The Intra and Extracellular Mechanisms of Microbially-Synthesized Nanomaterials and Their Purification

by Ery S. Retnoningtyas

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Abstract

Nanotechnology is the most important scientific breakthrough in the 21st century which has led to changes and advances in various fields of application. Generally, nanomaterials (NMs) with specific shapes, sizes, and compositions are required for nanotechnology. Synthesis of NMs using conventional chemical and physical methods involves high costs, the use of hazardous substances, and environmental damage. In contrast, the green synthesis approach provides a sustainable method for synthesizing NMs such as the utilization of biodegradable waste and microorganisms. Nowadays, microbially-synthesized NMs have been recognized as an effective and eco-friendly method suitable for the large-scale fabrication of biocompatible nanostructures. Various microorganisms such as yeast, fungi, algae, and bacteria can serve as potential stabilizing and reducing agents for synthesizing NMs. This chapter contributes to recent developments in the green synthesis of various NMs using microorganisms, focusing on intracellular or extracellular mechanisms and the purification of NMs. The characterization, applications, and prospects for NMs biosynthesis are also discussed in this chapter.

Keywords: *Nanomaterials, green synthesis, microbes, intracellular, extracellular, purification*

1. Introduction

Nanotechnology, which involves creating functional systems at the molecular level, is one of the scientific and technology fields that is growing the fastest. The word "nanotechnology" has gained enormous traction in recent years due to its numerous uses in agriculture, health, food, textiles, cosmetics, and electronics industries. Nanotechnology is linked to the production of nanomaterials (NMs) with improved properties that distinguish them from bulk materials. NMs consist of one or more components having at least one dimension between 1 and 100 nm, for example, nanoparticles, composite materials, nanofibers, and nano-structured surfaces (Borm et al., 2006; Verma et al., 2019, 2018). NMs have become more prominent in technological breakthroughs due to their superior performance compared to their bulk counterparts in terms of mechanical, electrical, and magnetic behavior, as well as chemical characteristics (Jeevanandam et al., 2018; Lloyd et al., 2011). These NMs can be classified into the following types based on their size and characteristics i.e., carbon-based NMs, composite-based NMs, organic-based NMs, and inorganic-based NMs (Kolahalam et al., 2019; Zhang et al., 2012). Currently, metal-based NMs such as silver (Ag), zinc (Zn), lead (Pb), gold (Au), iron (Fe), carbon (C), and copper (Cu) have attracted great interest among researchers (Khan et al., 2021; Zhang et al., 2023).

The synthesis of NMs can be prepared by various techniques, including a top-down approach and a bottom-up approach (self-assembly). These techniques are further divided into subclasses based on the operation and reaction conditions. The bottom-up approach also known as a building-up process involves constructing a structure atom by atom, molecule by molecule, or by self-arrangements. Techniques such as sedimentation and reduction through green synthesis, spinning, and biochemical synthesis serve as examples of this method. In the top-down approach, physical and chemical techniques are used to reduce the size of the appropriate starting components. NMs have been synthesized using conventional physical techniques such as electrospinning, radiolysis, spray pyrolysis, ultrasonication, and photoirradiation (Bhardwaj et al., 2019, 2018, 2017; Khan et al., 2019) However, chemical techniques have attracted more interest than physical techniques due to their greater ability to control the size and structure of NMs. Sol-gel, solvothermal, co-precipitation, and template-based approaches are the major chemical techniques. The accessible and widely used physical and chemical methods for producing NMs are energy-intensive, contain hazardous chemicals, and require a high temperature for reaction (Abid et al., 2022; Nasaruddin et al., 2021). Although there are many physicochemical ways to synthesize NMs, it is still necessary to develop non-toxic, low-cost, high-yield, low-energy, and eco-friendly methods particularly for applications in the fields of human health and medicine. Therefore, numerous strategies for the bio-based synthesis of NMs have been explored to establish sustainable and cost-effective bioproduction alternatives. For instance, various flavonoids found in biomass waste produced from fruit residues can chelate metal ions and reduce them into nanoparticles (Aswathi et al., 2022; Putro et al., 2022). Several researchers have reported the production of graphene utilizing pulp waste and biodegradable waste from paper cups (Shukla et al., 2020.; Singh et al., 2021).

Other biosynthesis pathways of NMs using microbes involving bacteria, fungi, yeast, and algae have been widely reported due to their reducing characteristics, which are often responsible for reducing metal compounds in particular NMs. Microorganisms can be used in nanotechnology as a green technology for sustainable development strategies due to the use of cleaner production as well as the preservation of natural resources. For instance, fungus-mediated methods include simple procedures for the nano-synthesis of inorganic substances such as $CuAlO_2$ which requires low-temperature conditions (Ahmad et al., 2007). Moreover, fungal biomass was also essential for chemically synthesized BiOCl nanoplates with sizes between 150 and 200 nm to break down into extremely tiny particles (<10 nm) without affecting their crystalline structure (Chung et al., 2016). Researchers have recently exploited a variety of biological extracts to synthesize metallic NMs by following direct techniques and employing microbial extracts as a source of reductants. With the use of biological resources, it is feasible to get the specific size, shape, and monodispersity of NMs either extracellularly or intracellularly (de Jesus et al., 2021). This chapter reviewed the current works in green synthesis of NMs by microbes that focused on their intra and extracellular mechanisms, purification techniques, characterizations, and applications. The difficulties of elaborating this technology at a large-scale level and the prospects of biological synthesis approaches are also highlighted in the last section.

2. Microbially-synthesized of NMs

2.1. Intracellular and extracellular mechanisms

Since the formation of the Earth, biological organisms and inorganic materials have been in continual touch with each other. The interactions between inorganic substances and living things have drawn more attention from scientists in recent years. Numerous microorganisms produce various inorganic compounds either extracellularly or intracellularly, and the mechanisms vary from one organism to another (Fariq et al., 2017; Hulkoti and Taranath, 2014). By using several synthesis components, including microorganisms, plant extracts, and other biological components, NMs are synthesized through biological processes (Saravanan et al., 2021). Due to their ease of cultivation, rapid growth, and potential to thrive under ambient conditions, microbes such as bacteria, algae, yeast, and fungi are typically selected for synthesis in NMs. Interestingly, microbes can detoxify and accumulate heavy metals in the presence of reductase enzymes, which play a crucial role in reducing metal salts into NMs (Ovais et al., 2018). Different biological agents and various metal solutions have varying effects on the production of NMs.

There are two categories for microbial production of NMs. The first category is biosorption, which does not require energy use and involves the attachment of metal ions found in aqueous solutions to the cell wall. Stable NMs are formed as a result of interactions with the cell wall or peptides (Egan-Morriss et al., 2022; Pantidos, 2014). The prospective processes for the biosorption of the metal on microbes consist of physical processes including ion exchange, complexation, precipitation, and physisorption. Microbes typically secrete lipopolysaccharide, glycoprotein, and other exopolysaccharide compounds that have anionic structural groups for positive metal adhering to negative charges of the cell wall. Chitin was shown to be the primary component of the fungal cell wall and it is associated with the complex formation of heavy metals, which leads to the synthesis of NMs (L. Wang et al., 2018). Few researchers have reported the biosynthesis of copper NMs via the biosorption method from *Rhodotorula mucilaginosa* biomass. The spherical form of the produced NMs made them accessible for simultaneous pollution removal and NMs synthesis. The formation of metallic molybdenum NMs by *Clostridium pasteurianum* has also been the subject of another investigation (Nordmeier et al., 2018; Salvadori et al., 2014).

Meanwhile, bioreduction occurs when metal ions are chemically reduced by living organisms into more stable forms. Numerous species can utilize metabolism metal reduction, in which the reduction of a metal ion is linked to the oxidation of an enzyme. As a consequence, stable and inert metallic NMs are formed which may be removed safely from a polluted material. The synthesis of NMs may be triggered by several substances found in microbial cells, notably amides, amines, alkaloids, carbonyl groups, proteins, pigments, and other reducing agents (Quintero-Quiroz et al., 2019; Sable et al., 2020). Some microbes usually release chemicals with a high capacity for oxidation or reduction of metal ions to produce zero valent or magnetic NMs. Additionally, these organisms are easy to handle and susceptible to genetic manipulation (Puspitasari et al., 2021; Puspitasari and Lee, 2021).

It is well known that both intracellular and extracellular proteins, enzymes, lipids, and chelating activity of DNA subunits are actively involved as reducing agents throughout the biosynthesis process. These bioactive substances have high reduction potential and can donate H^+ ions to reduce metal ions from a higher oxidation form to a lower oxidation form (Dauthal and Mukhopadhyay, 2016; Srivastava et al., 2021). According to the site where NMs are generated, extracellular and intracellular synthesis become the most common processes of biosynthesis (Fig.1). NMs can be accumulated in the periplasm, cytoplasmic membrane, and cell wall when observed under a microscope.

In the extracellular approach, NMs are produced outside cells by capturing metal ions on their surfaces and reducing ions in the presence of microbe-secreted enzymes (Li et al., 2011). Cofactors such as reduced nicotinamide adenine dinucleotide (NADH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH) reliant enzymes both have crucial roles as reductants via electron transfer from NADH through NADH-reliant enzymes. For example, the release of NADH and NADH-reliant enzymes is an important process in the extracellular biosynthesis of silver nanomaterials (AgNMs) by microbes. The bioreduction of silver is initiated by NADH-reliant reductase enzymes found in microbes by electron transfer from NADH (He et al., 2007). As a result, silver ions (Ag^+) receive electrons and are reduced (Ag^0), resulting in the generation of enlarged metal nuclei and the formation of stable AgNMs within cell-free supernatant. Precursor concentration, pH, temperature, and reaction time are some limiting factors affecting the size and properties of NMs.

The intracellular approach includes transporting ions into the inner space of microbial cells to produce NMs when the enzymes are present. Microbial cells and sugar molecules are primarily involved in the intracellular process of metal bioreduction. The interactions between intracellular enzymes and positively charged groups are the main mechanism for the trapping of metal ions from the media and their subsequent reduction within the cell. This resulted from

NMs being produced as a result of enzymatic reduction and metal ion transport across membranes (Dauthal and Mukhopadhyay, 2016). In order to release the biosynthesized NMs from intracellular production, additional processes are needed such as ultrasonic treatment or interactions with the appropriate detergents. In contrast, extracellular biosynthesis is inexpensive, requires less complex downstream processing, and supports large-scale production of NMs to investigate its possible uses. Therefore, the extracellular method for biosynthesis of NMs has been the main subject of several studies compared to the intracellular method (Das et al., 2014). An extensive list of the microbes used in synthesizing NMs is provided in Table 1.

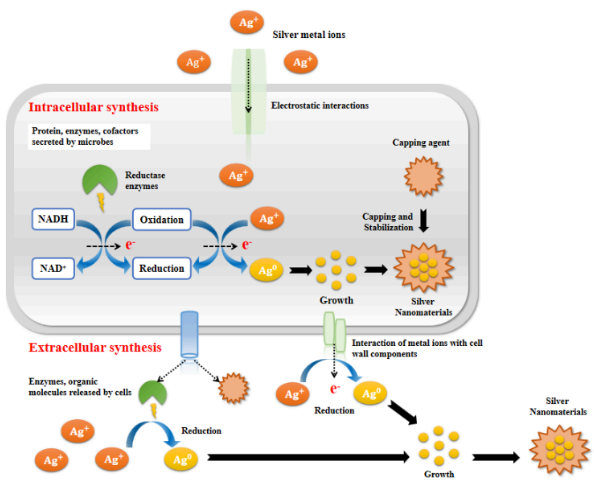

Fig. 1. Biosynthesis of silver nanomaterials via intra and extracellular mechanisms

Table 1. Biosynthesis of various NMs using microbes and their applications

No.	Microbe	Type of nanomaterial	Synthesis location	Physicochemical parameters			Size (nm)	Shape	Application	Reference
				Temperature	pH	Incubation time				
	Bacteria									
1.	*Geobacillus spp*.	Silver (Ag)	Extracellular	55°C	7.5	48 h	<100	Spherical	-	(Cekuolyte et al., 2023)
2.	*Vibrio alginolyticus*	Gold (Au)	Extracellular	40°C	7	14 h	100-150	Irregular	Anticancer and antioxidant	(Shunmugam et al., 2021)
3.	*Marinomonas sp*. ef1	Cooper (Cu)	Extracellular	22°C	-	48 h	10-70	Spherical / ovoidal	Antimicrobial	(John et al., 2021)
4.	*Shewanella loihica* PV-4	Palladium (Pd)	Extracellular	30°C	7	72 h	4-10	Spherical	Catalyst for Cr (VI) reduction	(W. Wang et al., 2018)
5.	*Nocardiopsis flavascens* RD30	Silver (Ag)	Extracellular	30°C	-	72 h	5-50	Spherical	Cytotoxicity	(Ranjani et al., 2018)
6.	*Pseudoalteromonas lipolytica*	Silver (Ag)	Extracellular	28°C	6.5-7	72 h	5-15	Spherical	Dye decolorization	(Kulkarni et al., 2018)
7.	*Shewanella loihica* PV-4	Platinum (Pt)	Extracellular	30°C	7	48 h	2-6	-	Dye decomposition	(Ahmed et al., 2018)
8.	*Desulfovibrio sp*. LS4	Maghemite (Fe_2O_3)	Extracellular	30°C	7.8	35 days	18	Round	Iron nanoparticle formation in saltpan sediment	(Das et al., 2018)
9.	*Enterococcus faecalis*	Selenium (Se)	Extracellular	37°C	7	24 h	29-195	Spherical	Antibacterial	(Shoeibi and Mashreghi, 2017)

10.	*Pseudomonas aeruginosa* JP-11	Cadmium sulfide (CdS)	Extracellular	50°C	-	20 h	20-40	Spherical	Cadmium removal from aqueous solution	(Raj et al., 2016)
	Fungi									
1.	*Penicillium oxalicum*	Silver (Ag)	Extracellular	28°C	-	24 h	10-50	Spherical	Antimicrobial, anticancer, antioxidant	(Gupta et al., 2022)
2.	*Trichoderma longibranchiatum*	Silver (Ag)	Extracellular	55°C	7	24 h	5-50	Spherical	Biosafety assessment	(Cui et al., 2022)
3.	*Periconium sp.*	Zinc oxide (ZnO)	Extracellular	45°C	5	24 h	16-78	Quasi-spherical	Antioxidant, antibacterial	(Ganesan et al., 2020)
4.	*Lignosus rhinocerotis*	Gold (Au)	Extracellular	65°C	4.5	2.5 h	49.5-82.4	Spherical	Antibacterial	(Katas et al., 2019)
5.	*Trichoderma asperellum*	Copper oxide (CuO)	Extracellular	40°C	-	24 h	110	Spherical	Photothermolisis on human lung carcinoma	(Saravanakumar et al., 2019)
6.	*Rhodotorula mucilaginosa*	Silver (Ag)	Extracellular	25°C	7	168 h	13.7	Spherical	Antifungal, catalyst, cytotoxicity	(Cunha et al., 2018)
7.	*Aspergillus niger*	Zinc oxide (ZnO)	Extracellular	32°C	6.2	48 h	53-69	Spherical	Antibacterial, dye degradation	(Kalpana et al., 2018)
8.	*Penicillium chrysogenum*	Platinum (Pt)	Extracellular	100°C	-	12 h	5-40	Spherical	Cytotoxicity	(Subramaniyan et al., 2017)
9.	*Cladosporium cladosporioides*	Gold (Au)	Extracellular	30°C	7	48 h	60	Round	Antioxidant, antibacterial	(Joshi et al., 2017)
10.	*Rhizopus stolonifer*	Silver (Ag)	Extracellular	40°C	-	48 h	2.86	Spherical	-	(AbdelRahim et al., 2017)
	Yeast									

1.	*Saccharomyces cerevisiae*	Iron oxide (Fe_2O_3)	Extracellular	30°C	-	2-3 days	70-100	Spherical	Antimicrobial	(Asha Ranjani et al., 2022)
2.	*Pichia kudriavzevii*HA	Silver (Ag)	Extracellular	30°C	-	72 h	29.6-30.14	Round /cubic	Anticancer	(Ammar et al., 2021)
3.	*Saccharomyces cerevisiae*	Silica	Intracellular	29°C	6-11	1 h	40-70	Spherical	Oil recovery	(Zamani et al., 2020)
4.	*Saccharomyces cerevisiae*	Silver (Ag)	Intracellular	25°C	7	24 h	2-20	Spherical	Biocatalyst	(Korbekandi et al., 2016)
5.	*Magnusiomyces ingens* LH-F1	Gold (Au)	Extracellular	30°C	-	24 h	80.1	Sphere/ triangle/ hexagon	Catalytic reduction of nitrophenols	(Zhang et al., 2016)
	Algae									
1.	*Spirogyra hyalina*	Silver (Ag)	Extracellular	60°C	-	24 h	52.7	Spherical	Antimicrobial	(Abdullah et al., 2022)
2.	*Coelastrella aeroterrestrica*	Silver (Ag)	Extracellular	30°C	-	24 h	14.5	Hexagon	Antimicrobial, anticancer, antioxidant	(Hamida et al., 2022)
3.	*Padina sp.*	Silver (Ag)	Extracellular	60°C	-	48 h	25-60	Spherical /oval	Antibacterial	(Bhuyar et al., 2020)
4.	*Colpomenia sinuosa*	Iron oxide (Fe_3O_4)	Extracellular	30°C	2	1 h	11.24–33.71	Nano spheres	Antibacterial, antifungal	(Salem et al., 2019)
5.	*Spirulina platensis*	Palladium (Pd)	Extracellular	70°C	-	20 min	10-20	Spherical	Adsorbent	(Sayadi et al., 2018)

2.2. Synthesis of NMs using bacteria

Bacteria have become one of the most useful research subjects due to their abundance in the environment and their ability to endure harsh circumstances. Additionally, they can grow rapidly and their cultivation is easy to control, such as temperature, pH, oxygenation, and incubation time. Optimizing these conditions is crucial since different sizes of NMs are needed for various applications including optics, catalysts, and antimicrobials (He et al., 2007). Bacteria typically produce intracellular or extracellular inorganic substances, which can be employed for the biosynthesis of NMs. *Bacillus marisflavi* was shown to produce AuNMs with a particle size of 14 nm. AuNMs synthesis from bacterial cell-free extract occurred extracellularly and the color changed from light yellow to bluish-purple. The production of AuNMs was indicated by the presence of bluish-purple color caused by surface plasmon resonance (Nadaf and Kanase, 2019).

2.3. Synthesis of NMs using fungi

Researchers across the world frequently utilize fungi for NMs synthesis using both intracellular and extracellular processes. It is well known that using fungi to produce metal oxide or NMs is an effective technique with clear morphology (Ijaz et al., 2020). Fungi produce more NMs than bacteria because their intracellular enzymes function as biological substances that increase the bioaccumulation capacity and metal resistance (Kalpana and Devi Rajeswari, 2018). Significant advantages include the ease of scaling up and downstream processing, economic feasibility, and the presence of mycelia which supplies a high surface area (Mohanpuria et al., 2008). The most well-known fungi for synthesizing silver and gold nanomaterials are *Fusarium sp., Penicillium sp.,* and *Aspergillus sp*. (Shah et al., 2015). The extracellular production of AgNMs was carried out using *Penicillium sp*. The enzyme induction was facilitated by the existence of silver nitrate in the cell culture broth and optimal synthesis was shown at pH 6 with a substrate concentration of about 1.5 mM (Shareef et al., 2017; Spagnoletti et al., 2019).

2.4. Synthesis of NMs using yeast

Due to their improved function and stability, yeasts have been considered a highly efficient source of NMs synthesis. Additionally, they can capture large amounts of potentially toxic metals. The present study on yeast focuses mostly on the production of nanocrystalline quantum semiconductors, notably cadmium sulfide (CdS) and zinc sulfide (ZnS) nanomaterials. The biosynthesis of silver and gold NMs was mainly carried out by *S. cerevisiae* and other silver-resistant yeast strains (Korbekandi et al., 2016). The production of silica NMs is another use of *S. cerevisiae* in the nanomaterial generation process. The NMs were produced when yeast extract and sodium silicate (precursor solution) were added. One potential mechanism involves the interaction of yeast extract and sodium silicate in an aqueous medium to generate sodium hydroxide and silica oxide NMs (Zamani et al., 2020).

2.5. Synthesis of NMs using algae

It has been reported that algae play a significant part in the biological synthesis of NMs and the buildup of certain toxic metals. Large-scale algae production is mostly utilized to synthesize gold, silver, and possibly zinc oxide NMs. Algae are recognized for their capacity to transform toxic metals into their harmless equivalents (Ong et al., 2021). For example, *Sargassum muticum* was employed in the production of ZnO NMs and was found to have anti-apoptotic and anti-angiogenesis properties in $HepG_2$ cells (Yang and Cui, 2008). Furthermore, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were effectively inhibited by the NMs, with inhibition zones of 13.33 mm and 15.17 mm, respectively (Bhuyar et al., 2020).

3. Purification methods of biosynthesized NMs

The biosynthesized NMs can be purified by several methods including chromatography, magnetic fields, density gradient centrifugation, and electrophoresis (Table 2).

3.1. Chromatography

Chromatography is a method for separating mixtures of substances based on variations in how fast the different components spread through a given media. These media are the stationary phase and mobile phase. The stationary phase can be solid or liquid while the mobile phase can be liquid or gas. This chromatography can be used for purification and separation in the biosynthesis of NMs. Several uses of chromatographic methods in the purification of NMs synthesis are described. Current researchers widely use intracellular enzymes in producing AuNM for various applications (Gholami-Shabani et al., 2015). The enzyme is an agent in reducing the metal NMs to be stable material. Enzymes produced by microbes (e.g., *Acinetobacter sp*.) extracellularly and intracellularly after purification by anion exchange and gel filtration chromatography were used to produce Au and Se nanomaterials (Wadhwani et al., 2018).

3.2. Magnetic fields

Magnetic fields are purification methods that use magnetic properties to separate and purify NMs, particularly iron (Fe) NMs. One magnetotactic bacteria is *Magnetospirillum gryphiswaldense*, which can move along magnetic field lines due to magnetosomes (MagMn). Magnetosomes produced by intracellular bacteria are membrane-enclosed single-domain ferromagnetic NMs (Rosenfeldt et al., 2021). The purification of synthetic materials containing Fe by bacteria consists of 2 stages: (1) cell wall breakdown and (2) separation-purification. For the breakdown of cell walls, sonification and ultracentrifugation methods can be used, while column-based magnetic (neodymium magnet) can be used for the separation-purification method (Hamdous et al., 2017; Raschdorf et al., 2018; Rosenfeldt et al., 2021).

3.3. Density gradient centrifugation

Density gradient centrifugation is the simple purification method of NMs extracellular synthesis. The process of centrifugation is used to separate particles from a solution based on their size, shape, density, medium viscosity, and rotor speed. The density gradient centrifugation method may be required more than once in some cases. For example, *Nocardiopsis sp*. cultures were centrifuged at 10,000x g, 4ºC for 10 min up to three times after incubation, and 5 ml of each strain's cell-free supernatant was then subjected to 50 ml of an aqueous solution containing $1x10^{-3}$ M $HAuCl_4.3H_2O$. Subsequently, the samples were centrifuged again at high speed after the reaction for a certain time to separate the produced AuNMs (Manivasagan et al., 2015). Extracellular purification of AgNMs synthesized using *Bacillus subtilis* can be performed by centrifugation method at 10,000 rpm for 5 minutes twice (Alsamhary, 2020).

3.4. Electrophoresis

Electrophoresis is the term used to describe the movement and separation of charged particles (ions) caused by electric fields. Two electrodes (anode, cathode) with opposing charges are joined by a conducting liquid known as an electrolyte to form an electrophoretic system. Agarose gel electrophoresis is usually used to purify and separate NMs based on size and shape. For example, one percent agarose gel electrophoresis (Bio-Rad) was used to purify AgNMs generated by fungi isolated from mangroves (Rodrigues et al., 2013). Another work on AgNMs that utilized amplified DNA fragments from *Streptomyces sp*. was separated using TBE buffer containing ethidium bromide (1 g/mL) on 1% agarose gel electrophoresis

(Mabrouk et al., 2021). The synthesis of AgNMs by *Staphylococcus aureus* can be carried out intracellularly and extracellularly so that the purification process requires cell wall lysis (Triton-X100), as well as separation using centrifugation and gel electrophoresis (Amin et al., 2019).

Table 2. Purification methods of biosynthesized NMs by various microbes

Type	Microbe	NMs	Synthetic location	Purification method	Application	Reference
Chromatography						
Fungi	*Talaromyces purpurogenus* (pigment)	Ag	Extracellular	Two steps: -Centrifugation (6,700xg, 4°C, 20 min) - Thin Layer Chromatography	Biomedical	(Bhatnagar et al., 2022)
Bacteria	*Acinetobacter sp*. (lignin peroxidase)	Au, Se	Extracellular	Two steps sequentially: - Anion exchange chromatography - Gel filtration chromatography (lignin peroxidase)	Biocatalyst	(Wadhwani et al., 2018)
Bacteria	*Escherichia coli* (sulfite reductase)	Au	Extracellular	Two steps: - Column chromatography (sulfite reductase) - Centrifugation (80,000xg, 20 min) (mixed sulfite reductase AuNMs)	Biocatalyst	(Gholami-Shabani et al., 2015)
Bacteria	*Pseudomonas aeruginosa* (rhamnolipids)	Ag	Extracellular	Two steps: - Gel column chromatography (rhamnolipids) - Centrifugation (mixed rhamnolipids - AgNMs)	Biosurfactant	(Ganesh et al., 2010)
Magnetic Fields						
Bacteria	*Magnetospirillum magneticum*	Mag Mn	Intracellular	Two steps: - Centrifugation (8,000xg, 10°C, 20 min) - Neodymium magnets	Magnetic tumor targeting	(Designed Research; K, 2022)

Bacteria	*Magnetospirillum gryphiswaldense*	Mag Mn	Intracellular	Two steps: - Column-based magnetic - Ultracentrifugation	Biomedical and Biotechnology	Rosenfeldt et al., 2021)
Fungi	*Mixed fungi*	Fe_3O_4	Intracellular	Two steps: - Centrifugation (500 rpm, 10°C, 20 min) - Permanent magnets	Cleaning agent	(Sayed et al., 2021)
Fungi	*Aspergillus niger*	FeS and Fe_3O_4	Intracellular	Permanent magnets	Biomedical	(Abdeen et al., 2016)
Density gradient Centrifugation						
Fungi	*Aspergillus flavus*	Fe	Extracellular	Centrifugation (5000 rpm, 5 min)	Extraction and Clarification	(Hassan et al., 2022)
Bacteria	*Bacillus subtilis*	Ag	Extracellular	Centrifugation twice (10,000 rpm, 5 min)	Antibacterial	(Alsamhary, 2020)
Bacteria	*Actinomycetes sp.*	Ag	Extracellular	Centrifugation (15,000 rpm, 15 min)	Antimicrobial	(Al-Dhabi et al., 2018)
Fungi	*Pleurotus ostreatus* (Laccase)	Au	Extracellular	Centrifugation (2415xg, 15 min, 4°C)	Decolorization	(El-Batal et al., 2015)
Electrophoresis						
Bacteria	*Streptomyces spiralis; Streptomyces rochei*	Ag	Extracellular	Agarose gel electrophoresis 1%	Antibacterial	(Mabrouk et al., 2021)
Fungi	*Aspergillus tubingensis; Bionectria ochroleuca*	Ag	Extracellular	Electrophoresis (sodium dodecyl sulfate-polyacrylamide gel)	Antimicrobial	(Rodríguez-González et al., 2020)
Bacteria	*Staphylococcus aureus*	Ag	Intracellular and Extracellular	Agarose gel electrophoresis 0.7%	Biosensors	(Amin et al., 2019)

4. Characterization of biosynthesized NMs

Biosynthesized nanomaterials characterizations were determined by various techniques, such as spectroscopic technique, microscopic technique, and diffraction technique. Nanomaterials characterization play a huge role in various application of nanomaterials. Each technique has a different purpose, methods, and instruments, which will be discovered below.

4.1. Spectroscopic techniques

The spectroscopic technique is a measurement to examine the content of the materials, specifically nanomaterials and the surface properties in a mixture solution. It uses various types of instruments, such as UV-Vis Spectroscopy, Fourier Transform Infra-Red (FTIR), and Raman Scattering which have distinctive methods. UV-Vis Spectroscopy aims to detect and monitor the size and shape of metal ions of NMs with particle sizes between 2 nm to 100 nm (Begum et al., 2018; Kumar et al., 2020). Another spectroscopy technique commonly used in NMs is FTIR, to observe the functional group, composition, and inter interaction of molecules (Alessio et al., 2017; Kamnev et al., 2021). In addition, FTIR could identify and classify several microorganisms, such as *Bacillus* (Procacci et al., 2021), *Escherichia coli* (Farouk et al., 2022), Pseudomonas (Lee et al., 2019), and *Staphylococcus aureus* (Hong et al., 2022).

4.2. Microscopic techniques

The microscopic technique is used to determine the physical morphology, texture, and size of the NMs. Several instruments included microscopic techniques, such as the optical microscope, Scanning Electron Microscope (SEM), and Transmission Electron Microscope (TEM). SEM performs morphology, size, and shape of nanoparticles between 0.001 to 5 μm (Maheshwari et al., 2018). In addition, compositional information could be collected by Energy Dispersive X-Ray (EDX) and mapping analysis with an SEM instrument. TEM could observe material with a particle size of up to 1 nm due to high image resolutions, thus real size and structures are detected (Sierra, 2019). The NMs microbially synthesized keep developing with various raw materials, microorganisms, and methods to acquire wider and better applications of NMs. Moreover, High Resolution-TEM (HR-TEM) can provide the morphology of the samples and identify the crystal structure from the atomic scale to thin layer of samples (Javed et al., 2018). All SEM, TEM, and HR-TEM perform best in solid samples, usually powder, fiber, and membrane.

4.3. Diffraction techniques

One of the diffraction techniques well-known in NMs characterization is X-Ray Diffraction (XRD), which provides data on the crystallography and structure of the material, also the lattice parameter of samples (Mourdikoudis et al., 2018). Various peaks in the 2θ range show different molecules, for example, Ag nanoparticles appear at 27.81º, 32.16º, 38.12º, 44.3º, 46.21º, 54.83º, 57.39º, 64.42º, and 77.45º (Meng, 2015); while TiO_2 nanoparticles show peaks at 25.23º, 37.71º, 47.72º, and 62.54º (Toro et al., 2020). XRD performs well in solid, dry, and homogeneous materials. However, for suspension of NMs, measurement of hydrodynamic diameter could be conducted by Dynamic Light Scattering (DLS). Liquid NMs with high viscosity, such as liposomes (Zong et al., 2022), polymeric micelles (Ghezzi et al., 2021), nano gels (Ahmed et al., 2020; Pourjavadi et al., 2020), and microemulsion (Gunarto et al., 2020) are required for dilution to have an accurate measurement.

5. Challenges and limitations

The NMs are produced from various sources of microbes and have been developed rapidly since the 21st century. Over the years, different methods, sources, and analyses have been carried out and resulted in different types of NMs based on their structure and sizes. However, obtaining homogeneous NMs with the same methods and type of microbe is still challenging due to the unpredictable growth and ability of the microbes. Therefore, more experiments are essential in determining and observing the microorganism in NMs systems. Purification steps of NMs by either intra or extracellular are considered expensive on an industrial scale as the process requires advanced equipment like nanofiltration to enhance the purity of NMs. Another limitation in NMs microbially-synthesized is an insufficient yield. However, the discovery of a cost-effective NMs biosynthesis alternative can be carried out by utilizing waste materials.

6. Conclusions and future outlook

In this chapter, green and sustainable approaches of microbially-synthesized nanomaterials was summarized, as well as the intra-extracellular mechanisms and purification methods of NMs. Nanomaterials are synthesized by several types of microbes, such as bacteria, fungi, yeast, and algae. Several researchers are manipulating the DNA of microbes to improve the yield of NMs. In addition, the combination of synthesis mechanism, intra-extracellular in a system is likely to produce a higher amount of nanomaterial. However, it required an established and complete process of purification for industrial production. On the other hand, utilization of NMs specifically in medical applications is possibly over-absorbed due to their tiny size and excellent efficient absorption towards the human body.

7. References

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