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Microbially induced calcite precipitation through urolytic organisms – A Review

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ABSTRACT

Bio cementation is a novel process in a geotechnical engineering that deal with the application of microbiological activity to improve the properties of soil. The most common process is through microbiologically induced calcite precipitation (MICP). This technique also known as bio mineralization process in which urolytic bacteria hydrolyzes urea via metabolic pathway through various reactions which raises the pH of the system. In the presence of the calcium ions, as pH shifts the saturation state of the system, which allowing the solid calcium carbonate formation. The ubiquity and importance of microbes in inducing calcite precipitation make "Bio cement" a most important metabolic product of Bio mineralization. This review presents the detailed mechanism of bio calcite production, factor effect on the formation bio calcite, list of organism involve in formation of bio calcite, and their application in various field. Finally we discuss about the advantages and disadvantages of Bio cement.

Keywords: Bio mineralization, Carbonate precipitation, Urolysis, Bio cementation, Bioconsolodation.

INTRODUCTION

The constant developments in the field of civil engineering and the growth of industrial activities have created growing demand of materials for the construction industry (Varalakshmi & Devi; 2014). Concrete is mainly use as construction material play an indispensable role in many field like construction of building, dams, storage tank, sea-ports, roads, bridges, tunnels, subways and other infrastructures. Concrete is mainly a combination of water, aggregate and cement. Cement is the most important part of the concrete material. Property like High compressive strength, availability, as well as compatible behavior with reinforcement bars, low price, simple preparation and possibility of casting in desired shape and sizes make concrete the material of choice for many application (Seifan *et* *al; 2016*), but the porosity of all the building material along with moisture and other harmful chemicals such as acids, chlorides and sulfates, that affect the material and reduce their strength and life. The Urease producing organisms addresses a solution to the growing problem in a natural way by precipitating the Bio cement (Varalakshmi & Devi; 2014).

Bio cement is a minerals synthesized by bio mineralization process. Bio mineralization is a process in which an organism creates a local micro environment with conditions that allow optimal extracellular chemical precipitation of mineral phases; Minerals include silica, iron-oxide, hydroxyapatite and calcium carbonate (Dhami *et al*; 2013). Calcium carbonate ($CaCO_3$) is one of the most common minerals on earth. The increase in the concentration or decrease in the solubility of the calcium or carbonate in the solution causes the natural precipitation of $CaCO_3$. Abiotic change (e.g. evaporation or change in the temperature or pressure) or Biotic action (microbial action) participates in the natural precipitation of $CaCO_3$. The rate of microbial $CaCO_3$ precipitation correlated with cell growth (Al-Thawadi; 2011).

MICROBIALLY INDUCED CALCITE PRECIPITATION

Precipitation of calcite is a bio mineralization process. The biological mode of synthesis of the minerals is known as the bio mineralization process (Saryu *et al;* 2013), which induces the fallowing two different mechanisms such as biologically induced and biologically controlled bio mineralization. In the biologically induced mechanism, production of $CaCO_3$ is somewhat dependent on the environment condition. On the other hand, in the biologically controlled mechanism, the organism controls the nucleation and growth of mineral particles (Saryu *et al;* 2013, Mujah *et al*; 2016). Bacterial $CaCO_3$ precipitation under appropriate condition is a general phenomenon (Al-Thawadi; 2011). Bacteria are able to produce a wide range of minerals such as carbonates, sulphides, silicates and phosphates. Calcium carbonate precipitations by microorganisms extra cellularly occur through two metabolic pathways namely autotrophic and heterotrophic (Seifan *et al; 2016*).

Autotrophic pathway happens in the presence of carbon dioxide for which microbes convert carbon dioxide to carbonate through three distinct ways, namely (i) non-methylotrophic methanogenesis (by Methanogenic archaea); (ii) oxygenic photosynthesis (by *Cynobacteria* and microalgae)(Marjadi; 2016); (iii) anoxygenic photosynthesis (by purple bacteria). Non-methaylotrophic methanogenesis pathway converts carbon dioxide and hydrogen to methane (Eq.1). Accordingly, anaerobic oxidation of methane by electron acceptors such as sulfate (Eq.2) results in the production of bicarbonate (Seifan *et al*; 2016). Produced carbonate will then result in calcium carbonate precipitation in the presence of calcium ions (Eq.3). This pathway is more common in marine sediments. In present-day environments, while aerobic methanotrophs have been identified (Zhu & Dittrich; 2016).

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \quad (1)$$
$$CH_4 + SO_4^{-2} \rightarrow HCO_3 + H_2O \quad (2)$$
$$Ca^{2+} + 2HCO_3^{-} \leftrightarrow CaCO_3 + H_2O \quad (3)$$

Photosynthesis process is also an autotrophic pathway to produce calcium carbonate in presence of calcium ions. Oxygenic and anoxygenic photosynthesizing organisms utilize different type of electron donor, like water and hydrogen respectively (Seifan; *2016*). Cynobacteria, are recognized as being responsible for massive carbonate precipitation ((Uma *et al;* 2014, Zhu & Dittrich; 2016).

The precipitation of $CaCO_3$ is also done by heterotrophic organism. This microorganism produces carbonate or bicarbonate and modified the system so that the carbonate precipitation may occur (Marjadi; 2016). The sulphur cycle and nitrogen cycle are other mechanism of producing calcium carbonate. Sulphate reducing bacteria reduce sulfate while oxidizing organic carbon to bicarbonate, during which pH and saturation state are increased (Zhu & Dittrich; 2016). Production of carbonate through nitrogen cycle can be established through three main pathways namely (i) urea degradation (ureolysis), (ii) ammonification of amino acids, and (iii) dissimilatory nitrate reduction ((Sarayu; 2013, Dhami *et al*; 2013, Seifan *et al., 2016*). Ammonification of amino acid through microbial metabolisms produces CO_3^{2-} and NH_3. The subsequent hydrolysis of NH_3 generates OH^- around the cells and leads to a high local supersaturation with respect to calcium carbonate and increase in a pH (Nazel; 2016), and, consequently, precipitates calcite(Zhu & Dittrich; 2016). In nitrogen cycle, minerals are precipitated through oxidation of organic compounds by the reduction of nitrate (NO_3^-) via denitrifying bacteria, carbon dioxide, water, and nitrogen are produced(Eq.4). According to Eq.5, an increase in pH due to consumption of H^+ during the denitrification process. The final reaction of calcium source and carbonate results in precipitation of calcium carbonate (Eq.6) (Seifan *et al; 2016*, Zhu & Dittrich; 2016).

$$\text{Organic compound} + NO_3^- + H^+ \rightarrow CO_2 + H_2O + N_2 \quad (4)$$

$$CO_2 + 2OH^- \rightarrow CO_3^{2-} + H_2O \quad (5)$$

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad (6)$$

The urease enzyme is present in a great diversity of microorganisms enabling the cell to use urea as source of nitrogen (Arias *et al*; 2017). There are bacteria that produce urease in the cytoplasm of the cell for ATP generation (Al-Thawadi; 2011, Cuzman *et al*; 2015). The urolytic bacteria are catalyze the hydrolysis of urea to produce carbonic acid and ammonium. These products, in solution have as final result to induce change of pH in the medium (Eq.7-10):

$$CO(NH_2)_2 + H_2O \rightarrow NH_3 + CO(NH_2)OH \quad (7)$$

$$CO(NH_2)OH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (8)$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad (9)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (10)$$

The increase in a pH leads to an adjustment of the bicarbonate equilibrium to form carbonate ions, further favoring the formation of CO_3^{-2} from HCO_3. A high carbonate concentration induces $CaCO_3$ precipitation around the cells and the presence of calcium ions in the surrounding environment (Eq.11-13) (Arias *et al*., 2017):

$$2HCO_3^- + 2H^+ + 2NH_3 + 2OH^- \leftrightarrow 2CO_3^{2-} + 2NH_4^+ + 2H_2O \quad (11)$$

$$Ca^{2+} + Cell \rightarrow Cell{:}Ca^{2+} \quad (12)$$

$$Cell{:}Ca^{2+}CO_3^{2-} \rightarrow Cell{:}CaCO_3 \quad (13)$$

Fungi are also involve in bio mineralization. This activity is actually related, since products released as a result of $CaCO_3$ dissolution can further re-precipitate as $CaCO_3$ depending on physicochemical condition. In order to precipitate $CaCO_3$ two factors are critical: carbonate alkalinity and calcium (Ca^{2+}) concentration (Bindschedler *et al;* 2016).

BIOCEMENT AND THEIR TYPE

Bio cement is a product innovation from developing bioprocess technology called bio cementation. Bio cement refers to a $CaCO_3$ deposit that formed due to microorganism activity in a system rich of calcium ion. Bio cement is the generation of particle-binding materials through microbial processes in situ so that the shear strength of soil can be increases (Marjadi; 2016). There are two types of bio cement being reviewed, such as bio carbonate cement and bio phosphate cement. Calcium ions surrounding the cells are attracted to the bacterial cell wall due to the presence of several negative charge groups, favoring nucleation. The precipitation of calcium carbonate on the cell surface is shown as eq. 12 and 13 (Qian *et al;* 2018). The morphology of calcium carbonate minerals are depend on oversaturation, temperature, pH, $(Ca^{2+})/(CO_3^{2-})$ ratio and the concentration of urea(Arias *et al;* 2017).

Like the bio mineralization of calcium carbonate, phosphate precipitation can also occur induce by the bacteria. Bacteria use organic phosphate monoester and release PO_4^{3-}/HPO_4^{2-} (Eq.14) (Qian *et al;* 2018). Both calcium phosphate formation and calcium carbonate deposition are exergonic processes, but are strictly controlled in a biochemical system through the activation energy barriers that prevent a chemical reaction from occurring at physiological condition (Wang; 2014). Calcium Phosphate Cement (CPC) is widely used in biomedical applications such as Bone regeneration. Very few researches being done on an application of CPC in construction as the cost increase a lot when CPC is used (Parmar & Marjadi; 2017).

$$\text{Substrate} + H_2O \xrightarrow{\text{Bacteria}} PO_4^{3-}/HPO_4^{2-} \quad (14)$$

MICROORGANISMS INVOLVE IN BIOCEMENTATION

A number of diverse microorganisms participate in the precipitation of the carbonate minerals including the heterotrophic and autotrophic organisms in various natural environments like soil, water, geological monuments, ocean, and saline or soda lakes (Saryu *et al*; 2014).These microorganisms are currently the subject of extensive studies, which are yielding a fresh understanding about their role in carbonatogenesis, and aiding the development of new technologies for the bio conservation and consolidation of monument and stone work of art (Marjadi; 2016).

Table 1: enumerates the microorganisms that have been reported to precipitate biologically induced calcium carbonate

No.	Microorganisms	Isolation site	Reference
A	**Bacteria**		
1.	*Bacillus subtilis*	Calcerious soil from Andhra Pradesh	Anbu *et al; 2016*, Perito *et al; 2014.*
2.	*Bacillus megaterium*	Alkaliphic soil	Krishnapriya *et al*; 2015, Anbu *et al*; 2016
3.	*Bacillus thuringieinsis*	Calcerious soil	Anbu *et al*; 2016
4.	*Bacillus aerius* U2	Israfil river in Turkey	Sensoy *et al*; 2017
5.	*Bacillus sphaericus* LMG22257	Belgian culture collection	Shirakawa *et al*; 2015
6.	*Bacillus pseudifirmus*	German collection of microorganism & cell culture	Dhami *et al*; 2012
7.	*Bacillus cohnii*	German culture collection	Dhami *et al*; 2012
8.	*Bacillus amyloliquedaciens*	-	Seifan, *et al*; 2016
9.	*Bacillus massiliensis*	-	Seifan, *et al*; 2016
10.	*Bacillus cereus*	Old building at CSIR campus, Chennai	Maheswaran *et al;* 2014
11	*Bacillus pasteurii*	CSIR-IMT, Chandigarh	Maheswaran *et al*; 2014
12.	*Klebsiella aerogenes*	-	Cuzman *et al*; 2015
13.	*Halomonassp.* SR4	Mine tailing, Chaina	Anbu *et al;* 2016
14.	*K.flava* CR1	Mining ore soil, Chaina	Anbu *et al;* 2016
15.	*K.pnemoniae*	Sewage sample	Varalakshmi & Devi; 2014
16.	*Proteus vulgaris*	Sewage sample	Varalakshmi & Devi; 2014
17.	*Straphylococcus aureus*	Sewage sample	Varalakshmi & Devi; 2014
18.	*Sporosarcina pasteurii* ATCC6453	China center of industrial culture, national chemical laboratory	Yu *et al;* 2019, Achal et al; 2009, Sensoy *et al;* 2017.
19.	*Rhodococcus*	-	Arias *et al*; 2017
B	**Fungi**		
20.	*P.chrysogeny* CS1	Cement sludge	Fang *et al*; 2018
C	**Cynobacteria**		
21.	*Myxococcus xanthus*	-	Saryu *et al;* 2013
22.	*Osillatoria willei BDU130791*	-	Uma *et al*; 2014
23.	*Phormidium valderianum* BDU20041	-	Uma *et al*; 2014

FACTERS INFLUENCING THE CALCIFICATION PROCESS

The $CaCO_3$ crystallographic patterns (i.e. size, shape and distribution) play a significant role in determining the engineering response of MICP treated soils (Mujah *et al;* 2016). $CaCO_3$ precipitation mediated by microorganisms is basically decided by following factors:

The pH of Environment

The environmental conditions surrounding the microorganisms are the important aspect of any bio mineralization. The pH of the environment of the organism is an important factor that controls the survival and the metabolic activity of the microorganisms that indirectly controls the secretion of the products (Saryu *et al;* 2014).The change in pH level, which is due to the formation of the hydroxyl ions (OH^-) generated from the production of ammonium ions (NH_4^+), helps to create an alkaline environment suitable for $CaCO_3$ precipitation. The presence of OH^- ions raises the pH around the cells. Uniformly distributed $CaCO_3$ crystals across bio cemented soils are desirable in MICP treatment because it produces uniformly well-cemented samples that possess greater strength (Mujah *et al;* 2016).

Temperature

The effect of temperature on MICP is complex as it affects the urease activity of microorganisms, growth and nucleation rate of $CaCO_3$ crystals and solubility of $CaCO_3$. Increase in the temperature from 20°C to 50°C enhanced the production rate of $CaCO_3$ from the enzymatic reaction and hence affected the size and shape of the formed $CaCO_3$ crystals. Temperature higher than 60°C, the $CaCO_3$ production ceased to occur due to the death of microorganisms. Hence, it is vital to know the most optimum temperature for formation of $CaCO_3$ crystal, as it contributes to the highest strength. It was found that about three times more $CaCO_3$ crystals were precipitated at 50°C, the strength of bio treated soil samples was 60% less than that of the samples treated at 25°C (Mujah *et al;* 2016).

The Presence of Nucleation Sites

The presence of nucleation sites is important as it governs the homogeneity of the carbonation and also the strength of the carbonate being produced. Depending on the type of microorganism being used the precipitation and material properties of the $CaCO_3$ produced (Marjadi; 2016).

Concentration of Calcium Ions

The concentrations of the calcium ions in the environment play an important role in the MICP. Bicarbonate synthesized by the bacterial cell is generally excreted out of the cell where it combines with the calcium available in the environment to precipitate MICP. So, the calcium involved in this mechanism is supplied either by the medium or may result from the support material to which the bacteria is attached to (Saryu *et al;* 2014).Urea hydrolysis generates carbonate ions at a 1:1 molar ratio. Hence with increased urea, carbonate concentration can be increased to facilitate $CaCO_3$ saturation (Marjadi; 2016).

Genetic Factors

Any activity of a bacterium is always coded by a gene responsible and specific for that activity. Similarly, the metabolic activity of the bacteria is also controlled by the gene responsible. The enzyme urease that initiates the calcium carbonate precipitation by bacteria is a trimer of three subunits encodes by *ure*A, *ure*B, *ure*C (Saryu *et al*; 2014).

ADVANTAGES OF BIO CEMENTATION

The bio cementation process is advantageous over the ordinary cementation processes by:

1. It is possible to reuse cells in situ, which is a cost saving process as cost of culturing the cells is not considered (Marjadi; 2016);
2. Reactant are aqueous in nature, hence less energy required as low injection pressure is require as they easily infiltrate into pores (Marjadi; 2016);
3. Its economical effective process as compared to Calcite In situ Precipitation System technology (Marjadi; 2016);
4. Bio cementation process dependent on bacteria which are more tolerant to the cementation condition than the plant source (Marjadi; 2016);
5. It is an ecological, environmentally friendly alternative to other traditional consolidation treatments (Nazel; 2016);
6. Cost effective (Mujah *et al*; 2017);
7. Promoting sustainability (Mujah *et al*; 2017).

DISADVANTAGES

There are several limitations of soil bio clogging and bio cementation in comparison with chemical grouting which are summarized as follows;

1. Bio cement is microbial process which is usually slower; (Marjadi; 2016, Arias *et al;* 2017);
2. Microbial process is more complex than the chemical reaction because the microbial activity depends on many environmental factors such as temperature, pH, urea concentration, salts present (Marjadi, 2016, Arias *et al;* 2017);
3. One of the main disadvantages of MICP is that it can only be utilized for specific soil sizes; the technique is currently only suitable for treating sands of particle sizes equal to 0.5-3 mm (Mujah *et al*; 2017);
4. The implementation of technologies based on bio mineralization for water treatment requires the expertise of many disciplines including the efforts

of engineers, microbiologists, biochemist, among others (Arias *et al; 2017*);

5. In bio cement production mainly ureolytic organism used, as byproducts of the metabolism, ammonium and nitrate are produced, which could be toxic and harmful to human health in high concentration (Arias *et al*; 2017, Mujah *et al; 2017*);
6. Economic limitations, especially in replacement of reagents and nutrients of analytical grade used in most studies by other of lower cost (Arias *et al*; 2017);
7. Bio consolidation has not proved effective for stones with lower porosity and smaller pores (Nazel; 2016);
8. The accumulation of newly formed calcite as a thin and denser crust on the stone surface decreases the absorption properties of the stone dramatically (Nazel; 2016).

CONCLUSION

MICP is a complex biochemical process that utilizes the urea and hydrolysis of urea takes place between the sand particles for improvement of soil engineering properties. Microbial concrete technology has proved to be better than many conventional technologies because of its eco-friendly nature, self-healing abilities and increase in durability of various building materials. The use of bacteria for remediation of building materials seems to mimic nature. This biotechnological aspect of using microbes for calcium carbonate deposition or microbial concrete can be used for solving various durability issues of construction material. As the microorganism can penetrate and reproduce themselves in soil or any such environments, there is no need to disturb the ground or environment unlike that of the chemically synthesized cement. This technology also offers the benefit of being novel and eco-friendly.

REFERENCES

Achal V, Mukherjee A, Basu PC & Reddy MS (2009) Lactose mother liquor as an alternative nutrient source for microbial concrete production by *Sporosarcina pasteurii*. *Journal of industrial microbiology & biotechnology*, 36(3), 433-438.

Al-Thawadi SM (2011) Ureolytic bacteria and calcium carbonate formation as a mechanism of strength enhancement of sand. *J. Adv. Sci. Eng. Res,* 1(1), 98-114.

Anbu P, Kang CH, Shin YJ & So JS (2016) Formations of calcium carbonate minerals by bacteria and its multiple applications. *Springerplus*, 5(1), 250.

Arias D, Cisternas L & Rivas M (2017) Biomineralization mediated by ureolytic bacteria applied to water treatment: *A review. Crystals*, 7(11), 345.

Bindschedler S, Cailleau G & Verrecchia E (2016) Role of fungi in the biomineralization of calcite. *Minerals,* 6(2), 41.

Cuzman OA, Wittig L, Abancéns FR, Herrera C, Anastasi NR & Alonso LS (2015) Bacterial "masons" at work with wastes for producing eco-cement. *International Journal of Environmental Science and Development*, 6(10), 767.

Dhami NK, Reddy SM & Mukherjee A (2012) Biofilm and microbial applications in biomineralization concrete. In Advanced topics in Biomineralization. IntechOpen.

Dhami NK, Reddy MS & Mukherjee A (2013) *Bacillus megaterium* mediated mineralization of calcium carbonate as biogenic surface treatment of green building materials. *World Journal of Microbiology and Biotechnology,* 29(12), 2397-2406.

Fang C, Kumari D, Zhu X, & Achal V (2018) Role of fungal-mediated mineralization in biocementation of sand and its improved compressive strength. *International biodeterioration & biodegradation*, 133, 216-220.

Krishnapriya S & Babu DV (2015) Isolation and identification of bacteria to improve the strength of concrete. *Microbiological research*, 174, 48-55.

Maheswaran S, Dasuru SS, Murthy ARC, Bhuvaneshwari B, Kumar VR, Palani GS, ... & Sandhya, S (2014) Strength improvement studies using new type wild strain *Bacillus cereus* on cement mortar. *Current Science*, 50-57.

Marjadi DS (2016) Conservation and restoration of cultural heritage: A biotechnological approach. *Advances in Applied Science Research*, 7(4), 159-167.

Mujah D, Shahin MA & Cheng L (2017) State-of-the-art review of biocementation by microbially induced calcite precipitation (MICP) for soil stabilization. *Geomicrobiology Journal*, 34(6), 524-537.

Nazel T (2016) Bioconsolidation of Stone Monuments. An Overview. Restoration of Buildings and Monuments, 22(1), 37-45.

Parmar S & Marjadi D. Bio Cementation: A Novel Technique and Approach Towards Sustainable Material.

Perito B, Marvasi M, Barabesi C, Mastromei G, Bracci S, Vendrell M & Tiano P (2014) A *Bacillus subtilis* cell fraction (BCF) inducing calcium carbonate precipitation: biotechnological perspectives for monumental stone reinforcement. *Journal of Cultural Heritage*, 15(4), 345-351.

Qian C, Yu X, & Wang X (2018) Potential uses and cementing mechanism of bio-carbonate cement and bio-phosphate cement. AIP Advances, 8(9), 095224.

Sarayu K, Iyer NR & Murthy AR (2014) Exploration on the biotechnological aspect of the ureolytic bacteria for the production of the cementitious materials—a review.

Applied biochemistry and biotechnology, 172(5), 2308-2323.

Seifan M, Samani AK & Berenjian A (2016) Bioconcrete: next generation of self-healing concrete. *Applied microbiology and biotechnology*, 100(6), 2591-2602.

Sensoy T, Bozbeyoglu N, Dogan N, Bozkaya O, Akyol E (2017) Characterization of Calcium Carbonate Produced by ureolytic bacteria (*Sporocarcina pasteurii* ATCC 6453 and *Bacillus aerius* U2) and Effect of Environmental Conditions on Production of Calcium Carbonate.15th International Conference on Environmental Science and Technology.

Shirakawa MA, John VM, De Belie N, Alves JV, Pinto JB & Gaylarde CC (2015) Susceptibility of biocalcite-modified fiber cement to biodeterioration. *International Biodeterioration & Biodegradation*, 103, 215-220.

Uma VS, Dineshbabu G, Subramanian G, Uma L & Prabaharan D (2014) Biocalcification mediated remediation of calcium rich ossein effluent by filamentous marine cyanobacteria. J. Bioremed. Biodeg, 5(257), 10-4172.

Varalakshmi AD & Devi A (2014) Isolation and characterization of urease utilizing bacteria to produce biocement. IOSR-JESTFT, 8, 52-57.

Wang X, Schröder HC & Müller WE (2014) Biocalcite, a multifunctional inorganic polymer: Building block for calcareous sponge spicules and bioseed for the synthesis of calcium phosphate-based bone. *Beilstein Journal of Nanotechnology*, 5(1), 610-621.

Yoosathaporn S, Tiangburanatham P, Bovonsombut S, Chaipanich A, & Pathom-Aree W (2016) A cost effective cultivation medium for biocalcification of *Bacillus pasteurii* KCTC 3558 and its effect on cement cubes properties. *Microbiological research*, 186, 132-138.

Yu X, Qian C & Jiang J (2019) Desert sand cemented by bio-magnesium ammonium phosphate cement and its microscopic properties. Construction and Building Materials, 200, 116-123.

Zhu T & Dittrich M (2016) Carbonate precipitation through microbial activities in natural environment, and their potential in biotechnology: a review. Frontiers in bioengineering and biotechnology, 4, 4.s