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Review

From Microbes to Molecules: Synthetic Biology Approaches for Advanced Materials Design

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Abstract: Traditional materials synthesis often involves energy-intensive processes with significant waste generation and limited control over material properties. This review examines synthetic biology as a sustainable alternative for designing advanced materials with enhanced precision and versatility. It explores microbial biomineralization, detailing how microorganisms influence the formation of mineral deposits and participate in key biogeochemical cycles. It highlights recent research advancements in using a wide variety of microorganisms for the synthesis of inorganic materials such as metal and metal oxide nanoparticles, quantum dots, magnetic nanoparticles, and thin films. The review also discusses the production and properties of various biopolymers. Important factors that can influence the size, morphology, and uniformity of these biomaterials are covered in detail. Emphasis is placed on advancements utilizing synthetic biology tools, such as protein engineering and genome editing, and recent research for creating smart and responsive materials. Considering the present limitations of synthetic biology, challenges related to scale-up, yield, and uniformity are discussed, and suggestions for future research are detailed.

Keywords: synthetic biology; biopolymer; nanotechnology; biomineralization; MICP

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1. Introduction

The ongoing quest for materials of improved performance, designed for particular functionality and applications, has been achieved traditionally through synthetic routes that are high in energy demand, extreme in chemical conditions, and detrimental in environmental impact [1–5]. From the elevated temperatures of ceramic processing to the multi-step, intricate chemical syntheses of polymers, traditional material synthesis is geared to generate large amounts of waste, greenhouse gas emissions, and copious use of solvents and catalysts [2,6,7]. Furthermore, the degree of control over the properties of materials, including nanoscale architecture and precise chemical composition, may be lost because of the intrinsic limitations of these top–down approaches. These challenges necessitate a revolutionary change toward sustainable and specific material synthesis approaches. Synthetic biology is a new discipline that takes advantage of the natural capacity of living systems to construct biological entities with specific functions [8]. This strategy has the potential to transform materials science through the facilitation of a green, bottom–up route to the production of new materials with higher versatility and precision [5]. Microorganisms are very efficient biofactories that have the capacity to synthesize an incredibly large number of organic and inorganic materials under mild conditions and from renewable feedstocks. The potential to apply biological processes in the fabrication of materials presents appealing avenues of inquiry culminating in the development of sustainable alternatives for

conventional paradigms of manufacture and addressing acute concerns in the area of environmental sustainability and depletion of resources [2,9,10]. Theoretical underpinnings of biofabricated materials are often directly inspired by nature. Living systems have evolved sophisticated methodologies for the synthesis of intricate architectures with extremely high precision and efficiency. From the complex silica structure of diatoms to the durable nacre of mollusc shells, nature provides the template for developing materials with specific properties [11]. Through imitation of these biological processes, researchers are developing bio-inspired materials with performance characteristics that exceed their traditional synthetic counterparts [12,13]. The intersection of bio-inspired design and synthetic biology is therefore catalyzing an exponential growth in research interest in the area of biofabrication and elevating it to a forefront position in materials science research [14].

The "biofabrication paradigm" is a fundamental departure from traditional materials synthesis, initiating an era where living organisms, particularly microorganisms, are conceptualized as programmable factories [12]. This paradigm shift moves away from conventional, often environmentally expensive, chemical processes and towards biologically mediated manufacturing, taking advantage of the inherent efficiency and diversity of living systems [15–17]. It envisions a future in which microbes, with their genetic and metabolic pathways exactly controlled, produce all kinds of advanced materials, from complex nanomaterials to elaborate biopolymers, in a sustainable manner [18–20]. Inspired by the efficiencies of nature's solutions and driven by the accuracy of synthetic biology, the biofabrication paradigm will transform materials science, creating a future where design and fabrication are inseparably intertwined with biological processes. Nature has created processes for the synthesis of materials with complex properties, as in the case of bone's capacity for self-healing and spider silk's combination of rigidity and lightness [21]. Researchers are working on the creation of materials that can mimic natural processes to exhibit unprecedented properties. Microorganisms are at the forefront of this effort since they can be utilized to create bio-inspired materials with desired properties. The growing emphasis on naturally derived materials highlights the promise of synthetic biology to help advance materials science.

This article seeks to present a review of recent developments in microbial-mediated synthesis of inorganic and organic materials—in particular, how recent advancements in synthetic biology tools that utilize genetic and metabolic engineering have led to robust methods for industrial use. We will explore the ways in which microorganisms create molecules through biomineralization and focus on the microbial synthesis of inorganic nanostructures and biopolymers. We will discuss pathways microbes undertake to fabricate materials and highlight how microbes can be engineered to synthesize materials with control over their composition. We will discuss the prospects of microbes being used to engineer enhanced properties, improved biocompatibility, and biodegradability. We will deliberate the challenges that currently hinder the widespread adoption of these technologies, including issues related to scale-up, yield, product uniformity, and environmental considerations, and postulate future directions for research and development, highlighting the potential of emerging synthetic biology tools and synergistic approaches to overcome existing limitations.

2. Biomineralization

2.1. Overview

Biomineralization is the process by which living organisms produce minerals. Microbial pathways contribute to the formation of mineral deposits on the earth, such as carbonates, sulfides, and oxides [22]. Microbes are the key drivers of biogeochemical cycles, influencing the transformation of elements such as carbon, nitrogen, sulfur, and metals.

Microbial biomineralization plays a vital role in these cycles by affecting the solubility and mobility of various elements [23]. Microbial processes such as respiration, fermentation, and photosynthesis can alter the pH of their surroundings; for example, the hydrolysis of urea by ureolytic bacteria releases ammonia, thus increasing pH and promoting the precipitation of carbonate minerals [22,24–26]. Conversely, the production of organic acids during the degradation of organic matter can decrease the pH, leading to mineral dissolution [27,28]. Microbes can also catalyze redox reactions by changing the oxidation state of metals and other elements, thereby forcing the precipitation or dissolution of minerals. For example, the reduction in ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by iron-reducing bacteria can lead to the formation of Fe^{2+}-based minerals like siderite and magnetite [29,30]. Magnetotactic bacteria (MTB), which are capable of synthesizing magnetic nanoparticles (Fe_3O_4 or Fe_3S_4) using specialized organelles called magnetosomes, are an especially fascinating example of highly stereotyped and robust biomineralization with a lot of potential for industrial applications [31]. Similarly, diatoms, a group of photosynthetic algae, construct elaborate cell walls composed of amorphous silica (SiO_2) called frustules. These frustules display hierarchical porosity and nanoscale patterning—features that have inspired the development of biomimetic materials for filtration, optics, and catalysis [11]. Furthermore, the microbial production of CO_2 or H_2S gases can influence mineral formation; CO_2 production can increase carbonate saturation, while H_2S production by sulfate-reducing bacteria (SRB) can lead to metal sulfide precipitation [32,33]. This influences sulfur cycling in nature and the availability of metals in different environments.

2.2. Bioremediation

A well-studied application of biomineralization is bioremediation, i.e., the use of microbes to remove pollutants from contaminated environments [34,35]. The origins of human-caused heavy metal contamination stem from both industrial and domestic sources. The metal ion contaminants that disperse into the environment experience difficulty degrading, introducing potential issues for human health and physical systems. In an effort to combat the bioaccumulation of these contaminants, many technological advancements, such as induced chemical precipitation, physical adsorption, ion exchange, and membrane filtration, have been in production but experience difficulties with progression due to financial constraints. Therefore, there is a focus on the advancement of biomineralization technology towards the goal of providing a stable and sustainable sequestration of heavy metals. One method of bioremediation is through microbial-induced carbonate precipitation [22,35]. This process produces the carbonate anion, CO_3^{2-}, through microorganisms such as bacteria, fungi, or enzymes, which then precipitates the heavy metal ions into free, stable carbonates that do not leave harmful impacts on the environment. The precipitation of heavy metals can also be completed through other methods such as urea hydrolysis, denitrification, or sulfate reduction. Urea hydrolysis stands as the most advantageous method due to its time and money efficiency. Within this process, microorganisms decompose urea into compounds, which increases the overall pH, lessening the residual impact the contaminant would originally have on its surroundings [35–37]. Urea hydrolysis is driven largely by urease-producing bacteria (UPB), which can be easily ordered through commercial collection centers, making it an overall more preferable method of remediation.

Another method is phosphate precipitation, which utilizes microbial activity during the dissolution of inorganic phosphate and organic phosphorus to create an enzyme-induced phosphate precipitation reaction [38–40]. The process enables the precipitation of free metal ions into stable heavy metal phosphates, mitigating their toxic effects. Among the most well-studied methods of bioremediation, phosphate-solubilizing bacteria (PSB) play a crucial role in making organic phosphorus and inorganic phosphate soluble through the

usage of enzymes and organic acids, which speed up the process [35]. Moreover, scientists have discovered that the addition of an increased amount of organic acids and microorganisms (such as *Bacillus*, *Ochrobactrum*, *Pseudomonas*, *Marcescens*, and *Rahnella*) improves the efficiency of the reaction, as they demonstrate increased decomposition rates [34,35]. The method of phosphate-based remediation introduces many advantages over carbonate precipitation, such as its greater thermodynamic stability, tolerance to lower pH levels, and lack of emission of harmful gases [38,41–43]. However, it remains an unattainable method to practice, as the cost is out of range for sustainable, long-term use. The efficiency of microbial precipitation is largely influenced by multiple conditions concerning its environment and the bacterial species involved in the heavy-metal deterioration process. In regard to phosphate precipitation-based bioremediation, differing species of bacteria exhibit varying degrees of tolerance to heavy metal ions [35]. As for carbonate precipitation, certain fungal species inherit higher capabilities of urea hydrolysis [44]. The situational differences in heavy-metal removal, lack of funding towards research, provision of materials, and overall high costs inhibit large steps towards technological advancements.

While bioremediation represents an important application of harnessing microbe-driven mineral formation, the processes involved in biomineralization also underpin their ability to synthesize a wider range of inorganic materials. Building on the understanding of how microorganisms control mineral precipitation for bioremediation, researchers have explored their potential in the synthesis of novel inorganic materials. Figure 1 highlights two mechanisms of biomineralization-induced precipitation.

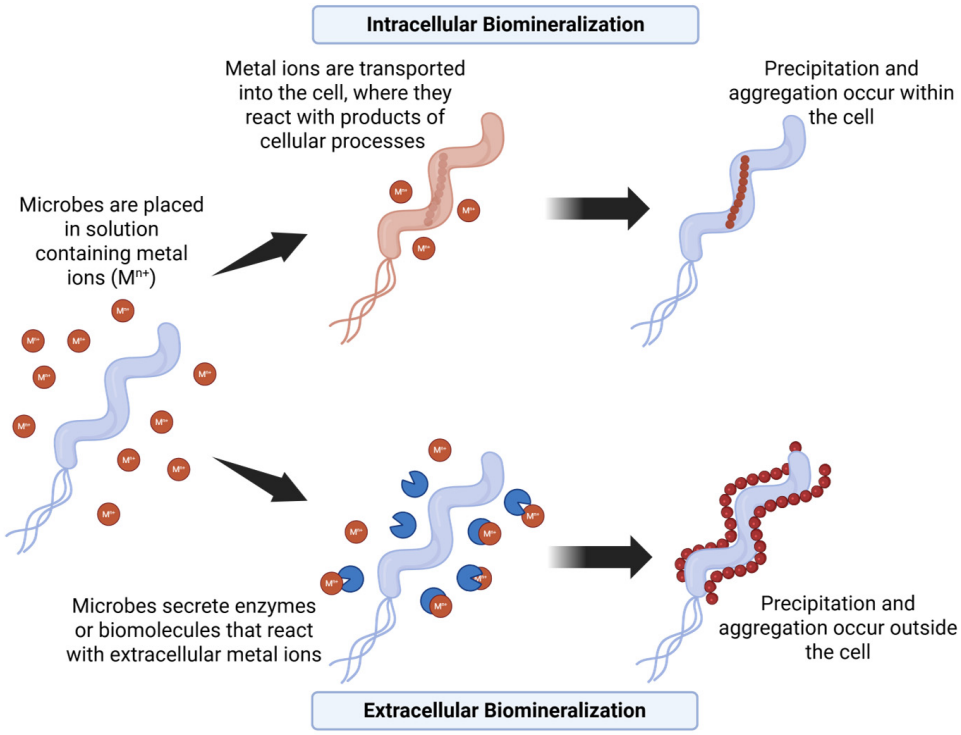


Figure 1. Mechanism of biomineralization using microbial-induced precipitation.

3. Synthesis of Advanced Materials

Biomineralization has long inspired materials scientists. By using synthetic biology to design materials, researchers are moving beyond traditional top–down approaches toward bottom–up, self-organizing systems with new functionalities. By accessing the metabolic potential of microorganisms, scientists have engineered biological systems that synthesize materials under less harsh conditions, using renewable biomass feedstocks and reducing toxic waste [1,3,45,46]. Another significant advantage of microbial-mediated synthesis is

its versatility. Microorganisms are able to readily adjust to changing environments and can metabolize substrates with broad diversity, thereby enabling the production of a wide range of products [47].

3.1. Inorganic Nanostructures

Microorganisms can be genetically designed to synthesize inorganic nanostructures with defined size and shape, eliminating the necessity for toxic chemical reductants and high-temperature ovens. Naturally occurring microbial metabolic processes like photosynthesis and respiration can alter the chemical environment and lead to mineral or metal precipitation.

3.1.1. Metal Nanoparticles

Metal nanoparticles, especially those involving noble metals, are ubiquitous in catalytic converters [45]. Some metal nanoparticles are also utilized in drug delivery and therapeutics, making biocompatibility an essential requirement [48]. Due to their inherent ability to reduce metal ions, microorganisms have the ability to synthesize a range of metal nanoparticles [49]. By controlling the biological systems and reaction conditions, the size, shape, and composition of the nanoparticles can be precisely tuned [49,50].

The reduction in metal ions by microorganisms can occur both intracellularly and extracellularly (Figure 1). In intracellular synthesis, metal ions are transported into the microbial cell, where they are reduced and assembled into nanoparticles (NPs). This process involves specific enzymes (e.g., reductases) that act as bioreductants and stabilizing agents [3,51]. NPs are typically formed within the cell cytoplasm or periplasmic space. Bacteria like *Shewanella oneidensis* can transport gold (Au^{3+}) and silver (Ag^{+}) ions into the cell where they are reduced by intracellular reductases to their metallic form (Au^{0} and Ag^{0}), leading to the accumulation of metal NPs [1,52]. It also utilizes nitrate reductase on its cell surface and in the periplasmic space to catalyze the reduction of gold and silver ions [53,54].

Extracellular synthesis occurs outside the cell, where metal ions are reduced by enzymes or biomolecules secreted by the microbe. Extracellular synthesis simplifies the purification process for obtaining the final product when compared to intracellular synthesis. *Shewanella oneidensis* employs extracellular electron shuttles, such as riboflavin, to transfer electrons to gold and silver ions in the surrounding environment [55,56]. *Pseudomonas aeruginosa* secretes enzymes such as NADH-dependent reductases extracellularly [49,52]. The bacterium also releases organic molecules, such as polysaccharides and proteins, which prevent aggregation of the resulting nanoparticles [54]. Similarly, certain fungi secrete a high concentration of enzymes and proteins from their mycelia, which act as primary reducing agents [57,58]. The proteins and polysaccharides in the fungal filtrate can act as capping agents and stabilize the synthesized NPs.

3.1.2. Metal Oxide Nanostructures

Metal oxide materials have diverse applications in fields such as catalysis, electronics, and medicine [46]. The mechanisms involved in microbial synthesis of metal oxide NPs often overlap with those in the synthesis of metal NPs. However, a key difference is that the metal ions are not reduced to their zero-valent state and instead form complexes with oxygen. Bio-precipitation is the most common mechanism for metal oxide formation within the cell [23,32,59]. Enzymes such as phosphatases or oxidases that are produced can alter the local chemical environment, leading to the precipitation of metal oxides. Similarly, metabolic activities can alter the pH of their surroundings, leading to the precipitation of metal ions as oxides or hydroxides. One example is the precipitation of zinc oxide (ZnO) NPs under alkaline conditions using fungal biomass (*Aspergillus niger*) [60,61]. Phytochemi-

cals present in Azadirachta indica (neem) leaves were also used to synthesize metal oxide nanoparticles [8,62].

Microbial cell surfaces can also bind to metal ions through various functional groups (e.g., carboxyl, phosphate, hydroxyl). This creates a nucleation site for nanoparticle formation. Algae like *Spirulina platensis* have been successfully used as templates for the formation of titanium dioxide (TiO_2) NPs through binding of titanium ions to the algal cell wall and its subsequent oxidation [54,63]. Here, the microbes do not actively mediate synthesis via metabolic processes but serve as structural scaffolds for abiotic mineral formation. This highlights the distinction between true microbial biosynthesis, where the organism plays an active biochemical role, and templated synthesis, where the organism's surface facilitates material deposition. Some bacteria produce siderophores, which chelate with iron ions and facilitate their transport into the cell [64]. In certain cases, the iron ions are further processed to form iron oxide NPs, such as goethite (α-FeOOH) or hematite (α-Fe_2O_3) [23,65]. Microbial enzymes, such as reductases, can also support the synthesis of iron oxide (Fe_2O_3) nanoparticles [66].

3.1.3. Magnetic Nanoparticles

Microbial synthesis of magnetic nanoparticles, especially magnetite (Fe_3O_4), has been researched for producing biocompatible nanomaterials with targeted applications in drug delivery, magnetic hyperthermia therapy, and MRI contrast agents [29,67]. Magnetotactic bacteria (MTB) can naturally synthesize magnetic nanoparticles within specialized organelles called magnetosomes that contain magnetite or greigite (Fe_3S_4) crystals. The magnetosomes can control the size, shape, and crystal structure of the magnetic nanoparticles with precision [68]. *Magnetospirillum magnetotacticum* and *Magnetospirillum gryphiswaldense* are well-studied MTBs that produce uniform magnetite crystals within magnetosomes [69,70]. The process of magnetosome formation involves several steps: uptake of iron, formation of a membrane vesicle, regulation of iron transport and redox state, nucleation and growth of the magnetic crystal, and arrangement of magnetosomes in chains within the cell [68,71,72]. Specific pH conditions, oxygen availability, and the presence of other minerals have an influence on the transformation process [29]. The ability to control the properties of synthesized magnetic nanoparticles via genetics has allowed MTBs to play an outsized role in engineering chimeric magnetic nanoparticles necessary for novel research applications. Magnetogenetics is an emerging technology in biomedical research, allowing for noninvasive manipulation of cell signaling pathways via magnetic fields; an enduring challenge is developing a viable fusion of proteins and magnetic probes tailored to individual research needs. A recent study described a robust method for generating engineered ferritin fused to green fluorescent protein (GFP) as an efficient and biostable in vivo technique to manipulate cellular processes remotely [73].

Some bacteria and fungi can also produce magnetic nanoparticles extracellularly. These microorganisms secrete organic molecules that can reduce Fe^{3+} to Fe^{2+}, leading to the formation of magnetite nanoparticles. Elblbesy et al. explored the extracellular production of nanoparticles by microbes, specifically using *Magnetospirillum* strain AMB-1 to produce magnetic nanoparticles [67]. The experiment showed that this nitrate-reducing Fe (II) oxidizing bacteria produced paramagnetic magnetite nanoparticles of ~50 nm size that possessed a low coercivity. Interestingly, even minute changes to incubation conditions under which biosynthesis was conducted caused small variations in the magnetic characteristics of the produced nanoparticles [67].

Shewanella oneidensis does not possess magnetosomes, but it can perform an extracellular reduction of iron ions [30]. It forms magnetite nanoparticles during the process of anaerobic respiration, where it uses iron oxides as electron acceptors. Sulfate-reducing

bacteria are capable of producing magnetite nanoparticles during the process of sulfate reduction [74]. The mechanism involves the production of sulfide, which can react with iron ions to form iron sulfides. These iron sulfide minerals undergo transformation to form magnetite through processes like dissolution, recrystallization, and oxidation–reduction [74].

3.1.4. Semiconductor Nanocrystals

Quantum dots (QDs) are semiconductor nanocrystals ranging in size from 2 to 10 nanometers. Their unique optical and electronic properties arise from quantum confinement effects due to their small size, making them suitable for photocatalysis, optoelectronics, and bioimaging. Both intracellular and extracellular processes have been explored for the microbial synthesis of semiconductor nanocrystals [52,75–79].

Sulfate-reducing bacteria can react with metal ions to create nanocrystals of cadmium sulfide and zinc sulfide [4,80]. Through an intracellular process, they take up metal ions (Cd^{2+}, Zn^{2+}) into their cells. Sulfide ions (S^{2-}), which are generated through sulfate reduction, then react with metal ions within the cell. This leads to the formation of CdS or ZnS QDs within the microbial cell. As expected, the size and properties of the QDs are influenced by the metal ion concentration, sulfide ion availability, temperature, pH, and enzymatic activity [78]. *Escherichia coli* has been engineered to synthesize CdS QDs intracellularly by introducing genes encoding gamma-glutamylcysteine synthetase (gamma-GCS) and glutathione synthetase (GS), which are involved in sulfate reduction [81]. Mi et al. reported the production of sulfide ions within the cells, leading to efficient CdS QD formation [77]. *E. coli* was also reported to synthesize CdTe quantum dots through an extracellular mechanism [76]. The fungus *Fusarium oxysporum* produces proteins and enzymes when exposed to a zinc ion-containing solution [82,83]. These secreted biomolecules convert Zn^{2+} to ZnS. The Gram-negative bacterium *Pseudomonas putida* can perform both intracellular and extracellular synthesis methods to make CdS QDs [75,84].

It is important to recognize that quantum dots are environmentally harmful due to the toxicity of their constituent materials [85]. Quantum dots can degrade and release heavy metals (e.g., Cd, Zn, Te) when improperly disposed of into soil and water [86]. This makes techniques like microbial bioremediation increasingly relevant; however, some studies have shown that quantum dots can be toxic to bacteria and algae. Nonetheless, microbial synthesis is a promising avenue for producing less toxic QDs. For example, a study investigating the effects of biogenic CdSQDs generated using *F. oxysporum* on bacterial viability and lettuce germination found that concentrations had to be at their highest to display antibacterial activity, while biogenic CdSQDs did not inhibit seed germination [87].

3.2. Thin Films Through Microbial Deposition

Microbial deposition leverages the ability of microorganisms to precisely control the nucleation and growth of inorganic materials [55]. It relies purely on biomineralization to precipitate the desired inorganic materials. Unlike traditional chemical vapor deposition or sputtering techniques, microbial deposition occurs under mild conditions, often requiring only room temperatures and neutral pH. For example, *Bacillus subtilis* precipitates $CaCO_3$ through urea hydrolysis [25]; this process can be employed to create thin films of calcium carbonate for concrete reinforcement [88–90].

Some bacteria and fungi can actively transport metal ions into their cells, leading to intracellular precipitation. *Pseudomonas* sp. can accumulate uranium within their cells through active transport mechanisms where uranium ions are taken up and then precipitated as uranium phosphate [91]. *Aspergillus niger* and *Paecilomyces javanicus* also produce uranium phosphates on fungal mycelia in combination with extracellular processes [92]. These insights can be used to create thin biofilms of uranium compounds [93]. Another

method is through passive absorption, where the microbial cell surfaces act as a template for nucleation and growth of minerals, owing to the presence of certain functional groups (e.g., carboxyl, phosphate) that bind to ions. A well-known example is diatoms, a type of algae that possess silica cell walls (frustules) with highly ordered nanoporous structures [11]. Research has shown that coating diatom frustules with other materials can fabricate thin films with tailored optical properties [94]. We have summarized the inorganic materials that can be synthesized microbially in Figure 2.

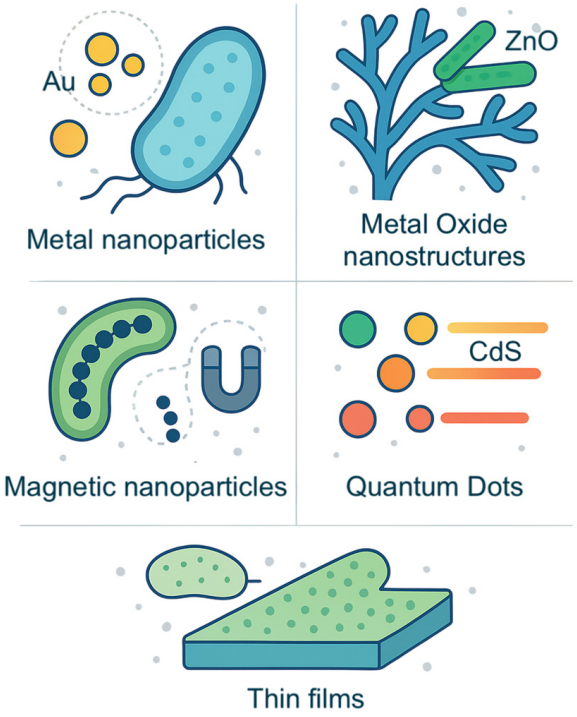


Figure 2. Infographic representing the inorganic nanostructures described in Section 3.

3.3. Biopolymers

Microorganisms offer sustainable alternatives to traditional petroleum-based polymers. These microbial biopolymers are biodegradable, biocompatible, and synthesized from renewable resources. The production of microbial biopolymers involves (i) microbial selection and strain development, (ii) fermentation, i.e., nutrient consumption under controlled conditions, and (iii) extraction of biopolymer from the microbial cells [2,20].

Polysaccharides are made up of sugar monomers, which makes them an ideal avenue for biosynthesis. Some bacteria, such as *Komagataeibacter xylinus*, can synthesize bacterial cellulose with high purity and tensile strength [5,95–97]. *K. xylinus* converts glucose from its surroundings into UDP-glucose, which is then polymerized into glucan chains by cellulose synthase, an enzyme complex located at the bacterial cell membrane. The cellulose nanofibers produced have applications in wound healing, tissue engineering, and high-strength materials [2,20,98,99]. Similarly, xanthan gum, which is widely used in the food and cosmetic industry, can be produced by the bacterium *Xanthomonas campestris* from glucose starting materials [100].

Bacteria from the genera *Azotobacter* and *Pseudomonas* are capable of producing alginates, which are extensively used in wound dressings and drug delivery [5]. The enzymes alginate synthases and epimerases play a key role in determining the final structure and properties of the alginate product. An interesting example to demonstrate the versatility of microbial biopolymers is the production of polyhydroxyalkanoates (PHA) by *Cupriavidus necator* [101,102]. The type of PHA produced can be influenced by the carbon source

provided to the bacteria—using glucose leads to polyhydroxybutyrate (PHB) production, while adding valerate leads to the production of polyhydroxybutyrate-co-hydroxyvalerate (PHBV) copolymer [103].

Bacteria are also capable of producing monomers for specific polymers, such as the synthesis of lactic acid by the *Lactobacillus* species [104]. These bacteria ferment carbohydrates like glucose or starch to produce lactic acid, which is then polymerized into polylactic acid. Similarly, succinic acid can be produced by bacteria like *Actinobacillus succinogenes* [105,106]. These bacteria can ferment renewable feedstocks containing glucose, glycerol, and agricultural byproducts to produce succinic acid [104,107,108].

Genetic alterations in microbes can also facilitate the synthesis of biopolymers with designed properties, including enhanced mechanical properties and biodegradability [96]. Researchers can genetically design bacteria to synthesize cellulose from alternative carbon sources, including waste streams [97]. Yeast can be made to secrete lipids with specific kinds of fatty acids, thereby offering a renewable feedstock for biopolymers [109]. Recently, researchers engineered bacteria and yeast to produce spider silk-like proteins [21]. These protein fibers have a high strength and elasticity, with potential to be used in textiles, biomedicine, and composite materials. Table 1 summarizes.

Table 1. Summary of advanced materials discussed in Section 3.

Material	Microbe	Category	Reference
Alginate	*Azotobacter* sp. *Pseudomonas* sp.	Biopolymer	[5]
Bacterial cellulose	*Komagataeibacter xylinus*	Biopolymer	[95–97]
CdS	*Escherichia coli*	QD	[81]
CdS	*Pseudomonas putida*	QD	[75,84]
CdTe	*Escherichia coli*	QD	[76]
Fe_3O_4	*Magnetospirillum strain AMB-1*	NP	[67]
	Magnetospirillum gryphiswaldense		[70]
	Magnetospirillum magnetotacticum		[69]
	Shewanella oneidensis		[30]
Gold	*Shewanella oneidensis*	NP	[52–55]
Polyhydroxyalkanoates	*Cupriavidus necator*	Biopolymer	[101]
Polylactic acid	*Lactobacillus* sp.	Biopolymer	[104]
Silver	*Pseudomonas aeruginosa*	NP	[49,52]
	Fusarium oxysporum	NP	[51,58]
	Shewanella oneidensis	NP	[54,56]
Succinic acid	*Actinobacillus succinogenes*	Monomer	[104–108]
TiO_2	*Bacillariophyceae* (diatoms)	Thin films	[94]
	Spirulina platensis	NP	[54,63]
Uranium phosphate	*Pseudomonas* sp.	Thin films	[93]
Xanthan gum	*Xanthomonas campestris*	Biopolymer	[100]
ZnO	*Aspergillus niger*	NP	[60,61]
ZnS	*Fusarium oxysporum*	QD	[82,83]

4. Factors Affecting Material Properties

By manipulating biological and experimental parameters, we can tailor the properties of the resulting materials for specific applications. There are several factors that influence the morphology and properties of the resulting material (Figure 3), which are discussed in detail below.

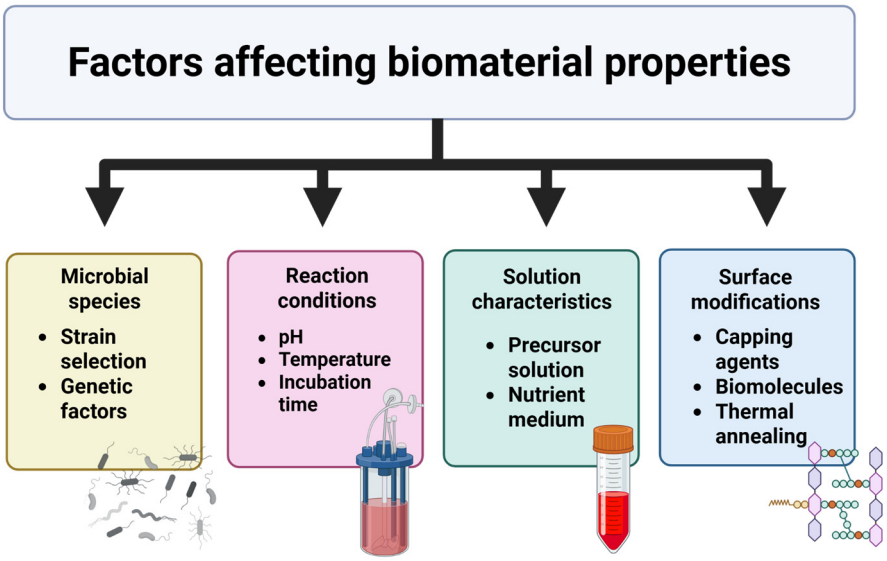


Figure 3. General factors affecting the properties of biosynthesized materials.

4.1. Microbial Species/Strain Selection

Different organisms possess distinct enzyme machinery and metabolic processes, which directly influence the formation of the product. For example, researchers have found that careful selection of the strains of *Fusarium oxysporum* is required to produce monodisperse, spherical silver nanoparticles [15,48,79]. Other fungal species, or even different strains of *Fusarium*, could produce different shapes or a wider size distribution [3,45,49]. Also, *S. xiamenensis* was found to produce a higher yield of gold nanoparticles when compared to *S. oneidensis* [53].

Magnetotactic bacteria (MTB) clearly demonstrate how the choice of strain can affect the desired properties of the material produced. Genetic factors within MTB control iron uptake and crystal growth [71], leading to monodisperse Fe_3O_4 NPs. The size of magnetosomes (typically 35–120 nm) as well as their shape (cuboidal, elongated, and bullet-shaped) is genetically determined [29,68]. Specific proteins within the magnetosome guide crystal growth along preferred crystallographic planes, governing the final shape of the inorganic nanoparticles formed [29,68]. In contrast, sulfate-reducing bacteria (SRB) produce irregularly shaped Fe_3O_4 NPs extracellularly during anaerobic respiration [74].

4.2. Precursor and/or Nutrient Characteristics

As established with solution-based synthesis of inorganic nanoparticles, lower concentrations of metal salts lead to smaller nanoparticles, while higher concentrations of metal salts lead to larger nanoparticles due to agglomeration [62,110]. Carefully controlling the ratio of microbes and cations (e.g., Zn^{2+}) in the growth medium will produce nanoparticles with a narrower size distribution [110,111]. In the synthesis of gold nanoparticles using *Shewanella oneidensis*, increasing the concentration of gold chloride (the metal precursor) in the growth medium generally led to an increase in the average size of the resulting gold nanoparticles [53,112]. Similarly, the size of palladium nanoparticles was controlled by fine-tuning the Pd/cell dry weight [65,112]. Additionally, the presence of other organic

matter and salts in the growth medium can play a role in the nanoproduct [113]. Depending on the chemical composition of the product, the presence of specific ligands or additives can guide its morphology.

Biopolymers are also affected by the nutrient medium. In the synthesis of bacterial cellulose (BC) nanofibers, using glucose as the sugar source leads to a high yield of product [96]. In some cases, it has been shown that using glycerol can also lead to high BC yields [96,97]. Use of peptone in the medium improved BC yields from mannitol and sucrose [114]. The addition of polysaccharide pullulan can affect the bundling behavior of BC and improve the mechanical strength of BC [115]. In the biosynthesis of xanthan gum by *Xanthomonas campestris*, the choice of carbon source and composition of the nutrient medium controls the yield and molecular weight of the biopolymer [100,116,117].

4.3. Reaction pH, Temperature, and Time

Reaction pH, temperature, and time influence microbial enzyme activity and the kinetics of nanoparticle nucleation and growth [118]. Optimal ranges for synthesis vary depending on the microorganism and the target material. Therefore, these factors exhibit considerable variability across published research [15]. All factors are interactive, and therefore, they have to be carefully considered together in order to determine the best suitable conditions for a particular microorganism [119].

An initial increase in temperature generally increases the rate of synthesis; however, very high temperatures can denature microbial enzymes and lead to poor-quality products. In general, temperatures of 25–37 °C are ideal for the formation of uniform nanoparticles [46]. Higher temperatures can cause nanoparticle aggregation or loss of capping agent. Similarly, longer incubation times can lead to a higher yield; however, the product may be polydisperse with a larger size distribution due to continued growth or aggregation [111]. For biopolymers, longer fermentation times can lead to increased polymer chain elongation and higher molecular weight [116,120,121], but very long times may lead to degradation.

Synthesis of Ag nanoparticles by *Bacillus* sp. is more efficient at slightly alkaline pH, resulting in smaller, spherical nanoparticles [59,122–124]. This is because acidic pH could lead to delayed nucleation and agglomeration [125]. However, a pH that is too high may cause undesirable precipitation of hydroxides [126]. On the other hand, a slightly acidic pH promotes Fe_3O_4 precipitation by *P. aeruginosa* [72]. The formation of xanthan gum was increased at a pH of 7.0–7.5; however, further increase in the pH decreased the production [127]. For the production of bacterial cellulose, the ideal conditions are a lower pH of 3–7 and a temperature range of 25–30 °C [95].

4.4. Biomolecules and Capping Agents

Proteins and polysaccharides secreted by extracellular processes can act as capping and stabilizing agents, influencing product morphology. For example, specific proteins can act as templates, directing the growth of Ag NPs into different shapes like spherical and triangular [128,129]. Altering the reaction pH can affect the charge of these biomolecules, influencing their interaction with Ag^+ ions and the final particle shape. In the biosynthesis of Au nanorods, some proteins bind to gold ions in a way that directs their assembly into rod-shaped structures rather than spheres [130]. Post-synthesis modifications such as thermal annealing or capping with additional ligands can be employed to fine-tune nanostructure properties.

5. Synergistic Approaches

The field of synthetic biology is naturally interdisciplinary, and its utility in material science has increased by its convergence with other technologies. The integration of synthetic biology with existing tools enables researchers to develop a robust approach to design and fabricate novel materials with increased complexity and functionality.

5.1. Protein Engineering and Genome Editing

One interesting approach is the combination of protein engineering with biomineralization. This entails creating proteins with specific shapes and chemical functionalities, acting as templates for mineral deposition [131–133]. These protein scaffolds can bind specific ions, thereby controlling the nucleation and growth of minerals with desired size, shape, and crystalline phases. For example, genetically engineered proteins, such as silk proteins or peptides, can be used to template the formation of calcium carbonate, silica, or metal nanoparticles [18,134–139]. The resulting materials exhibited enhanced mechanical strength, optical properties, and catalytic activity. Studies have shown that silver nanoparticles prepared using genetically engineered bacteria demonstrated precise control over nanoparticle size and distribution [140].

Similarly, using genome editing such as CRISPR-Cas9, researchers can modify microorganisms to enhance the production of specific building blocks for polymers. This involves deleting competing pathways, introducing new genes, or altering regulatory elements to fine-tune gene expression. An example is the engineering of bacteria to overproduce monomers like lactic acid (for polylactic acid production) or isoprene (for rubber synthesis) [19,104,141,142]. Additionally, new advancements in Cas9-based genetic engineering have allowed for the development of synthetic gene circuits that (1) greatly reduce the leakiness of a system (i.e., the basal expression of a gene in the absence of an inducer) and (2) achieve high maximum expression of a target product. CASwitch is one such system that combines low leakiness with high maximum expression, resulting in high fold induction (i.e., the ratio between maximum expression and leakiness) by combining two established network motifs: the Coherent Feed-Forward Loop (CFFL) and Mutual Inhibition (MI) [143].

5.2. Smart and Responsive Living Materials

Living materials offer unique advantages such as the ability to respond dynamically to their environment [144]. Engineered living tissues with tailored mechanical and biological properties could be used to repair or replace damaged organs and tissues. For example, researchers are exploring the use of 3D-printed living constructs containing cells and extracellular matrix components to create functional bone, cartilage, or skin [13,145,146]. Self-healing hydrogels can use biosynthesis to repair damaged tissues [147,148]. Bacteria can be programmed to synthesize and release therapeutic proteins at tumor sites [149–151]. While the term 'living materials' encompasses a plethora of technologies that combine living cells with nonliving scaffolds, these are distinct from engineered cells (otherwise known as artificial cells), which are nonliving and designed to mimic biological processes [152].

In the field of engineering, an interesting example is bacteria-containing composite materials that are programmed to secrete a mineralizing agent in response to damage. For example, concrete can be engineered to incorporate bacteria that secrete calcium carbonate to repair cracks and restore structural integrity [25,89,90]. Living cells can be engineered to sense specific environmental cues, such as changes in pH, temperature, or the presence of pollutants, and then trigger a visual response or release a signaling molecule [147,153,154]. Microorganisms can be engineered to detect specific pollutants in water and emit a fluorescent signal [154–157].

5.3. Resource Management and Recycling

Environmental chemistry is another area of synergy with synthetic biology. Microorganisms can be engineered to produce biofuels from renewable feedstocks, reducing our reliance on fossil fuels. For example, bacteria can produce high-energy-density biofuels from agricultural waste [10,158,159], while yeast can be programmed to synthesize biodegradable polymers for packaging applications [109,160,161]. Living filters composed of microorganisms can be used to remove heavy metals or organic pollutants from contaminated water [1,16,162]. Synthetic biology can play a key role toward a circular economy by facilitating the recycling of materials [109,163]. For example, enzymes can be engineered to break down complex polymers into their constituent monomers, which can then be used to synthesize new materials [164,165]. Microorganisms can be engineered to convert waste streams, such as agricultural waste or CO_2 gas, into bioplastics or biofuels, closing the loop and reducing our reliance on non-renewable resources [1,22,109].

6. Challenges and Future Directions

Microbial synthesis has the potential to be a greener alternative to material synthesis by avoiding the use of toxic chemicals, generating less waste, and operating at mild conditions [14,16,33,54]. Microorganisms could be genetically engineered to produce substances with specific characteristics, such as enhanced strength, unique optical properties, or catalytic function, through the controlled synthesis mechanisms in the nanoscale. The process of microbial production supports the production of complex hierarchical structures through the self-assembly process, mimicking the biological formation processes for numerous materials. A variety of inorganic materials, ranging from metal nanoparticles, metal oxides, and sulfides to carbonates and phosphates, can be synthesized with the assistance of microorganisms [1,67]. However, the discipline also possesses numerous challenges that must be resolved in order to attain its full potential (Figure 4).

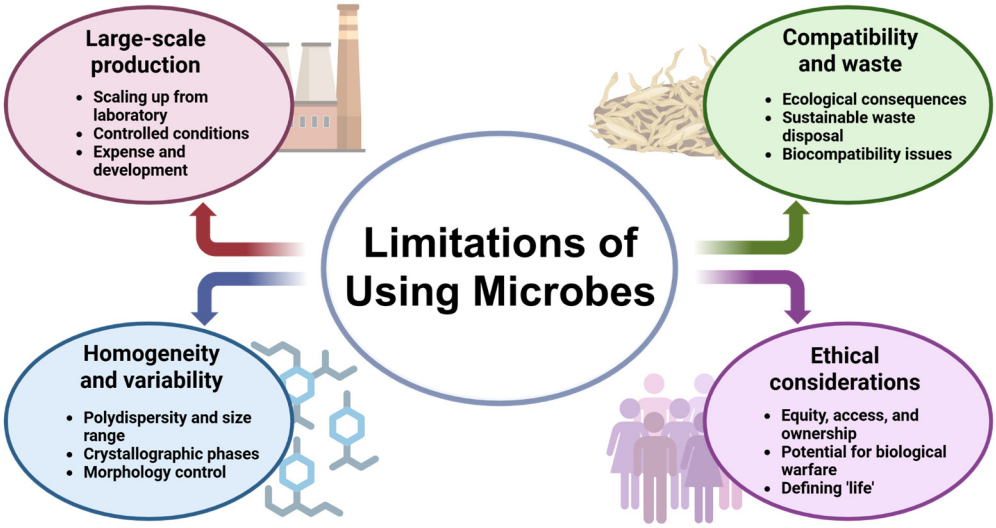


Figure 4. Flowchart depicting the limitations of using microbes for materials synthesis.

6.1. Large-Scale Production

A key challenge is the transition from laboratory experiments to large-scale production [22]. While microorganisms are capable of producing inorganic materials quite well under controlled laboratory conditions, scaling up this process is fraught with numerous issues. Large bioreactors must have extremely controlled temperature, pH, nutrient availability, and oxygen conditions to promote constant microbial activity and material synthesis. Such conditions could be hard to achieve uniformly in a large volume. Pro-

cess scale-up could increase the expense of bioreactor construction and operation, media preparation, and product purification. Moreover, growth conditions for microbes and synthesis of materials can be very different at the laboratory and production levels. A lot of process development is typically required to attain large scales and high yields and rates of production [14,54]. This can be achieved by creating novel bioreactors in which we can maintain control over important factors at large scales, for example, continuous-flow bioreactors, microfluidic bioreactors, or three-dimensional perfusion systems. In addition to technical scale-up challenges, regulatory frameworks and cost structures remain barriers to commercial adoption of microbial materials. Biomanufacturing of new nanomaterials must comply with biosafety and environmental guidelines, which vary across regions.

6.2. Polydispersity and Uniformity

Microbial growth and deposition rates can be slow compared to conventional techniques. Another problem is to regulate the properties and homogeneity of the produced materials. Microbe-produced materials, particularly nanoparticles, may be of varying sizes and shapes, which can alter their properties and performance [3,16,54]. It is desirable to have nanomaterials with a smaller size range for most applications in semiconductors and biology. Furthermore, regulating the crystal structure and shape of inorganic materials is extremely important. Microbial synthesis may sometimes produce amorphous or poorly crystalline material of non-uniform shape; batch and run consistency may be hard to obtain because of the inherent variability of biological systems [14,166]. Achieving uniform thin films over large areas can be challenging. Therefore, precise control over microbial metabolic pathways through genetic engineering techniques, optimization of precursor production, and designing microorganisms can aid uniform material synthesis [16,54]. Developing in situ methods of analyzing the synthesis process helps in controlling material properties and making them homogeneous.

6.3. Compatibility and Waste Production

For medical uses, the materials produced by the microorganisms must not cause adverse reactions in the host organism [14]. Certain microorganisms or their products can be harmful to human beings or other organisms. Non-toxic microorganisms must be chosen, and methods must be developed to remove any toxic byproducts [8,147]. Both in vivo and in vitro tests for biocompatibility must be conducted to ensure that the synthesized materials are safe and effective [5,98,99]. Finally, the synthesis of large amounts of material using microbes will generate enormous amounts of waste and biomass [1]. Disposal of these wastes must be conducted in a sustainable manner so as not to damage the environment. Even though microbial synthesis is generally greener than chemical synthesis, keen scrutiny of the degree to which the end materials are compatible with living things and their possible impact on the environment must be conducted. The release of engineered living materials in the environment, whether intentional or accidental, may have unexpected effects on ecosystems, biodiversity, and human health [167,168]. Strong risk assessments and control strategies are therefore crucial, but it can be difficult to predict long-term effects. Further research in the form of life-cycle assessments must be conducted to ascertain the effects of microbial synthesis on the environment.

6.4. Ethical Considerations

The emergence of technologies that enable the creation of living or biologically created materials brings forth ethical and societal considerations that require scrutiny. At the forefront is the question of defining “life” itself and the moral status of these novel entities. As we engineer organisms to produce materials with tailored properties, we blur the lines between living and non-living, potentially challenging traditional ethical frameworks. The

potential does arise for these techniques to be used in dual applications, such as the development of biological weapons, raising serious security concerns [17,168–171]. The ability to manipulate biological systems with increasing accuracy requires the establishment of strict regulatory structures and international cooperation to prevent abuse [47,169]. In addition, the social implications of large-scale adoption of live materials should be considered. There is a need to address issues of equity, access, and ownership of technologies to ensure that the benefits are widespread and existing inequalities are not increased [8,47,168]. Public commitment and transparent communication are important for promoting informed dialog in these new and emerging areas of research. It is necessary to move forward with a sense of responsibility and acknowledge that synthetic biology has the potential to reshape our world in profound ways, for better or for worse.

7. Conclusions

This review highlights the potential of synthetic biology for materials design, shifting from traditional, environmentally burdensome methods to sustainable, biologically driven approaches. In particular, this review describes a synthesis of natural mechanisms for material production and technological advancements that enhance endogenous mechanisms, either by scaling up production, reducing the toxicity of products, or developing new applications. By harnessing microbial processes, researchers can create advanced materials with precision and versatility, moving beyond conventional synthesis. Microorganisms can act as programmable biofactories to facilitate the production of inorganic nanostructures and biopolymers with tailored properties. For instance, we have described how magnetotactic bacteria (MTBs) have the ability to synthesize magnetic nanoparticles via both intracellular and extracellular mechanisms. This endogenous ability has further been enhanced by synergistic approaches in genetic engineering, culminating in applications in the field of magnetogenetics. The ability to manipulate microbial species, precursor concentrations, and environmental parameters allows for fine-tuning material characteristics for specific applications. However, challenges related to scale-up, yield, uniformity, and environmental impact remain. We have summarized these considerations and conclusions in Table 2. Future research should prioritize the development of robust biomanufacturing platforms, the expansion of genetic engineering tools, and the encouragement of synergistic approaches to create multifunctional biomaterials.

Table 2. Comparison of traditional and microbial methods of materials synthesis.

Factor	Traditional Synthesis	Microbial Synthesis
Energy Requirements	High temperatures and pressures	Ambient or mild conditions
Environmental Impact	High waste generation and toxic byproducts	Generally low waste and greener processes
Precision/Control	Moderate control over structure/composition	Polydispersity and uniformity remain ongoing challenges; high tunability via genetic/metabolic engineering
Raw Material Source	Petroleum-based and non-renewable	Renewable feedstocks (e.g., sugars and agricultural waste)
Scalability	Established large-scale industrial processes	Still challenging; limited by bioreactor design and yield

Table 2. *Cont.*

Factor	Traditional Synthesis	Microbial Synthesis
Cost	Higher due to being energy and purification intensive	Potentially lower, but requires optimization
Speed of Production	Fast, depending on the method	Slower and dependent on microbial growth and metabolic rates
Material Diversity	Broad, well-studied, and chemically intensive	Growing area of research, influenced by microbe strain
Biocompatibility	Often poor; requires surface modification	Often high, especially for medical/biological applications

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