} ion concentration, i.e., 0.6 mol/L, and the optimum L.B. concentration.

Thus, when the introduction of *B. mucilaginous* is done, the changes in the yield, concentration, environment factors, and properties are increased without even affecting the mineralised products. Mineralisation has a proved their vast range of applications, such as – when we use the minerals to repair the cracks in cement material as in concrete, with the help of minerals the repair can be done by their own, i.e., self-repair. In the Netherlands, the cement that contains the microbes has been implanted to repair the cracks that come at the surface (Hu *et al.,* 2020).

Uranium is such an element responsible for nuclear pollution, which releases the chemically toxic and radioactive particles that move to the environment and damage the human lifestyle and biodiversity. Uranium exists in different forms in the environment such as U (IV) and U (VI), which produces the UO_2^+ ion, which have the factors such as high insolubility and mobile due to this factor, it can travel into near surroundings from its source such as nuclear fuel, nuclear power plants or mills which ultimately lead to the uranium contamination in the environment in the large scale. Due to bioaccumulation, the uranium gets moving in the chain and gets ingested by humans, leading to some serious issues such as kidney failure, liver damage, and sometimes even death. Through the biomineralization by the minerals, the uranium can be fixed via the reaction between the microbes and the uranium, and the mineralisation led to low mobility of the uranium. It can be categorised into two parts. One is reductive, and the other is non-reductive, which depends on the last valence electrons of the uranium utilizing strains such as *Citrobacter sp*. *Bacillus subtilis* can be used to reduce the uranium's toxicity and make it soluble in the surrounding. *Bacillus subtilis* shows the property of good solubility, adsorption, resistance and affinity to uranium. The strain that is used is *Bacillus subtilis ATCC-6633* which mainly focus on two things, i.e., size of mineral and crystallisation degree also the uranium mineralisation, which depends on the factors such as P.H., the amount of U. SEM, TEM, FT-IR are used during the stages of mineralisation to observe the bacterial groups, i.e., Phosphoric acid group and amide group. The result shows that the *B. subtilis* ATCC-6633 can quickly be immobilised the U (VI) under aerobic conditions such as pH at 5 with an incubation period of 12 h (Song *et al.,* 2019).

A. Applications of Biomineralization

Engineering practices involves the high-end application of mineralisation (Joshi *et al.,* 2017; Seifan *et al.,* 2016). For instance, cement materials such as concrete can use biomineralization to achieve self-repairing of cracks. The implementation of biological cemented sand in repairing structural surface cracks has been successfully done by various researchers (Wang *et al.,* 2012; Van Tittelboom *et al.,* 2010). According to Bangs *et al*., 2001, microbes' enzymatic action can cause the precipitation of calcium carbonate. The study also included the demonstration of the use of microbes for repairing cracks over the surface of cemented materials. Biomineralization has also shown to be effective in crack repairs in decorative limestone surfaces (Rodriguez-Navarro *et al.,* 2003). Biomineralization is not limited to crack repairing only; it has various other applications as well.

Strength improvement of concrete. Concrete is highly in use today. Most of the constructions include the use of concrete as it provides excellent strength to the building structure. However, concrete also suffers from deterioration due to corrosion. Two major causes for such deterioration are carbonation and chloride-induced corrosion. Biomineralization stands outs to be a solution for such a problem. Researches are still undergoing this, but based on previously conducted studies, microbes that are added into the mortar or concrete for self-healing have also found to be effective in improving the overall strength of the mortar or concrete. According to an experimental study conducted by Vempada *et al.,* 2011, Several microorganisms were mixed with mortar mixture at a fixed concentration ratio (Vempada *et al.,* 2011). The microbes that are considered were involved in the disintegration of urea in ammonia and carbon dioxide via the action of enzymatic activity performed by urease, resulting in carbonate ions as calcium carbonate. The more urease production, the more the strength enhancement was observed. Also, according to the study, the choice of microorganism for strength improvement is very crucial. *E. coli* did not contribute anything to strength improvement. In contrast, all other microbes have shown some significant contribution to concrete strength improvement. The most substantial development in compressive power occurs at cell concentrations of 10^5 cells/ml for a long time. Several studies have shown that mixing bacterial cells into the mortar or concrete has increased the 28-d compressive strength by 9 to 25% (Achal *et al.,* 2012; Ghosh *et al.,* 2009). It is pretty challenging to prove the effect of living bacteria in strength improvement. In a mortar matrix, the bacterial cell is non-viable as their activities are limited due to lack of nutrition, oxygen and various other factors. Still, a reasonable explanation can be given. The reason is that because of accumulation on the microbe's cell surfaces not only on cell surfaces but also within the pores of cement–sand matrix, which occupied the pores which is present in the mortar thus strength increased (Vempada *et al.,* 2011).

Surface Bio consolidation. Biomineralization has been applied for conserving the historical decayed stones or monumental structures (Tiano *et al.,* 2006). A work conducted by Muynck *et al.,* 2014 used DRMS (Drilling resistance measurement system) to measure the strengthening effects of bio deposition on Maastricht Limestone. The study demonstrated the homogenous strengthening of limestone up to the depth of 30mm with at least better than traditional surface treatments. Microbial mineralisation has increased the drilling resistance by double, if not more (De Muynck *et al.,* 2014). The various researchers were also able to lower the cost of this treatment by taking some actions as minimising the level of the urease and carbonate precursor solutions. To date, we have not experienced much on-site bio consolidation, and it has been limited to porous calcareous stones of cultural heritage buildings. MICP treatment has been applied to cement-based materials, which has entirely revolutionised the process (Achal *et al.,* 2012; Chunxiang *et al.,* 2009). A study by Muynck *et al.,* 2014 showed that the exterior accumulation of calcium carbonate crystals on mortar drops the level of aqua immersion by 65 to 90%, which depends on the specimens' porosity. This resulted in decreased carbonation rate and chloride migration about 25–30% and 10–40%, respectively (De Muynck *et al.,* 2014).

Self-healing of cracks. The self-healing procedure is established on the principle of precipitating microbes, mainly bacteria. The term self-healing itself says that the fault or part that requires improvement heals itself into a better state. This whole self-healing process relies on carbonate precipitating bacteria which shows its effect when mixed into concrete. Via the procedure, the part of bacteria is simply initiating the precipitation of calcium carbonate in the in-situ condition to deal with the whole process. The self-healing process is helpful so that it leads to water-tightness, which further stops the penetration of corrosive-based materials in the concrete material, thus stabilising the concrete. Bacteria should able to extend the viability until the crack forms up (De Belie, 2016). For this purpose, researchers have proposed using spores instead of vegetative cells to viable for a longer life span (Setlow, 1994). The use of yeast removal is health-giving for *B. sphaericus* accelerates spore's shoot up and bio precipitation, especially in an unfavourable condition, like during lesser temperatures and in the existence of more concentrations of Ca^{2+} (Wang, 2013). According to Achal *et al.,* 2012, *Sporosarcinas pasteurii* decreases the level of depth of water precipitation. It also found that the calcium carbonate formed causes the low permeability of concrete due to the region formation of calcium carbonate interphase (Achal *et al.,* 2012). Studies also explain *Bacillus sphaericus* to heal the cracks with chemicals such as calcium nitrate or calcium acetate. The use of polyurethane and melamine capsules was in action that works when put in silica gel and the *Bacillus sphareicus* spores. It helps heal and level up the life span of bacteria (Wang *et al.,* 2014).

Bioaccumulation. The accumulation of toxic waste from industries is increasing day by day, and it is becoming a severe issue and attracted much attention these days. The discharge from the industries is causing severe harm, especially to the sea, as the metal-laden sewage sludge is dumped in that area. These heavy metals contain the chemical hazard of toxic radioactive materials. They may come from nuclear fuels, too, as the radioactive residuals are discharged into the environment. The chemical process involves the two most widely used techniques are solvent extraction and chemical precipitation. Other chemical processes such as ion exchange require costly material like absorption –cyclic desorption technique during their procedure. Biological based methods are a great alternative to the chemical removal of metal from an aqueous environment (Macaskie *et al.,* 1994).

The biological method falls into two ways: non-growing biomass, where they uptake the metal material and the other ways is bioaccumulation by living cells (Macaskie and Dean, 1989). In the Absorptive uptake method, there is a use of chemical strains and chemical adsorption isotherms. In the biomass method, the accumulation of toxic material can occur if their aerobic precipitation reactions. When bio precipitation occurs, the accumulation level increases by 15 % of dry biomass weight (Macaskie and Dean, 1989; Brierley *et al.,* 1986). The bio sorbents are introduced in the market, which absorbs the accumulation of the metal via biological process with microorganisms. The bioaccumulation metal uptake is the primary step in the water detoxification process, as biological fluidised beds have the growing biofilm (Remade and Houba, 1983). The biofilm-based beds are used in industries for their discard of waste into the water. A well-known example of the biological bed is the homestake wastewater treatment process (USA) also in Moscow, Ukraine and Kazakhstan, where chromate treatment-based process occurs on a large scale (Mudder and Whitlock, 1984; Koren'Kov *et al.,* 1979), where the whole process uses the cyanide –oxidising microbes (bacteria) where they can procreate at the expense of C.N. negative and can absorb the contamination of metal from the environment. This plant contains 48 rotating biological contractors with a large biofilm area, leading to the removal of metals by 95% metal such as N.I. Pb, Zn can be removed. The remaining half of the process involves the microbe based enzymatic chromate reductase activity, which can lead to the formation of Cr^{3+} and the condensation of insoluble $Cr(OH)_3$ onto disabling biofilm. This whole exercise is a continuous one and reduces the level up to 190 microgram ml at the flow rate of 7200 m^3/day (Koren'Kov *et al.,* 1979). Both processes are ideal for the specific removal of metals in situ. These processes have certain disadvantages in the discard of unwanted material with increased biomass to metal ratio. To overcome this disadvantage, the enzyme medicated metal precipitation (biomineralization). Hence, the enzymatic metabolic process can produce some metal –D.E. solubilising ligands in an unbroken manner. The exocellular deposition of metals occurs by metal-ligand precipitation; here, the metal precipitation occurs when the metal-ligand solubility outcome passes its average value. An example of that is *Alcaligenes eutrophus* bacteria, which is the metal resistant strain isolated from sediments from the decantation basin of the zinc factory. The CH_{34} also have the property to bear the large plasmid controlling resistance to heavy pollutants. *A. eutrophus* relies on the principle of alkalinisation of the periplasmic space with the hydrogen ion uptake counter to the metal efflux (Macaskie *et al.,* 1994).

Biomineralization of calcite. As Prokaryotes, bacteria are involved in microbiological biomineralization. The fungi are also well involved in the biomineralization process in many biochemical cycles. They are well known as the main ones in the following fields -- organic matter recycling, as nutrient suppliers via weathering. They are also significant producers of organic acids as oxalic acid and the formation of metal –oxalate. Calcite is an essential component of our ecosystem and plays a vital role in the carbon and calcium cycle. Fungi play a crucial action in $CaCO_3$. Biomineralization *Ascomycota, Purpureocillium, Illicium* are some important fungi engaged in the biomineralization of jarosite, a type of sulphur and iron minerals that are made during low pH conditions via its metabolite performance. The biomineralization of $CaCO_3$ depends on factors such as pH, temperature, PCO_2, Carbonate alkalinity and calcium concentration. All factors contributed to reducing the calcium and carbonate concentration in the environment, which fixes it via reactions (Bhina *et al.,* 2019).

B. Role of Microorganism in Biomineralization

Screening of Gold Biomineralization Mechanism in *Cyanobacteria*. Biomineralization named itself explains its meaning that potential of metal up taking process through the natural way. Most microorganisms that are involved are generally fungi, bacteria and yeast. They have been known to participate in the biomineralization process for some time. Through the biomineralization process, the microbes can extract the metals in a solid state very efficiently. The microbes extract the metals through metal ion stress response (Campbell and Martin, 1990). The procedure is also helpful for removing precious metal recovery and heavy metal bioremediation (Reith *et al.,* 2009). *Cyanobacteria* are a prokaryotic group of bacteria that have their energy source via photosynthesis. The other well-known name of cyanobacteria is blue, green algae. They are primarily found in the soil, water, marine water, and marine environment (Chakraborty *et al.,* 2009). In biomineralization, the cyanobacteria are pretty helpful; they have six strains with a high capacity for gold biomineralization. In the environment, the microbes cope up with the metal ion uptake process. They adjust to the situation so that they decrease the level of Metal ion stress in the environment. Microbes accumulate toxic metals in their cells in solid form (Campbell and Martin, 1990). One of the uses of biomineralization is bioremediation – it is a type of cleaning produce where the living entities are likely to clean up the area of contamination. Through bioremediation, the toxins from the environment can be removed, such as soil, water and more. In bioremediation, the microbes get their advantage, too, as they clean up the atmosphere. They utilise that contamination as the source of energy or food for them.

Another fascinating application of biomineralization is the recovery of precious metal from the environment with living organisms' help. They are known to collect some expensive metals such as gold deposited in the background with some treatment process or any procedure. Reith *et al.,* (2009) was able to find a gene among bacteria called *Cupriavidus metallidurans,* which is known to be useful for gold biomineralization (Reith *et al.,* 2009). The biomineralization process is found to be stressful procedure microbes are adapting in nature with the environment. They cope up and give responses concerning that condition. Cyanobacteria are one of the oldest groups among prokaryotes organism. They are known to be present in many places, such as Antarctic soil, volcanic hot springs (Ressom and Ressom, 1994). The bacterial species or blue, green algae have some properties. They can use oxygen via photosynthesis, using the water where the H_2^+ ions act as an electron donor for carbon dioxide reduction.

Not only this but also, they have wide ecological tolerance (Stal *et al.,* 2000). For the mechanism to start up, the screening process is initiated. A specially designed program language called PERL is used to form an instrument that will automatically pick up the target genome sequence. Here is 19 gold genome sequence. The NCBI standalone use the PERL program language for the BLAST application (BLAST + version 2.2.2.8 +). They act against each genome to find out the similarity in the genome sequence. For BLAST to act upon, the E- value is used, which is 10 to the power of -6. The two other things are also used: percentage of query and hit coverage of 50 %. After all this stepwise procedure, the BLAST result is collected and sum up, which tells us similar genes with the target (Dissook *et al.,* 2013). The strains of cyanobacteria that are potentially identified for screening the gold biomineralization are as follow –

Table 3: List of bacterial strain along with the protein sequence matched (Dissook *et al.,* 2013).

Name of Bacteria	**Strains**	**Protein sequence matched**
Chloroflexus aurantiacus	J-10-f1	11 protein sequence
Anabaenca cylindrica	PCC 7122	10
Calothrix sp.	PCC7507	10
Chamaesiphon minutus	PCC6605	10
Nostoc sp.	PCC7107	10
Rivularia sp.	PCC7116	10

C. Biomineralization Technique in Self-healing of Fly ash concrete

Bacteria have many properties; self-healing is one of them. The bacteria can act as bio-based agents, which mainly serve as a healing source in healing cracks by themselves in the fly as concrete. The functions of a microbes-based agent depend on two properties they are mechanical properties and durability properties. The cracks made due to external factors to heal them by selves, the different dosages of bacteria were given, and all the various bacteria at different cell concentrations. The research shows up in case of compressive strength by 15.6% with the depth of 1.2 inch and strains of bacterial cells by 105 cell/ml. The properties such as strength and durability of fly-ash concrete are improved also decrease the porosity. This all happens due to calcite formation, which fills up the pores. Here calcite act as a bio-based agent. Two different methods can detect the formation of calcite crystals. They act as a scanning electron microscope. The two different methods are SEM and XRD analysers. These bacterial strains are pretty suitable and act as a self-healing source that helps repair cracks and reduce maintenance. The other advantages are they are eco-friendly as they decrease the level of carbon dioxide emission. Also, a lesser amount of cement would be required to make the concrete sustainable. They are economical, i.e., they tend to save money (Kadapure *et al.,* 2017). Concrete is a robust construction material with a low cost, but the things that limit up the use of concrete are as follows: they have a limited lifespan and can easily be cracked (De Koster *et al.,* 2015; Jonkers *et al.,* 2010; Barbhuiya *et al.,* 2009). For the enrichment of the life of concrete, the cracks needed to be repaired. The older ways of fixing the cracks are a little unfriendly to the environment. They include epoxy systems, acrylic resins and silica-based polymers (Siddique and Chahal, 2011; Jonkers and Schlangen, 2008). Nowadays, the agents are applied to an external environment that will eventually penetrate the cracks but are hazardous to the environment and human health. Thus, there was a dire need for an eco-friendly, low-cost source that only repair the concrete and protect it. The bio-agents are insoluble in water (Wang *et al.,* 2012).

In the era of biomineralization, a new source called bio-based was introduced into the construction market. Though humans make these bio-based agents in industries with the help of biomineralization, the biological agents can fill up the cavities or cracks in concrete (Ghosh *et al.,* 2009). The biological concrete process is performed by the living entities, which perform their process by depositing inorganic solids into the pores of concrete or cavities. The extracted minerals are dense well as can block the cracks (Jonkers *et al.,* 2010). They are sometimes referring as Smart Bio-Material the reason behind this is the ability to accumulate calcite continuously. Some of the concrete bacteria have the features to form the spores, specifically endospores, which dominate nature and can bear extreme environmental conditions. More often, fly ash is used along with concrete which covers up the increasing demand for concrete (Barbhuiya *et al.,* 2009). The fly ash is referred to as a replacement for the cement. The other remarkable advantage of using fly ash in concrete is that it mainly occupies an ample space during disposal (Ahmaruzzaman, 2010). Bacterial species are unicellular organisms among microbes with 40 million cells in a gram of soil. Some bacterial features can produce at a very high rate and double their population under optimal conditions, only under 9.8 minutes. The concrete is alkali in nature, so the selected bacterial species should also survive under alkali conditions for a long time (Barbhuiya *et al.,* 2009).

When we say about surviving the extreme conditions, the Bacillus is one of the bacterial species that came forward. The bacillus species can produce the urease that further reacts to urea for transforming it to ammonia and carbon dioxide. Thus, urease act as a catalyst in the reaction. This leads to a rise in pH as minerals and ions accumulate as $CaCO_3$ (Chen *et al.,* 2020; Ahmaruzzaman, 2010; Sookie and Ramakrishnan, 2001).

The reaction:
Ca^{2+} + cell -→ cell – Ca^{2+} cell – Ca^{2+} + CO_3^{2-} → Cell --- $CaCO_3$

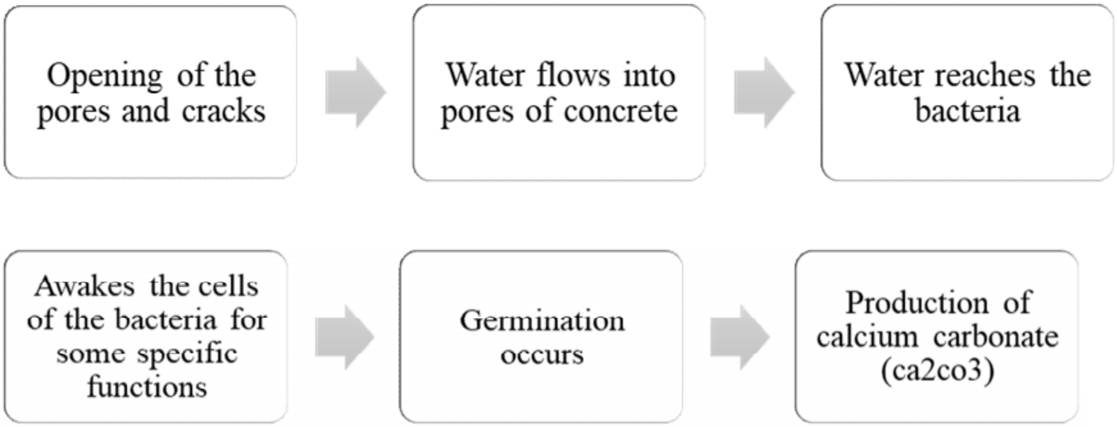

Fig. 3. Illustration of the process of Ca_2CO_3 production in Fly ash concrete.

D. Improving the durability of concrete by biomineralization

The area or zone present in concrete termed the Interfacial Transition zone (ITZ) is weak and frail. It possesses high porosity compared to the matrix, which sums up why it easily breaks under strain-full conditions and is counteractive to concrete's durability. However, the interfacial transition zone present in concrete is abundantly intact with crystals of calcium hydroxide that are efficient enough to be utilised to supply the calcium source for the process of biomineralization. In the study by Chen *et al.,* 2020, environmentally friendly bacterial strain *Sporosarcina pasteuri* and lightweight aggregate as a bacterial carrier were utilised. In the initial step, the bacterial strain was sporulated and fixed in lightweight porous aggregate for protection.

Further these lightweight aggregates also served the purpose of concrete aggregates. The study results revealed that the employment of lightweight aggregate as a carrier accompanying the inoculate of *Sporosarcina pasteuri* is successful in inducing biomineralization (Chen *et al.,* 2020). The method is efficient in repairing small internal concrete cracks and can strengthen the ITZ. Composite materials like concrete are constituted of certain essential elements. For such reasons, even though it comprises excellent properties, it still costs so cheap; concrete is considered the most common building in a significant part of the world. Nevertheless, the deformations and internal cracking in concrete occur due to its response to external loads, strained and stressful environmental condition. These factors can be categorised into two categories. The first category is of the internal causes, like aggregate alkaline reactions. The second category is external or environmental causes like physical strains, effects of chemical reactions and mechanical effects (Mehta and Monteiro, 2014).

Crack repair in concrete, acrylic resins, epoxy methods, or silicone-based polymer methods are the most commonly utilised methodologies (Sierra-Beltran *et al.,* 2014; Dry, 2000). Nevertheless, these methods are highly detrimental to the environment. The fact cannot be ignored that these substances are pretty insignificant for engaging them in repairing because of some sorts of compatibility issues with the concrete substrate, and Various researchers from all across the world have been working toward repair methodology which is not only environmentally friendly but is also sustainable repair technique and a large number of studies have pinpointed that minimal to small cracks present in concrete can be self-repaired by themselves with the help of phenomena such as continuous hydration and other physical and mechanical in cement and this phenomenon is referred to as "autogenous healing" or "concrete self-healing." (Castro-Alonso *et al.,* 2019; Xu and Wang, 2018). The biomineralization process is a quite extensive natural phenomenon and is referred to or defined as a process by which organisms, through their metabolic activities related to the environment, produce minerals (Iheanyichukwu *et al.,* 2018). Living organisms have biomineral phases with biopolymers as a part of this process, and microorganisms engaged in the process produce metabolites and their interaction with environmental ions or compounds, followed by mineral deposition as metabolites (Frankel, 2003). Biomineralization is remarkably dissimilar from geological mineralisation because the inorganic phase's crystallisation is rigidly under the control of organic matter produced through secretion by the organism so, the process of biomineralization often forms organic-inorganic composite material which is well ordered having excellent structure (Rivadeneyra *et al.,* 1998).

Microbiologically induced calcium carbonate precipitation (MICP) technology is generally based on microbial mineralisation and has become the point of contention in self-healing technology in concrete and successfully attracts considerable awareness in the academic community. As mentioned earlier most generally used a strain in biomineralization is *Sporosarcina pasteurii*, a Gram-positive bacterium (Minto *et al.,* 2018). This bacterial species is most commonly found in soil and can produce extensive intracellular urease. The urease produced can be utilised in catalysing the hydrolysis of urea to produce ammonia, carbon dioxide, and hydroxide ions. The pH gradually rises due to releasing of hydroxide ions, and then conversion of carbon dioxide into carbonate ions occurs. These ions react and then tend to combine with calcium ions present in the environment, and as a result, calcium carbonate tends to precipitate with other substances. Calcium carbonate precipitation is the pinpoint and primary behaviour of MICP.

In concrete's case, most of the researchers believe that the most critical factor that affects the process of its autogenous healing is calcium carbonate precipitation. MICP technology engages the urease secreted during microbial metabolism to hydrolysing urea for generating carbonate ions. In turn, these ions combine with calcium ions to form calcium carbonate with premium cementation quality, therefore fulfilling the aim to improve concrete's mechanical properties.

The process has proved to be successfully helpful to repair concrete or repair limestone surfaces and significantly increase the durability of concrete (Han *et al.,* 2019; Magaji *et al.,* 2019; Tsangouri *et al.,* 2019). However, in essence, the bacteria utilised in these studies as a part of the experiment were then applied externally to cracked concrete. In conclusion, we can say that the repair mechanisms discussed in these studies fail to meet the literal meaning or definition of self-healing. So, researchers worked by mixing bacterial spores and organic compounds at initial steps with fresh cement paste instead of going with the conventional method. It was observed that calcium carbonate precipitation from bacteria was evident in specimens of early concrete (days 1–7). Moreover, the conclusion showed that the colloidal pores gradually shirked and caused the crushing of spores during the cement hydration stage due to continuous hydration (Wiktor and Jonkers, 2011; Jonkers *et al.,* 2010).

In water permeability tests, the results showed that the permeation depth and total permeation area of lightweight aggregate concrete containing biological bacteria were smaller when compared to the control group. The chloride ion test results showed that the electric flux of lightweight aggregate concrete containing biological bacteria was low compared to that of the control group. Both these test results affirm that biomineralization plays a crucial role in strengthening the ITZ in concrete and can repair concrete cracks, thus increasing the compactness of concrete and providing prime quality of durability (Chen *et al.,* 2020).

E. Microbial Mechanism for Biomineralization

***C. metallidurans*-induced gold biomineralization.**

Au (III)-complexes are reduced to Au^0-particles via fast accumulation, which further leads to the formation of Au(I)-S complexes as intermediates. It follows a slow bio-reduction process which leads to intracellular and extra-cellular deposition of Au particles. As Au (III) gets absorbed to the cell surface, its reduction takes place rapidly to Au (I) because of the high redox potential of Au (III) complexes. This causes the uptake of electrons from suitable electron donors, which leads to induce oxidative stress in cells. The Au (I)- complex, thus formed, quickly binds with non-polar bases; for example, S, which are available in membrane and the upper cytoplasmic proteins. This leads to the formation of Au(I)-S species. Specific active mechanisms are responsible for the less quick reduction of Au(I)-S complexes to Au^0 particles. The accumulation potential and the deposition of nanoparticles in *C. metallidurans* were observed only after amendment with Ag(I)-thiosulfate complexes, which lead to Ag particles' formation associated with membrane (Reith *et al.,* 2009). Cyanobacterium *Plectonema boryanum* also showed a similar kind of bio-reducing activity. In this, the reduction from Au (III)-complex to metallic Au included a rapid formation of Au(I)-S intermediate and eventually a slow reduction to Au^0 (Lengke *et al.,* 2006).

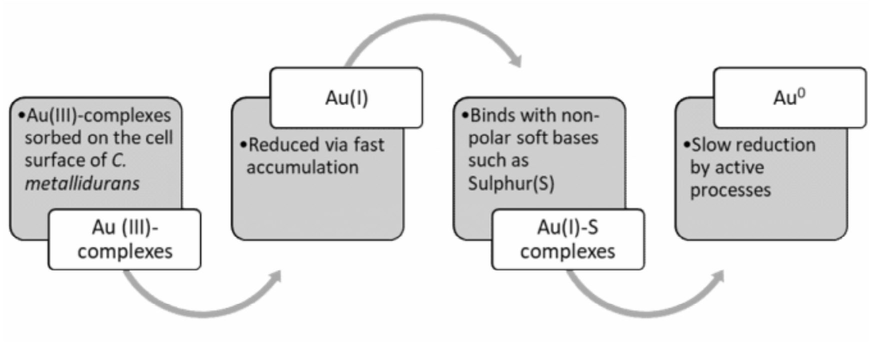

Fig. 4. Illustration of bio-reduction of gold in *C. metallidurans.*

Biomineralization of gold by Zygomycete Fungi Rhizopus oryzae

Reduction of gold chloride to particulate gold occurs both at the cell wall and the cytoplasmic region of fungi *Rhizopus oryzae*. To understand this process, Das *et al.*, conducted some experiments to get insights into the biomineralisation. When *R. oryzae* was incubated with a solution of $HAuCl_4$, a change in the mycelia colouration was observed. With time there was a colouration shift from pale white to purple. This shows that certain chemical processes are undergoing with the cell that accounts for the colour change. The purple colour represents the formation of nanoparticulate gold [Au^0], while the colour that occurs during the transition from pale white to purple represents bio-reduction from Au (III) to Au^0. Das *et al.*, (2010) also played with pH variation to understand its effect on bio-reduction. It was seen that at lower pH (<4.0), the overall reaction took less than 12 hours, whereas at higher pH (>8.0), the reaction took around 72 hours. At low pH, the pronation of function groups at the cell surface eventually resulted in a net positive charge, facilitating the sorption of the negatively charged gold chloride ions [$AuCl_4^-$]. The electrostatic interactions between the positively charged functional groups on the cell wall and the negatively charged $AuCl_4^-$ ions and then the bio-reduction lead to the formation of nanoparticulate gold [AuNPs]. Investigation with a transmission electron microscope shows that the number of nanoparticulate Au per unit area was more in the cytoplasmic region than the cell surface. This is probably because most of Au (III) is transported to the cytoplasm of the cell rather than staying at the cell surface for bio-reduction. The bio-reduction within the cytoplasm is protein-mediated. It is being confirmed via series of experimental studies by Das *et al.*, (2010). The XPS study results demonstrated that reduction of Au (III) to Au(I) was quick upon initial binding with mycelia via electrostatic interaction. In contrast, the complete reduction till Au^0 was slow. The process of reduction at the cell surface is similar to cytoplasmic reduction. However, in cytoplasmic reduction, Au (III) ions bind with cytosolic proteins via covalent interaction in place of electrostatic interaction (Das *et al.,* 2010).

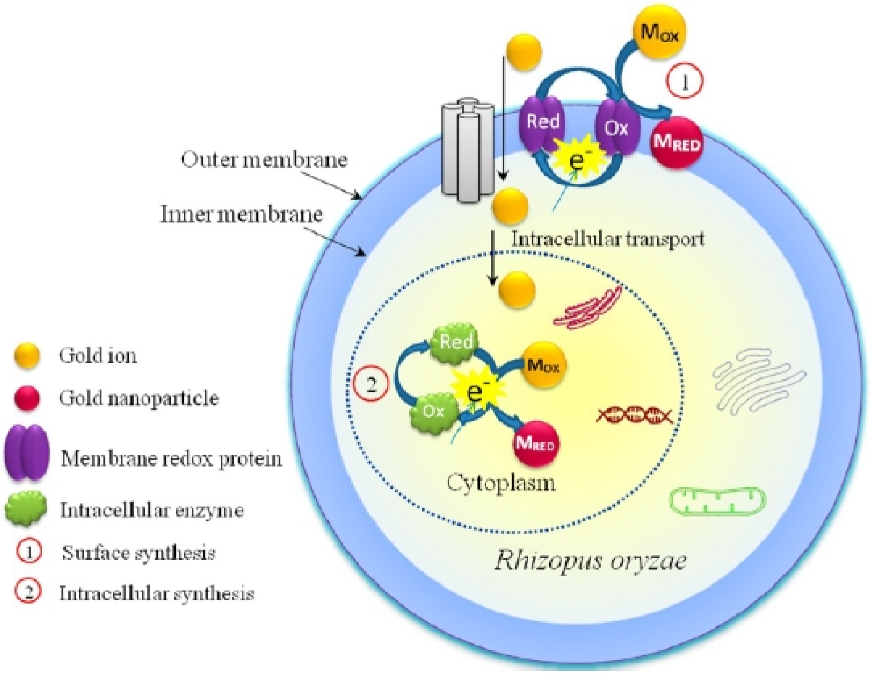

Fig. 5. Illustration of the mechanism of Gold Bio mineralisation in *R. Oryzae.*

Biomineralization of calcite by B. megaterium

The functional groups which are negatively charged on the cell walls of bacteria attract calcium ions as the first step to induce a local supersaturation so that calcite nucleation takes place on the cell surfaces (Schultze-Lam *et al.,* 1996). While, when considering gram-positive bacteria, the electronegativity is assured mainly from the secondary polymers, teichoic acids are the ones that contribute the excess negative charges from carboxyl and phosphates groups to the initially available peptidoglycan structure, and these two groups bind strongly with calcium ions (Hogg, 2013). Before crystallisation, the cells were not rinsed. The calcium ions assimilation on the cell surfaces should be attainable as a follow-up of any pre-sorbed ions destined to get washed off once the cells were immersed into the solutions used during experimental studies. The formation of microorganism begins to develop on the full-grown crystal faces and those synced with substrate cell surfaces. The formation of microorganisms on these crystal faces currently establishes two kinds of varieties of substrate for any growth: One being the colony itself within the centre space and the second being the remaining edge parts of the faces.

The kinetic relativeness of the two regions of the growth can decide the final morphology of the crystals. If the concentration of microorganism is high, it led to a microorganism scaffold that bears an outsized space of cell walls; epicellular and animate thing growth originated from the colony can outstrip the homo-epitaxial growth within the encompassing space. This may be the consequence of the native environment inside the colony having higher saturation states than the majority solutions. It was stated that the microbes associated with micro-environments in the aqueous regions are way dissimilar from those surrounding larger organisms, and one in each of the variations is that ionic solutes tend to concentrate within the fluid mechanics boundary layers encompassing the microorganism cells thanks to microorganism sequestration (Schultze-Lam *et al.,* 1996; Mera *et al.,* 1992; Thompson *et al.,* 1990). Quicker growth of the colonised section ends up in the continuous formation of colonies within the just grown area that brings a further extension in the central portion of the faces. However, due to the colony's centre-certain tendency, the growing projections gets thinner as the growth continues. After the bacteria die, the cytolysis of the cell clears up the space filled by the scaffold of the cell wall to reveal the pores in the crystals. In low bacterial concentration, it is not apparent why the vertical growth relinquishes its dominance. It is tended that more saturation ensuing from cell sequestration within the microenvironments wherever microorganism population is low might not be enough to beat the result of protons wired out from the cells throughout respiration. Urrutia *et al.,* (1992) showed that microorganisms like B. subtilis pump could pump out protons during respiration (Mera *et al.,* 1992). These pumped out protons are likely to acquire a negative charge. This can result in a low capacity for cation binding or low pH in the native environment. The epicellular growth is non-significant compared to homo-epitaxial growth when considering the areas that bacteria have not colonised. This further results in faster growth in the region of edges and corners of the faces, causing shallowness in the central region. The crystals resulting will have holes in their faces.

CONCLUSION

As we have discussed earlier that concrete is the most-used construction development material, and it pinpoints the fact that any damage and failure within a structure's design not only compromise its lifetime but also imposes a risk to infrastructure industries which will either directly or indirectly affect the developmental processes of urbanisation and industrialisation and these both platforms play a crucial role in determining the economic status of any country. So, to reduce the negative impacts of failed infrastructures, we need to adopt processes that will boost the Infrastructure industry, and self-healing concrete tends to have great potential to overcome this threat. *Sporosarcina pasteuri* with lightweight aggregate as a carrier can help induce biomineralization and strengthen the mortar structures. In this review paper, it's is mentioned that *E. coli* didn't contribute anything in strength, durability improvement but other microbes such as *Bacillus mucilaginous, Bacillus sphárecius, Bacillus subtilis, Ascomycota, Purpureocillium, Illicium* etc. all have shown some significant contribution to the improvement in the strength of concrete. Fungi tend to have calcite precipitation which is essential in improving Mortar strength, and this ability of fungi is naturally present in them. *Bacillus spharecius* and *Bacillus pasteuri* species showed a reduction in water absorption, ultimately increasing the durability of concrete. Also, spores are favoured instead of vegetative cells to increase the chances of lifespan improvement and increase. As a result of mixing bacterial cells into a standard mixture of concrete or mortar, there is a rise in 28 d compressive strength from 9% and 25%. Therefore, it is concluded that self-repairing and self-healing processes induced in mortar can replace the toxic and unfriendly methodologies such as epoxy methods, acrylic resins, or silicone-based polymer methods, which are a significant threat to our environmental and urban development.

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